

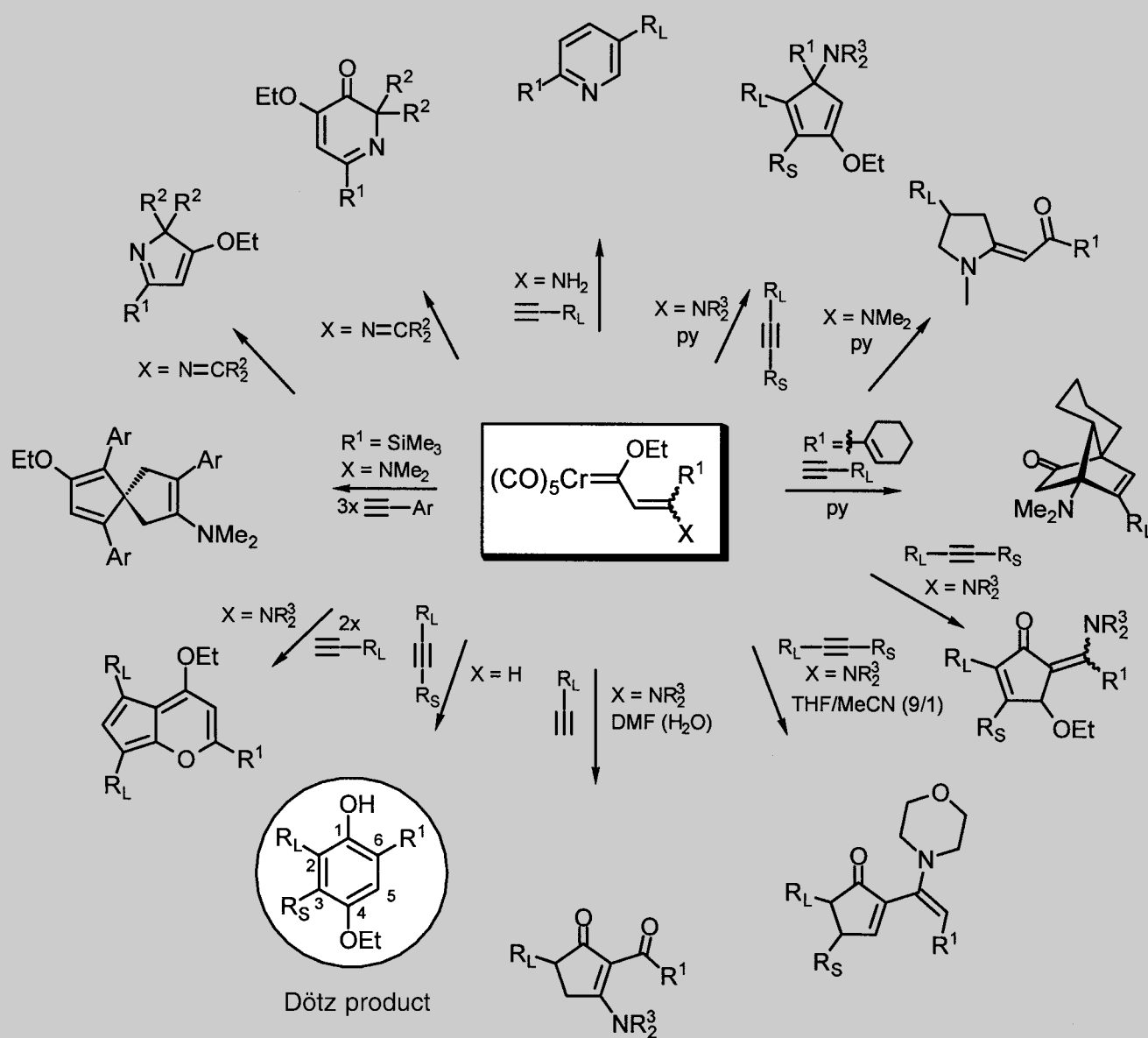
Cover Picture

Nguyet T. Tran, Douglas R. Powell, and Lawrence F. Dahl

The cover picture shows a remarkable Pd₁₄₅ nanocluster, whose metal-core geometry was unambiguously characterized from complete analyses of crystallographic X-ray data. This unprecedented close-packed multishell carbonyl metal cluster was isolated from the reduction of a monomeric square-planar palladium precursor [Pd(PEt₃)₂Cl₂]. The structure is made up of three distinct shells, the outermost of which possesses 60 equivalent vertices along with 12 pentagonal, 20 equilateral triangular, and 30 square faces; this semiregular (Archimedean) polyhedron, named rhombicosidodecahedron (with Schläfli symbol 3.4.5.4), is a hitherto crystallographically unknown stereoisomer of the universally familiar C₆₀ buckyball, an icosahedrally truncated semiregular polyhedron of *I_h* symmetry with 60 equivalent vertices and 12 pentagonal and 20 hexagonal faces. More about this fascinating nanocluster can be found in the communication by L. Dahl et al. on p. 4121 ff



Just a Section of the Incredible Range
of Products from α,β -Unsaturated
Carbenepentacarbonylchromium Complexes



Fischer Carbene Complexes as Chemical Multitalents: The Incredible Range of Products from Carbenepentacarbonylmetal α,β -Unsaturated Complexes

Armin de Meijere,* Heiko Schirmer, and Michael Duetsch

Dedicated to Professor Henning Hopf on the occasion of his 60th birthday

The metal carbene complexes, discovered by E. O. Fischer at the start of the 1960s and carrying his name, have since proved themselves to be irreplaceable building blocks for organic synthesis. In particular, since the discovery of the Dötz reaction, a formal cycloaddition of Fischer α,β -unsaturated carbene complexes to alkynes with CO insertion, this area of chemistry has become increasingly interesting to organic chemists. In spite of the considerable diversity of reactions performed with these complexes, proper selection of substrates and careful adjustment of the reaction conditions

have allowed, in most cases the perfectly selective preparation of individual compounds of this enormous range of products. The spectrum of new successes begins with the conventional Diels–Alder reaction of alkynylcarbene complexes and the formal regioselective [3+2] cycloaddition of alkenylcarbene complexes to alkynes. It extends much further, however, from cascade reactions with the formation of oligofunctional and oligocyclic products of impressive molecular complexity to complex, formal [3+6] cocyclizations in which six bonds are formed in a single operational step. Beyond doubt,

the methodological arsenal of preparative organic chemistry cannot be imagined any more without the valuable transformations of the Fischer carbene complexes; it only remains to be seen whether one or other of the numerous new types of cocyclization products of these complexes can establish itself as a lead structure in the search for biologically active compounds.

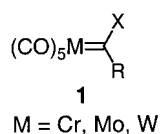
Keywords: alkynes • carbene complexes • cycloadditions • domino reactions • insertion

1. Introduction

A feature of a whole series of organometallic compounds is their ability, with the same type of substituent, to afford a spectrum of product types under diverse external conditions and with a range of different reaction partners. With proper selection of all variables the manifold reactions of such organometallic compounds can take place each time with high selectivity. Such classes of compounds can be justifiably described as “chemical multitalents”. Their transformations are particularly valuable for the repertoire of synthetic methods whenever these reactive intermediates are readily available and manipulable. One example of such a class of organometallic compounds is beyond doubt that of the Fischer carbene complexes, which were first described by

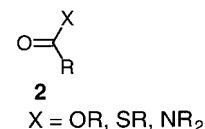
Fischer and Maasböl in 1964.^[1, 2] Initially they were only hesitantly introduced into preparative organic chemistry, but eventually began to achieve an established place for themselves in synthetic strategies, particularly through the discovery of the so-called Dötz reaction in the middle of the 1970s.^[3] Initially the Dötz reaction served mainly for the synthesis of naphthoquinone frameworks by the formal cycloaddition of phenylcarbene complexes to alkynes with CO insertion under very mild conditions. Since then a whole range of cocyclization products from the reactions of α,β -unsaturated carbene complexes with alkynes have been isolated and characterized. The range of 21 different product types obtained from the phenyl- and alkenylcarbene complexes of Group VI metals in reactions with alkynes mentioned by Wulff et al.^[4] is by far no longer up to date. Moreover, even though a small change to the carbene ligand, the reaction conditions, or the alkyne used, can lead to different products, it is a feature of these reactions that with the proper combination of all these factors only one type of product is formed, usually with high selectivity in each case. It is precisely this which makes the reactions of the Fischer carbene complexes so interesting and so valuable for organic synthesis.

[*] Prof. Dr. A. de Meijere, Dr. H. Schirmer, Dr. M. Duetsch
Institut für Organische Chemie der Georg-August-Universität Göttingen
Tammannstrasse 2, 37077 Göttingen (Germany)
Fax: (+49) 551-399475
E-mail: Armin.deMeijere@chemie.uni-goettingen.de



That with their help, that is, within the coordination sphere of the metals, the most varied of carbo- and heterocyclic compounds can be constructed has definitely contributed to the constantly increasing popularity of the Fischer carbene complexes **1**. However, it must not be overlooked that more conventional organic chemistry can also be readily carried out on the ligands. Because of the strong electron-withdrawing character of the pentacarbonylmethyl group—this is a charac-

teristic of the Fischer carbene complexes which at the same time differentiates them from the Schrock class of carbene complexes^[5]—depending upon the nature of the heteroatom at the carbon center they can be regarded as analogues **2** of esters, thioesters, or amides.^[6]



A few of the typically ester-like features are the pronounced α -CH acidity—with a $\text{p}K_{\text{a}}$ value of 8 for the pentacarbonyl(methoxymethylcarbene)chromium

Armin de Meijere was born in Homberg (Niederrhein) in 1939. He studied chemistry at the Universities of Freiburg and Göttingen, and he obtained his doctorate under the supervision of Professor Dr. Wolfgang Lüttke in 1966. He spent a period of two years as a postdoctoral research fellow at Yale University in New Haven, CT, USA with Professor K. B. Wiberg, and he obtained his Habilitation at the Universität Göttingen in 1971. In 1977 he was appointed *Ordentlicher Professor*



A. de Meijere



M. Duetsch



H. Schirmer

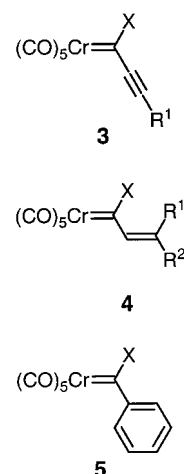
für Organische Chemie at the Universität Hamburg. In October 1989 he returned to the Universität Göttingen as Professor für Organische Chemie and successor to his former mentor. Over the years he has been Visiting Professor at the University of Wisconsin in Madison, WI, the IBM Research Laboratories in San José, CA, at the Technion in Haifa, Israel, Princeton University in Princeton, NJ, the Université d'Aix Marseille III, in Marseille, France, the Università degli Studi in Firenze, Italy, the Ecole Normale Supérieure in Paris, France, the Université de Paris-Sud in Orsay, France, the University of Colorado in Boulder, CO, and the University of Florida in Gainesville, FL. Until 1963 he was *Stipendiat der Studienstiftung des deutschen Volkes*, he was awarded the *Dozenten-Stipendium des Fonds der Chemischen Industrie* in 1972, and in 1992 he was appointed a member of the Norwegian Academy of Sciences and Letters. He was awarded the Gay Lussac-Alexander von Humboldt Award of the French Ministère de l'Éducation Nationale, de la Recherche et de la Technologie. In 1997 he was appointed as a member of the Braunschweigische Wissenschaftliche Gesellschaft, Honorary Professor of the St. Petersburg State University and Fellow of the Japan Society for the Promotion of Science. He is editor or member of the editorial board of a series of scientific journals, including *Chemical Reviews*, periodicals, and books. His scientific achievements have been published in over 420 original communications, review articles, and book contributions. His current research interests are: the development of new domino reactions for the efficient construction of complex structures and new small-ring compounds as synthesis building blocks including their use in the synthesis of natural and nonnatural compounds; new highly strained polycyclic compounds with interesting properties; the application of organometallic complexes and catalysts in organic synthesis, currently mainly palladium-catalyzed domino reactions as well as titanium induced cyclopropanations and other transformations of carbonyl compounds; carbon compounds and organometallic complexes with unconventional chemical and physical properties.

Michael Duetsch, born in Hamburg in 1966, concluded his studies at the Universität Hamburg under Professor Armin de Meijere with a *Diplomarbeit* on Michael additions to alkynylcarbene complexes. He followed Professor de Meijere to the Universität Göttingen where he received his doctorate with a dissertation on donor-substituted vinylcarbene and homovinylcarbene complexes of chromium and tungsten in 1993. He then held a postdoctoral research fellowship with Professor H. B. Kagan at the Université Paris-Sud with research work in the area of asymmetrically induced oxidation of sulfides to sulfoxides mediated by chiral titanium complexes. He joined the research department of Th. Goldschmidt AG, Essen in 1994.

Heiko Schirmer, born in Leinefelde (Thüringen) in 1971, studied chemistry at the Universität Göttingen and the Universidade Estadual de Campinas, in Campinas, Sao Paulo, Brazil. He gained his doctorate in 1999 under Professor Armin de Meijere with a dissertation on β -aminosubstituted α,β -unsaturated Fischer carbene complexes. He accepted a position at the Schering AG in Berlin.

complex^[7a] it is even considerably greater than that of ethyl acetate ($pK_a = 24.5^{[7b]}$)—and nucleophilic substitution by addition/elimination at the carbene C atom by amines,^[8] thiols, alcohols,^[9] and also allyllithium compounds.^[10] As would be expected, multiple bonds neighboring the carbene center, as in complexes **3–5**, are also activated towards attack by nucleophiles.

Particularly versatile are nucleophilic additions to the triple bond of the alkynylcarbene complexes **3** resulting in the α -alkenylcarbene complexes **4** which for their part can participate predominantly in formal cycloaddition reactions with alkynes with the inclusion of the carbene center and possibly a



CO ligand. Whereas the reactions of complexes **3** are dominated by the strong polarization of the triple bond, in complexes **4** and the analogous aryl complexes **5** the metal center also always plays a role by coordinating with possible reaction partners or stabilizing reactive intermediates by coordination.

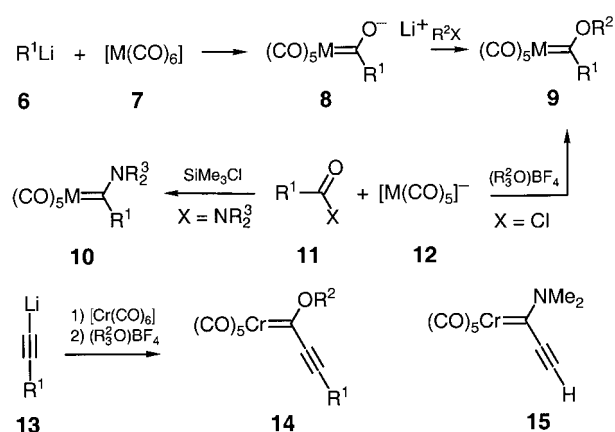
Thirty five years after the discovery of the Fischer carbene complexes and more than fifteen years after the last comprehensive review on this topic in this journal^[2b] it appeared appropriate to us to summarize the rapidly growing amount of work in this area in an orderly fashion. This contribution should serve to lift the confusion caused by the

manifold reactions of the multitasking Fischer carbene complexes from those less familiar with this chemistry, and to encourage others to consider the numerous novel possibilities of the readily assembled α,β -unsaturated Fischer carbene complexes for the elegant solution of synthetic problems.

Only the characteristic reactions of the three complex types **3**, **4**, and **5** will be treated in this review, and only those of the respective chromium complexes. On the one hand, their reactions are almost certainly the most extensively investigated, and on the other the change to molybdenum or tungsten complexes usually brings about only a change in the distribution of the product types known from the chromium complexes.^[4]

2. Alkynylcarbenechromium Complexes

Carbene complexes of the Group VI metals are prepared almost exclusively by two procedures. Either an allyllithium compound **6** is added to the respective hexacarbonylmetal complex **7**^[11] and the metallaacylate **8** thus formed is trapped with an electrophile,^[12] or an alkali metal or ammonium pentacarbonylmetalate **12** is reacted with the corresponding carboxylic acid chloride or amide.^[13] After addition of the Meerwein salt the adduct from the acid chloride eliminates chloride to form the alkoxy carbene complexes, whereas trapping the amide adduct with chlorotrimethylsilane leads to the aminocarbene complex. Alkynylcarbenechromium complexes of type **14** are accordingly obtained easily by reaction of the particularly readily available 1-lithio-1-alkynes

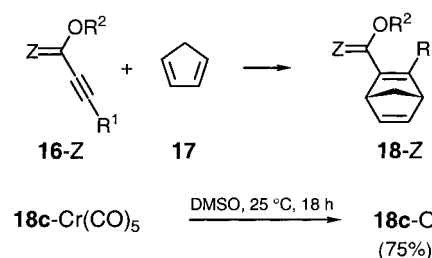


13 with hexacarbonylchromium with subsequent alkylation with trimethyl- or triethyloxonium tetrafluoroborate.^[14, 15] A large number of such α -alkynylcarbene complexes **14** with a wide variety of substituents R^1 have since been prepared, but the isolation of a terminally unsubstituted propynylidene complex has only relatively lately been achieved along an indirect route, and then only in the form of the 1-dimethyl-amino derivative **15**.^[16]

2.1. [4+2] and [2+2] Cycloadditions

2.1.1. With Dienes and Electron-Rich Alkenes

In view of the strong electron withdrawing influence of the pentacarbonylmetal moiety on the carbene ligand in Fischer carbene complexes it appeared obvious to use α,β -unsaturated complexes of this type as dienophiles in Diels–Alder reactions. The investigations concerning the dienophilic nature of 1-alkynylcarbene complexes and the regioselectivity of their Diels–Alder reactions with dienes extend until well into the 1990s.^[17] 1-Alkynylcarbene complexes are also significantly better dienophiles than the corresponding esters of propynoic acid (Scheme 1).^[18–20] Thus, the propynylidene-chromium complexes **16** [$Z = Cr(CO)_5$] add to cyclopentadiene (**17**) in yields of 73–91%, even at room temperature, whilst the esters **16** ($Z = O$) only react above 170 °C, if at

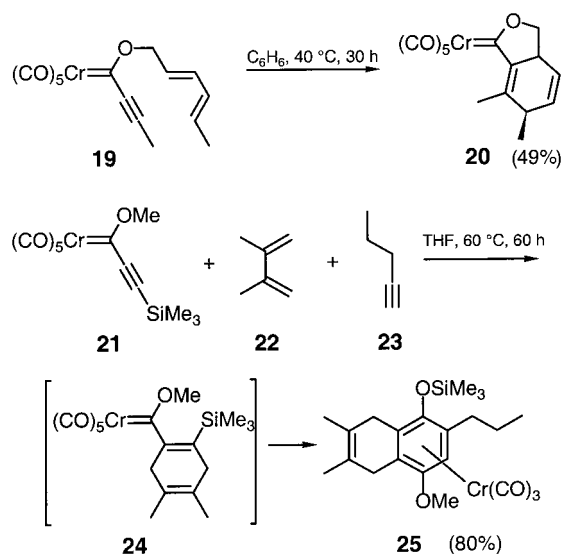


16	R^1	$T [^\circ C]/t [h]$	Z	18-Z		$T [^\circ C]/t [h]$	Z	18-Z	
					[%]				[%]
a	Me	170/1	O	22		25/2	$Cr(CO)_5$	85	
b	$SiMe_3$	190/24	O	66		25/0.75	$Cr(CO)_5$	91	
c	cPr	180/24	O	0		25/17	$Cr(CO)_5$	73	

Scheme 1. Comparative reactivities of 1-alkynylcarbene complexes and the corresponding esters. $R^2 = Me, Et$.

all.^[18, 19] Such comparisons are legitimate precisely because Fischer carbene complexes may be oxidized to the corresponding carboxylic acid derivatives under very mild conditions.^[21] Thus, for example the cyclopropyl substituted norbornadienylcarbenechromium complex **18c**-Cr(CO)₅ is transformed into the ester **18c**-O in a yield of 75% simply by stirring in dimethylsulfoxide.^[20a]

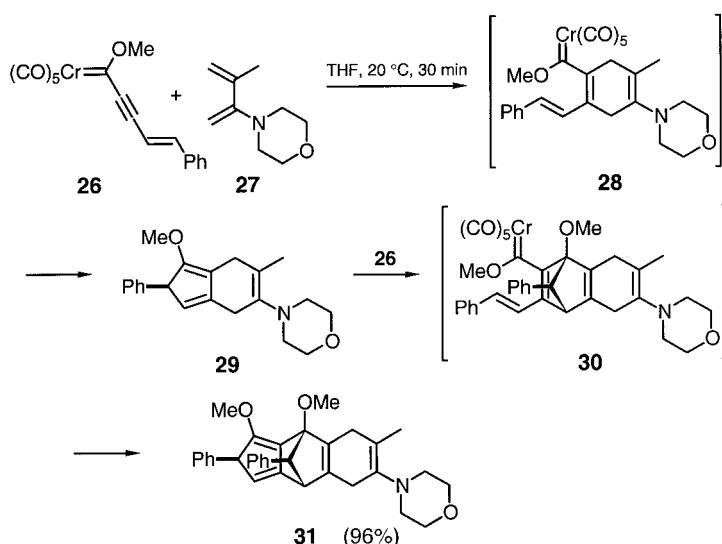
Intramolecular variants of such Diels–Alder reactions lead to bicyclic compounds with a pentacarbonylcarbenechromium moiety.^[17g–i, 21a, 22] In the example of the hexadienyloxypropynylidene carbene complex **19** the dienyl group is connected by way of the oxygen substituent on the carbene C atom; in benzene solution **19** gives the Diels–Alder product **20** in a yield of 49% after 30 h at 40 °C.^[19a] The combination of different types of reactions in reaction



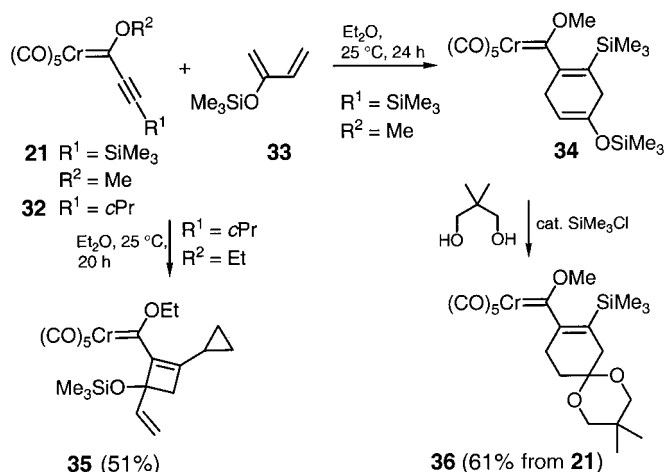
cascades is also possible with carbene complexes. Thus, cycloaddition of the alkynylcarbene complex **21** to 2,3-dimethylbutadiene (**22**) gives the α,β -unsaturated carbene complex **24** with a cyclohexa-1,4-diene moiety which immediately reacts with the added 1-pentyne (**23**) in a Dötz-type reaction. In this one-pot transformation the complexed dihydronaphthalene derivative **25** could be obtained by crystallization from the crude product in a yield of 80%.^[18]

A further increase in the number of interconnected reaction steps is demonstrated by the sequential transformation beginning with the [4+2] cycloaddition of 2-methyl-3-morpholinobuta-1,3-diene (**27**) to the alkynylcarbene complex **26** reported by Barluenga et al.,^[23] in which the initial product **28** cyclizes to the cyclopentadiene derivative **29**. This adds to a further molecule of **26** in a [4+2] cycloaddition, and the product cyclizes once more to afford the cyclopentadiene derivative **31**. The highly substituted tetracycle **31** results with formation of six new C–C bonds, and is isolated in 96% yield.

However, 2-(trimethylsilyloxy)butadiene (**33**), known for its high diene reactivity, can react differently with alkynylcarbene complexes depending on the nature of their terminal substituent. With the (trimethylsilylethynyl)carbene complex **21** it forms the expected [4+2] cycloadduct **34**,^[19a] which

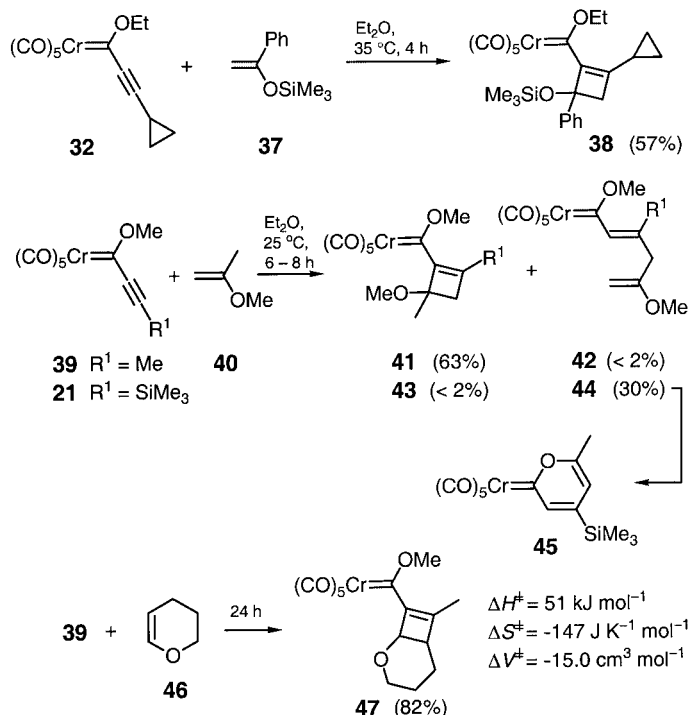


before further use in a Dötz reaction had to be converted into the acetal compound **36**. In contrast, the cyclopropyl-substituted complex **32** reacts regioselectively with the electron-rich double bond in **33** ($\text{R}^2 = \text{Et}$) in a [2+2] cycloaddition.^[20, 24] This is analogous to the [2+2] addition of 2,3-bis(*tert*-butyldimethylsilyloxy)butadiene to the complex **32** ($\text{R}^2 = \text{Me}$) observed by Faron and Wulff.^[25]

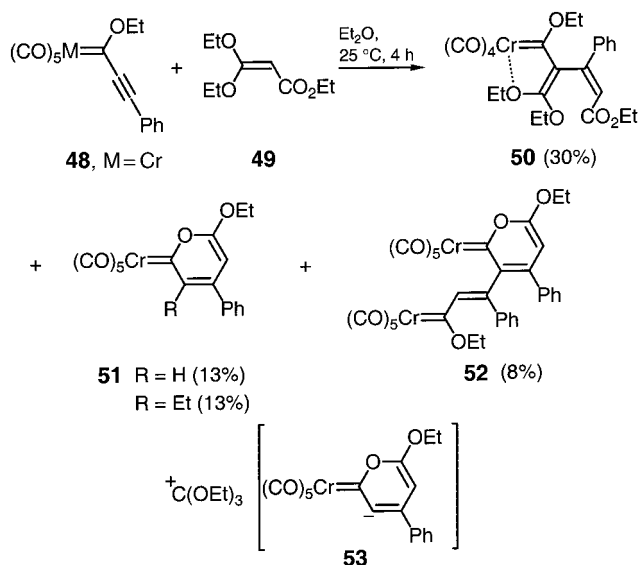


Meanwhile, it has been shown that enol ethers generally react with alkynylcarbene chromium and tungsten complexes by [2+2] cycloaddition.^[20–29] 2-Alkoxycyclobutenylcarbene complexes are formed with diverse enol ethers, as is demonstrated by the reaction of the cyclopropyl-substituted complex **32** ($\text{R}^2 = \text{Et}$) with α -trimethylsilyloxystyrene (**37**) to form **38**, and the reaction of the butynylidene complex **39** with 2-methoxypropene (**40**) to form **41**, as well as with dihydropyran (**46**) to afford **47**.^[25, 26] One exception arises again, however, with the trimethylsilyl-substituted complex **21**, which mainly undergoes an ene reaction with 2-methoxypropene (**40**) to form the (methoxypenta-1,4-dienyl)carbene complex **44**; compound **44** is for its part highly labile and cyclizes to the pyranylidene complex **45**.^[25] It was concluded

from kinetic measurements on the temperature and pressure dependence that the model reaction of dihydropyran (**46**) with **39** involves a synchronous, single-step process with a nonpolar transition state.^[28]

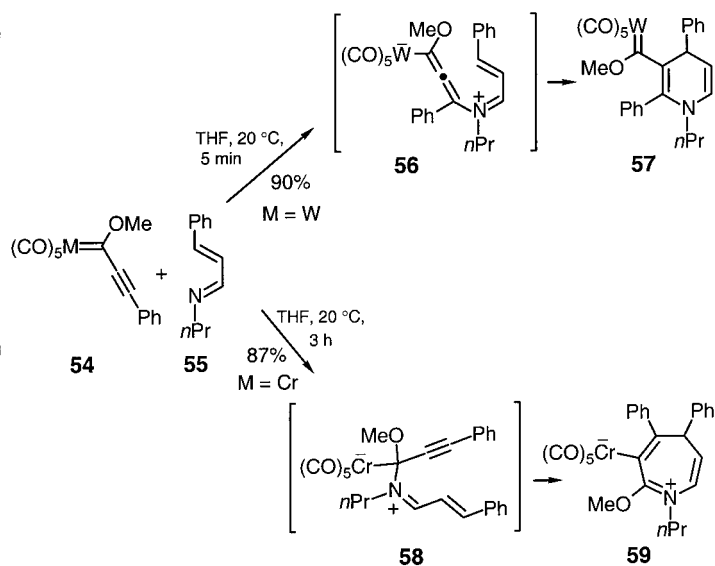


A spontaneous cycloreversion^[30] to butadien-2-ylcarbene complexes is sometimes observed after a successful [2+2] cycloaddition,^[25, 26, 29, 31] as for example in the reaction of **48** (M = Cr) with ethyl 3,3-diethoxyacrylate (**49**), which leads to the complex **50**.^[29] The total product spectrum from **48** (M = Cr) and **49** appears somewhat bizarre, for in addition to **50** two pyranilidene complexes **51** (R = H, Et) analogous to product **45** (see above), and the binuclear biscarbene complex **52** are found.^[31] It is assumed that the three compounds **51** (R = H, Et) and **52** arise from one and the same ionic intermediate **53**.

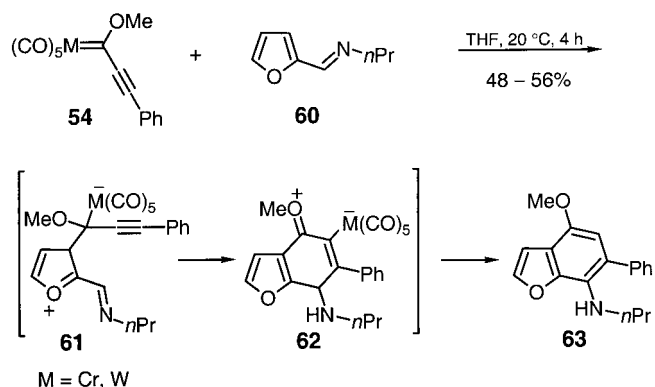


2.1.2. With Azadienes and Enamines

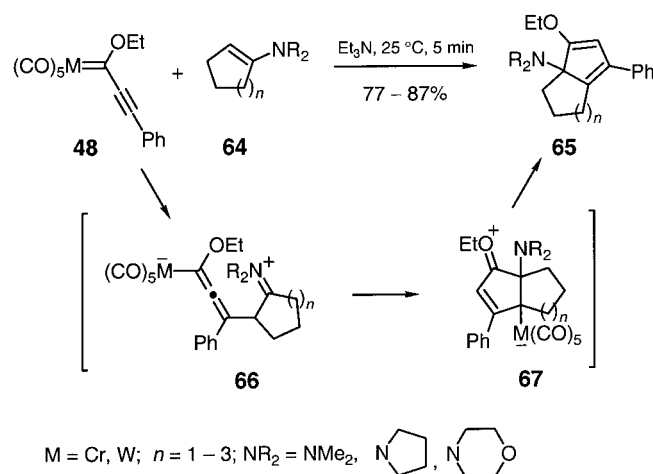
The reaction of alkynylcarbene complexes **54** with azadienes may take rather different courses depending upon the metal.^[32, 33] The alkynylcarbene tungsten complex **54** (M = W) affords the corresponding [4+2] cycloadduct **57** upon reaction with 4-phenyl-1-propyl-1-azabutadiene (**55**). The zwitterionic intermediate **56** was identified by NMR spectroscopy.^[32a] In contrast, with the identically substituted alkynylcarbene chromium complex **54** (M = Cr) cyclization of the zwitterion **58** with migration of the metal-complex fragment and formation of the azepine **59** follows a nucleophilic attack of the azadiene **55** at the carbene center.^[33]



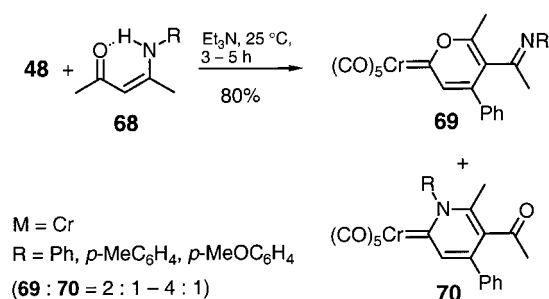
The direction of the reaction depends, however, not only upon the constitution of the alkynylcarbene complex,^[32, 33] but also upon the nature of the azadiene used.^[34] Thus, the tungsten and chromium complexes **54** described above do not react in a [4+2] or [4+3] cycloaddition with the N-substituted furan derivative **60**. Here too, the sequence begins with a nucleophilic attack, albeit of the furan fragment of **60** at the carbene center of **54**, and cyclization of **61** with [1,2] migration of the metal fragment leads to **62** with a furan-annulated six-membered ring. The trisubstituted benzofuran **63** results from the zwitterion **62** by protolysis.^[34]



According to these examples it does not require too great an imagination to think of a plethora of possible products for the reactions of alkynylcarbene complexes with enamines.^[35] Aumann and co-workers have shown impressively that alkynylcarbene complexes of type **48** react with cyclic enamines **64** in a formal [3+2] cycloaddition with the formation of ring-annulated cyclopentadienes **65**. A large number of products could be synthesized from five-, six- and seven-membered cyclic enamines using this reaction principle.^[35a,b]



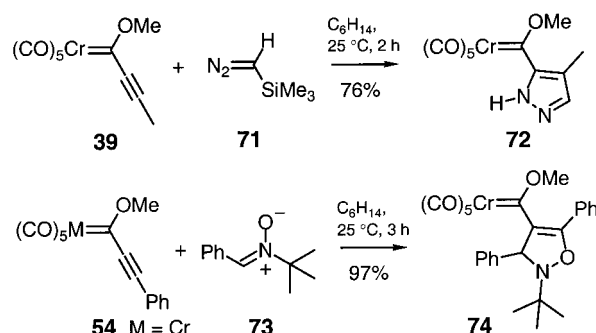
Solely the pyran-2-ylidene and 1,2-dihydropyridin-2-ylidene complexes **69** and **70**, respectively, were formed in the base-catalyzed reaction of the secondary enamin-3-ones **68** with chromium complex of **48**.^[35]



2.2. [3+2] Cycloadditions with 1,3-Dipoles

In view of the high reactivity of alkynylcarbene metal complexes in [4+2] and [2+2] cycloadditions the question of their behavior towards 1,3-dipoles is an obvious one. In fact, only a few investigations on this topic have been carried out. In an early study solely metal-free compounds were obtained in the reaction of pentacarbonyl(1-ethoxy-3-phenylpropynylidene) tungsten with diazomethane.^[36] This is not surprising since in reactions with ylids, metal carbene complexes also show a similar behavior to the esters and amides of carboxylic acids to form alkenes.^[37] It was over a decade later that Chan and Wulff were successful in observing a smooth cycloaddition of trimethylsilyldiazomethane (**71**) to **39**.^[14b] At 25 °C the

pyrazolyl(methoxy)carbenechromium complex **72** was obtained in a yield of 76 % with a strikingly high regioselectivity of more than 300:1. The almost quantitative formation of the 2,3-dihydroisoxazolyl(methoxy)carbenechromium complex **74** by addition of the nitrene **73** to the chromium complex of **54** was later reported.^[38]



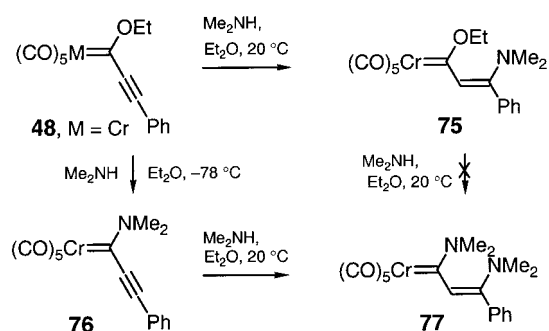
Azides, however, do not undergo 1,3-dipolar cycloadditions with alkynylcarbene complexes. Instead, after thermal release of nitrogen a nitrene is formed which, by hydrogen atom abstraction from the solvent, forms the primary amine. This then adds to the triple bond of the alkynylcarbene complex (see Section 2.3.1.).^[39]

2.3. Michael Additions

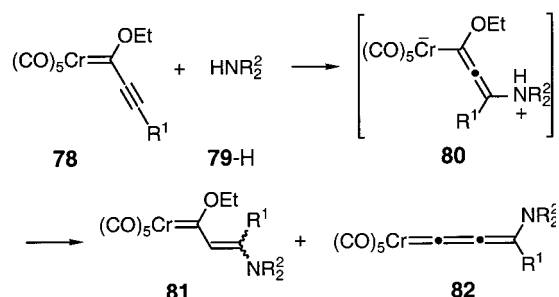
2.3.1. Addition of Amines, Ammonia, Imines, Hydrazine, and Phosphanes

In 1972 the facile 1,4-addition of a secondary amine to an alkynylcarbene complex was first described in the literature by Fischer and Kreissl.^[14a] Five years later came a report on the temperature dependence of the product distribution,^[40] for in addition to the 1,4-attack, 1,2-attack at the carbene C atom is also possible, which after rapid elimination of ethanol corresponds to simple substitution.

Fischer and Kalder found that the reaction of **48** (M = Cr) with dimethylamine at –78 °C gives solely the substitution product **76**, whereas at room temperature only the Michael adduct **75** is formed.^[40] However, at that time the configuration of the newly formed double bond was unclear. The further reaction of **76** with dimethylamine at 20 °C leads smoothly to the dimethylamino(dimethylaminoethenyl)carbene complex **77**, whereas **75** does not react further to **77** under these conditions, even over an extended period.



A series of rules were uncovered by de Meijere and co-workers for this type of reaction during the investigation of a large number of differently substituted complexes **78** with divers secondary amines **79-H**.^[15, 41] At 20 °C the reaction generally leads to the Michael adducts **81** in good, usually quantitative yields. The occurrence of the iminium ylid intermediate **80** could also be confirmed by isolation and X-ray structure analysis.^[42, 43]



Almost without exception the products **81** are formed as a single diastereomer, usually as the *E*-isomer, if R^1 is not too large and the amine is a secondary amine. In this case a coplanar arrangement with optimal conjugation including that of the lone electron pair on the nitrogen atom is possible (Figure 1). If R^1 is tertiary or otherwise extremely sterically demanding (e.g. $R^1 = \text{CH}(\text{Me})\text{OSiMe}_2\text{tBu}$), the products **81** with *Z*-configuration are formed.^[15] In (*Z*)-**81** the substituents on the amino group are rotated out of the plane so that the interaction of the lone electron pair with the 1-metalla-1,3-diene system is greatly reduced.

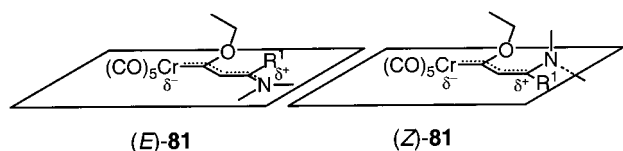
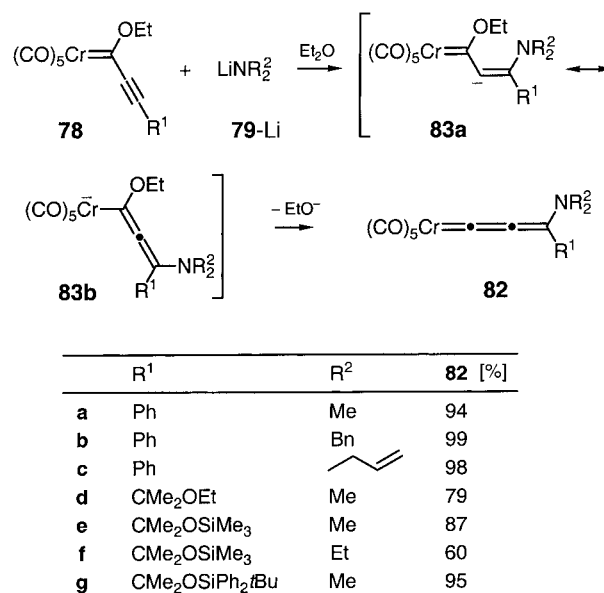


Figure 1. Steric relationships in *E*- and *Z*-configured pentacarbonyl(3-dialkylamino-1-ethoxyprop-2-enylidene)chromium complexes **81**.

Where R^1 represents large tertiary substituents in **78** side products are formed in the reaction with secondary amines which, with suitable choice of amine and/or temperature, can become the main products.^[41] In contrast to the findings of Fischer and Kalder with the phenyl-substituted complex **78** ($R^1 = \text{Ph}$),^[40] the 1,4 adduct is favored with all other substrates of type **81** when the temperature is reduced.^[41] The products of a new type formed at higher temperatures have proved to be the allenylidene complexes **82**,^[44] isomeric with the substitution products **76** (see above), as was recognized by X-ray structure analysis. They are even formed as sole products if the complexes **78** are treated with the lithium amides **79-Li** at low temperatures (Scheme 2).^[41]

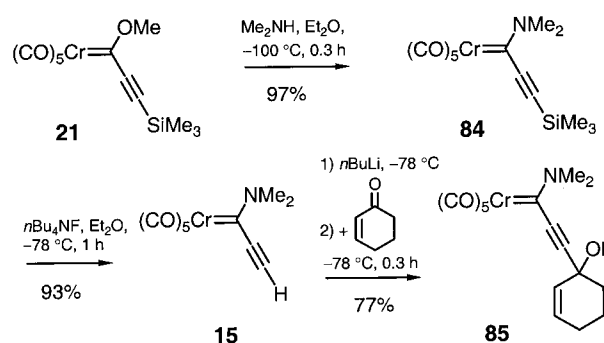
In this case the anionic complex **83** must be formed directly by nucleophilic attack of the amide at the alkyne terminus from which the 1-metallacumulene **82** then results by elimination of ethanolate. Allenylidene complexes of the type **82** are also formed by elimination of ethanol from the β -



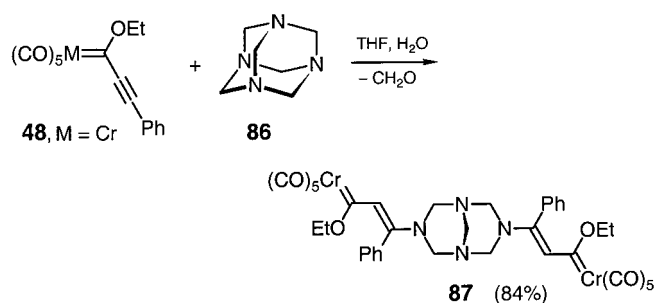
Scheme 2. Formation of allenylidene complexes **82** with the lithium amides **79-Li**.

amino-substituted alkenylcarbene complexes **78** by treatment with Lewis acids such as BF_3 or AlCl_3 .^[45]

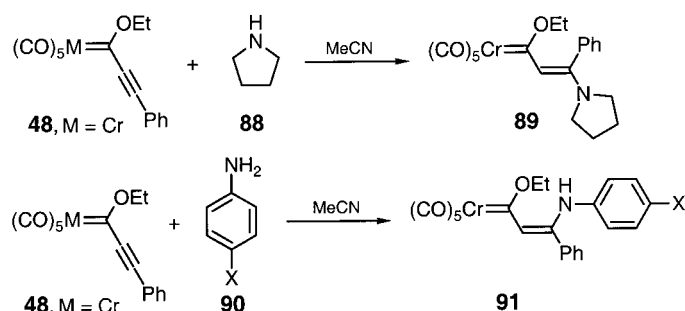
Because the trimethylsilyl substituent in **21** is sterically highly demanding, the 1-dimethylaminocarbene complex **84** is formed by reaction with dimethylamine, the addition product (28% at 20 °C) is also formed, but not the respective allenylidene complex of type **82**.^[16] The trimethylsilyl group may be removed from **84** by treatment with fluoride; in this way Wulff and co-workers obtained the first unsubstituted derivative **15** of this class of compounds. This could develop into a valuable synthetic building block, as it has been shown that **15** also reacts smoothly with electrophiles such as cyclohexenone to carbene complexes like **85** after conversion into the lithium derivative.^[16]



From any point of view, the result of the reaction of the phenylethynylcarbene complex **48** ($M = \text{Cr}$) with urotropine (**86**) is surprising.^[42] In aqueous THF the binuclear adduct **87** is formed in a surprisingly high yield (84%). Clearly the urotropine residue is partially hydrolyzed with removal of formaldehyde, and the resulting two secondary amino functions each add spontaneously to the triple bond in **48** ($M = \text{Cr}$) with formation of the binuclear complex **87**.

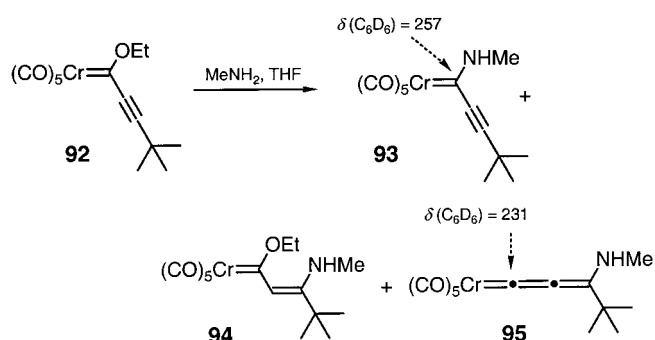


Kinetic investigations on the pressure dependence of the addition of pyrrolidine (**88**)^[43] and *para*-substituted anilines **90**^[46] to the phenylethynylcarbene complex **48** ($M = \text{Cr}$) by van Eldik et al. (Scheme 3) gave negative activation volumes with values between -16.6 and $-27.9 \text{ cm}^3 \text{ mol}^{-1}$; this and the increase of -2.95 ± 0.14 found in the Hammett plot for the addition of the aniline groups confirm a two-stage process with significant C–N bond formation in the polar transition structure (similar to the intermediate **83**) of the rate-determining reaction step.



Scheme 3. $X = \text{CN}, \text{MeCO}, \text{Cl}, \text{F}, \text{H}, \text{Me}, \text{MeO}$.

Michael adducts such as **94**, from the reaction of primary amines to alkynylcarbene complexes, are formed exclusively with *Z*-configuration.^[14, 47–50] The considerations regarding the configuration of the double bond and the orientation of the amino groups described above (see Figure 1) do not apply to complexes **94**. Rathermore these *Z*-configured complexes are



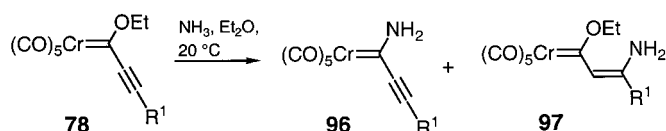
also coplanar with optimal conjugation precisely because of the hydrogen bond from the amine hydrogen to the oxygen atom of the 1-alkoxy group. Aumann obtained chirally modified 2-aminoethenylcarbene complexes by addition of

enantiomerically pure galactopyranosylamines;^[49] it has also been shown that the secondary amino group in such complexes may be acylated.^[50] Formal substitution (1,2-addition/elimination) occurs to a greater extent as a side reaction to the 1,4-addition of primary amines,^[47, 48] although allenylidene complexes are also found in reactions with amines such as isopropylamine.^[41] The reaction of the (3,3-dimethylbutynyl)-carbene complex **92** with methylamine is currently the only known example in which, in addition to Michael addition to give **94**, both formal substitution to give **93** and addition/elimination to give the allenylidene complex **95** occurs. The two isomeric compounds **93** and **95** are readily differentiated because of the considerable difference between the chemical shifts of the carbene C atoms in the ^{13}C NMR spectrum. The temperature dependence of the product distribution (Table 1) shows very clearly that low temperatures favor 1,2-addition/elimination (formal substitution), medium temperatures 1,4-addition, and higher temperatures 1,4-addition/elimination.

Table 1. The effect of temperature on product distribution in the reaction of **92** with methylamine.^[47]

$T [^\circ\text{C}]$	Fraction [%]		
	93	94	95
– 17	97	3	0
+ 3	88	12	0
+ 25	59	30	11
+ 41	26	46	28
+ 65	17	44	39

The reactions of various alkynylcarbene complexes **78** with ammonia always give considerable proportions of the respective formal substitution products **96** (Scheme 4). When $\text{R}^1 = \text{Ph}$, *t*Bu **96** is even the major product.^[15, 48, 51] The 1,4-addition products **97** exist as primary enamines, which is clearly shown by the two signals of the two NH_2 protons that are observed in the ^1H NMR spectrum, and exclusively as the *Z*-isomers in all cases.

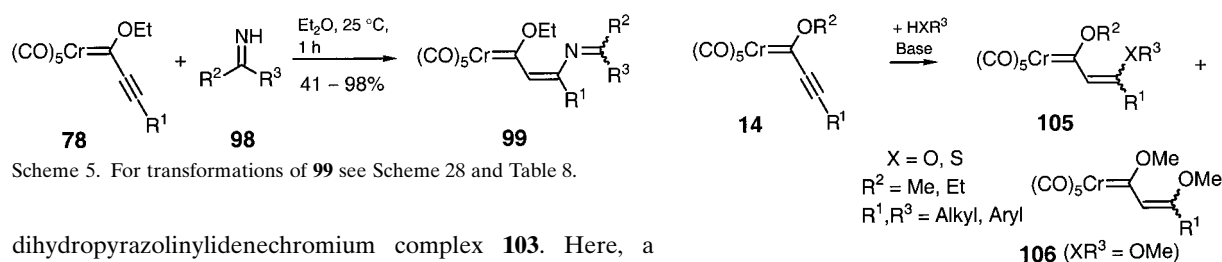


	R^1	96 [%]	97 [%]
a	Ph	86	13
b	<i>n</i> Pr	31	63
c	<i>c</i> Pr	28	70
d	<i>t</i> Bu	60	35

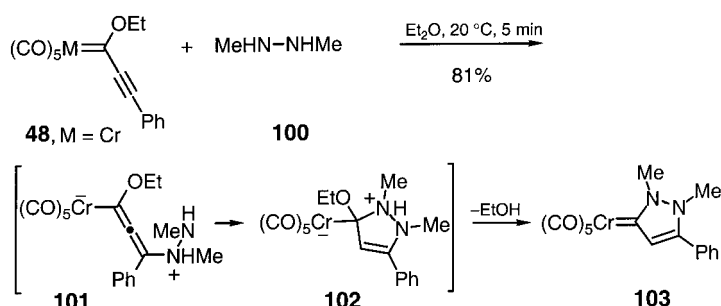
Scheme 4. Reactions of the alkynylcarbene complexes **78** with ammonia and product ratios.

The addition of imines **98** to the triple bond of **78** leads directly to the interesting 5-aza-1-chroma-1,3,5-hexatrienes **99** (Scheme 5).^[52] Unlike the additions of amines this reaction takes place distinctly more slowly, but totally selectively to the 1,4-adducts **99**.

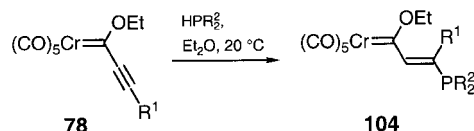
1,2-Dimethylhydrazine (**100**) reacts with alkynylcarbene-chromium complexes such as **48** ($M = \text{Cr}$) with formation of a

Scheme 5. For transformations of **99** see Scheme 28 and Table 8.

dihydropyrazolynylidenechromium complex **103**. Here, a formal substitution on the carbene carbon atom by the second secondary amino group to form the betaine complex **102** follows a Michael addition of **100** onto the triple bond of **48** ($\rightarrow$ **101**). The analogous tungsten complexes also gave formal substitution products as well as the pyrazolynylidene complexes on the addition of **100**.^[53]

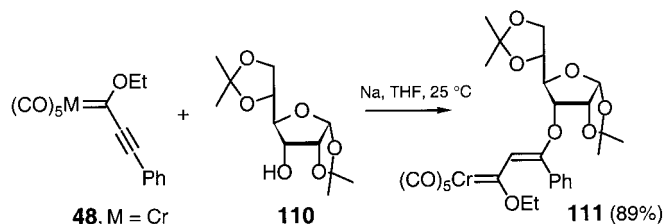


E-Configured 3-(diorganophosphanyl)propenylidenechromium complexes **104** could also be prepared for the first time by the Michael addition of secondary phosphanes to alkynylcarbene complexes (Scheme 6).^[54]

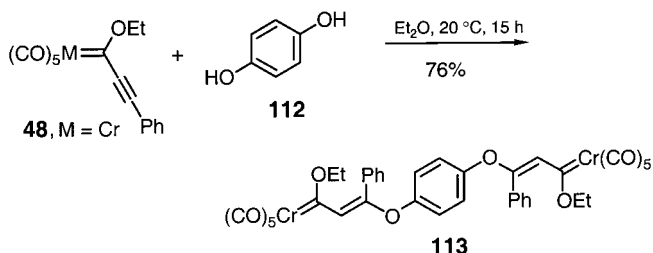
Scheme 6. $R^1 = \text{Ph}$; $R^2 = t\text{Bu}$, $c\text{-C}_6\text{H}_{11}$.

2.3.2. Additions of Alcohols, Thiols, and Carboxylic Acids

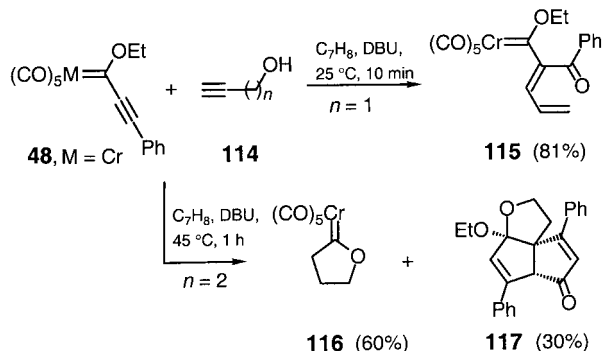
The addition of alcohols and thiols to alkynylcarbene complexes **14** generally furnishes *E/Z*-diastereomeric mixtures.^[15, 55, 56a] Furthermore, the addition of these nucleophiles takes place far more slowly than those of amines,^[15, 55] but can be accelerated by the addition of bases. Small amounts of the respective sodium alcoholate^[15, 56a] or 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU)^[55] have proved to be effective catalysts. In the reaction with methanol the 1,3-dimethoxypropenylidene complex **106** was also found as a byproduct in addition to the monoaddition product **105**.^[15, 55] The unsubstituted 1,3-diethoxypropenylidenechromium complex **109** was formed by the addition of ethanol to **107** and subsequent protidesilylation.^[15] In this way Aumann incorporated sugar moieties into carbene ligands for the first time.^[56b] The 1,2:5,6-protected α -D-allofuranose **110** gives the sugar adduct **111** as a single stereoisomer in a yield of 89% after addition of 0.2 equivalents of sodium in THF and subsequent addition of **48** ($M = \text{Cr}$).



Phenols react with alkynylcarbene complexes in an analogous 1,4-addition. In this way the binuclear complex **113** could also be obtained, prepared by the addition of hydroquinone (**112**) to **48** ($M = \text{Cr}$).^[57]

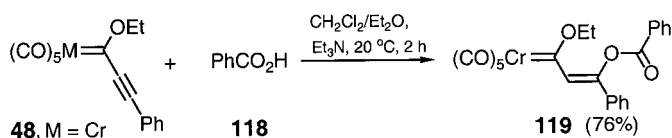


The reaction of **48** ($M = \text{Cr}$) with hydroxyalkyl-substituted alkynes leads to unexpected products. The Michael adduct was at best found in traces. Propargyl alcohol (**114**, $n = 1$) reacts with **48** ($M = \text{Cr}$) to form the butadienyl complex **115**, whereas 3-butyne-1-ol (**114**, $n = 2$) gives the tricycle **117** as a single diastereomer in a yield of 30%.^[58] Compound **115** is most probably formed from the Michael adduct by [3,3] sigmatropic rearrangement with a subsequent [1,3] proton



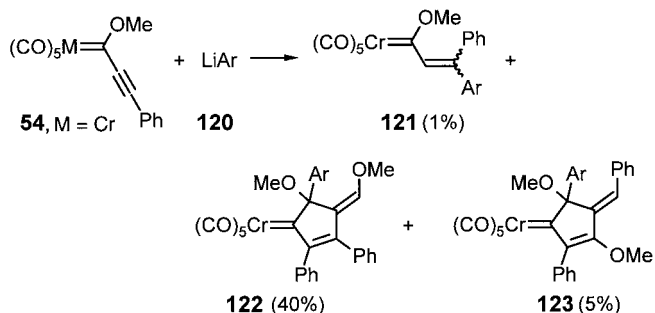
shift. At least two molecules of the starting complex **48** ($M = \text{Cr}$) are involved in the formation of **117**; the authors assume a sequence of intra- and intermolecular [4+2] cycloadditions. The major product in this case, however, was the tetrahydrofuranlydene complex **116**.

The Michael addition of carboxylic acids to alkynylcarbene complexes also proceeds smoothly.^[59] Benzoic acid (**118**) reacts uniformly with **48** ($M = \text{Cr}$) in the presence of triethylamine to form the *Z*-isomer **119** in 76 % yield.



2.3.3. Addition of Aryllithium Derivatives

The additions of secondary and primary amines, ammonia, imines, alcohols, and thiols^[55] are reactions which are easy to carry out and usually give the desired Michael adducts in good to very good yields, and frequently even in essentially quantitative yields. Adducts of type **82** from secondary amines can even be prepared in a one-pot reaction by deprotonation of terminal alkynes with *n*-butyllithium, addition of hexacarbonylchromium, addition of triethyloxonium tetrafluoroborate, and finally addition of the secondary amine. However, attempts at 1,4-addition of *p*-tolyllithium (**120**) proved to be rather disappointing (Scheme 7).^[60] Compound **121** was isolated in a yield of only 1%. It is difficult



Scheme 7. $\text{Ar} = p\text{-MeC}_6\text{H}_4$.

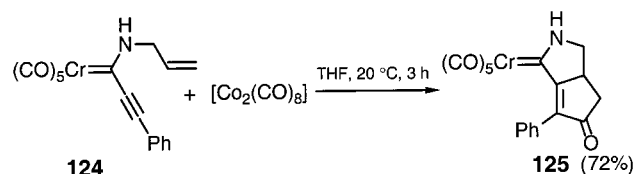
even to recognize the starting material in the main product **122** (yield 40 %). The intermediate formed after an initial 1,2-attack of the lithium compound has clearly attacked another molecule of the alkynyl complex **54** ($M = \text{Cr}$). The additional product **123** (5%) had to have formed analogously but with reversed regiochemistry during the attack on the second molecule of the starting complex.

The addition of organozinc compounds to the alkynylcarbene complexes has been reported. In this case a product of type **122** was formed selectively.^[61]

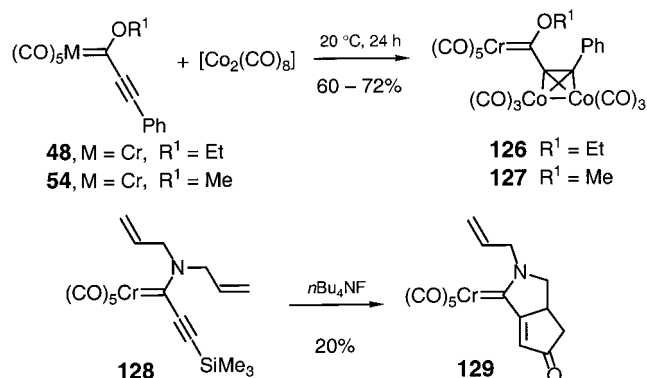
2.4. Reactions of Octacarbonyldicobalt—Intramolecular Pauson–Khand Reactions at Room Temperature

Simultaneously, but independently of each other, the groups of Moret  and D tz discovered that 1-(allylamino)al-

kynylidenechromium and -tungsten complexes undergo Pauson–Khand reactions at surprisingly low temperatures upon treatment with octacarbonyldicobalt.^[62, 63] After only 3 h at 20°C **124** had reacted completely, via the corresponding alkynehexacarbonyldicobalt complex, and the cyclization product **125** was isolated in a yield of 72%.^[62a]



Alkynylcarbenechromium complexes without an allyl group, such as **48** ($M = \text{Cr}$) and **54** ($M = \text{Cr}$), smoothly form the alkynehexacarbonyldicobalt complexes **126** and **127** which could be isolated and investigated by X-ray crystallography.^[64, 65] Complicated metal cluster compounds are obtained



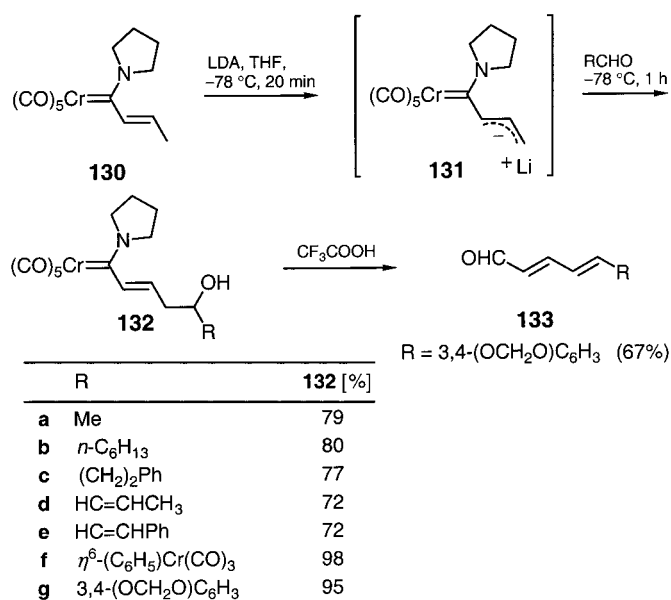
on heating these complexes.^[65] In this type of reaction too, a (trimethylsilyl)ethynylcarbene complex represents an exception. Compound **128** does not react, or at the best unconventionally, with octacarbonyldicobalt, although both intramolecular and intermolecular Pauson–Khand reactions usually give particularly good yields with trimethylsilyl-substituted alkynes. However, Moret  and co-workers obtained the cyclization product **129** in a yield of 20% by treatment of **128** with tetrabutylammonium fluoride without addition of octacarbonyldicobalt (the corresponding tungsten complex gave a better yield).^[66] The mechanism of this reaction is unclear, although the incorporated carbonyl group must have arisen from the decomposition of a further molecule of the carbenepentacarbonylchromium complex.

3. Aryl and Alkenylcarbenechromium Complexes

3.1. Aldol Reactions of Alkenylcarbenechromium Complexes

The 1-pyrrolidinylbutenylidenechromium complex **130** represents a synthetic equivalent of an α,β -unsaturated amide. As Maiorana and co-workers have demonstrated, the complex

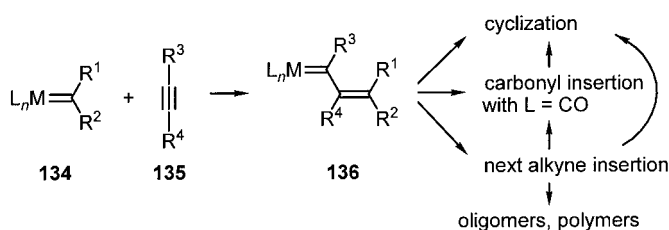
130 can be converted into the allyl anion **131**, equivalent to an amide enolate, with a base such as lithium diisopropylamide (LDA). This then reacts regioselectively with an aldehyde functionality with the formation of a δ -hydroxy-substituted alkenylcarbenechromium complex **132**. After removal of the chromium complex fragment with trifluoroacetic acid and concomitant dehydration, the substituted butadienylaldehydes **133** are obtained (Scheme 8).^[67]



Scheme 8. Reactions of the allyl anion **131** with aldehydes.

3.2. Reactions with Alkynes

Carbene complexes **134** of Group VI metals insert alkynes **135** into the metal–carbene double bond to form the alkenylcarbene complexes **136**. Depending upon the nature



of the complex, this alkyne insertion can be either repeated reiteratively or multiply, leading finally to alkyne oligomers or polymers.^[68] Alternatively, one of the intermediates cyclizes—possibly with the insertion of a carbonyl ligand—to afford an organic product after reductive elimination of the metal fragment.

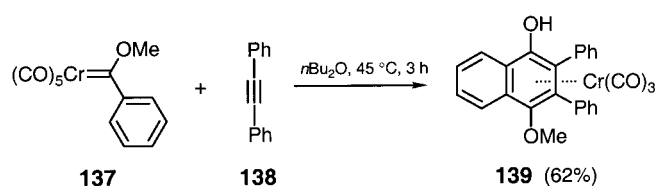
Examples of all three follow-up reactions after the first alkyne insertion illustrated above are known for alkenyl- and phenylcarbenepentacarbonylchromium complexes. They may be classified as formal $[k+m+n]$ cycloadditions, where k , m , and n represent the respective number of atoms from the carbene ligand (k), the alkyne (m), and the carbonyl ligand (n)

which form the ring. The following section will describe first those cases in which $k > 1$, namely where more than only the carbene C atom of the carbene ligand is found in the ring system of the product.

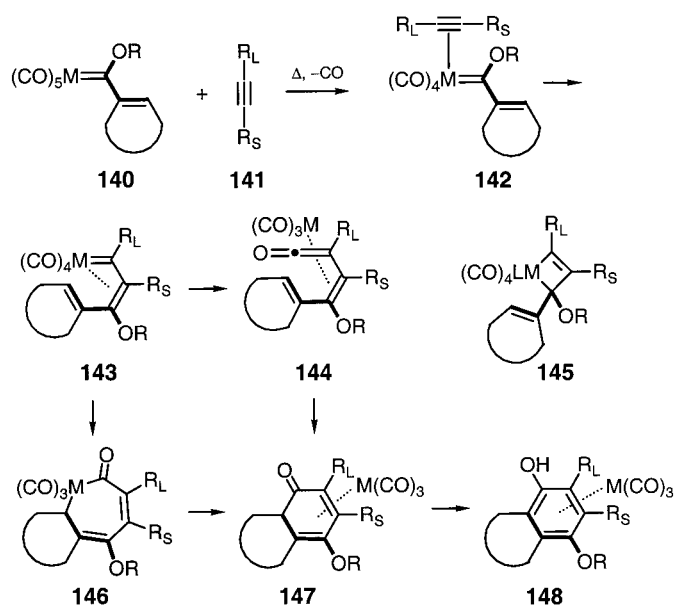
3.2.1. Formal $[3+2+1]$ Cycloadditions: The Now Classical Dötz Reaction

3.2.1.1. Arylcarbenechromium Complexes

In 1975 a benzene annelation by formal cycloaddition of the phenylcarbene complex **137** with diphenylethyne (**138**) was reported for the first time by Dötz.^[3, 69] In the meantime more than 100 reports on this reaction have been published.^[2] It is



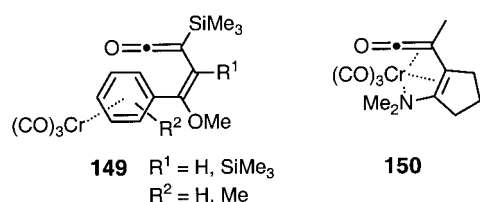
not possible to discuss all aspects within the scope of this review; instead, plausible ideas concerning the mechanism of the Dötz reaction will be discussed, followed by a number of substituent, solvent, and temperature effects. Finally, several new applications of this reaction in organic synthesis will be described (Scheme 9).



Scheme 9. The mechanism of the Dötz reaction (R_L = larger, R_S = smaller substituent).^[19a, 70–82]

The initiating and rate-determining step of this reaction must be the dissociation of one of the carbonyl ligands located *cis* to the carbene moiety.^[70] An alkyne molecule can then coordinate onto the coordinatively unsaturated complex, and then be inserted into the metal–carbon bond. The photochemically induced CO expulsion and the coordination of an

alkyne with subsequent phenol formation can be followed directly by on-line FT IR spectroscopy.^[71] According to this there is only one intermediate, which lies in a pronounced energy minimum and is thus relatively stable. The originally assumed pathway via the metallacyclobutene **145** was recognized as unfavorable by Hofmann et al. on the basis of semiempirical MO calculations.^[72, 73] The selectivity with which the diastereomer **143**, capable of cyclization, is formed during alkyne insertion was originally explained by the stereochemistry typical of an electrocyclic ring opening of a cyclobutene.^[74] However, this stereoselectivity can be explained satisfactorily by direct insertion via a η^3 -vinylcarbene complex, the pathway most favored by calculation.^[72b] Meanwhile the isolation and characterization of a carbene complex analogous to the intermediate **143** has been successful, as has that of the complex which is formed after the expulsion of a CO ligand from a 1-amino-substituted alkenylcarbene complex of type **140**.^[75] Two different pathways have been discussed for the formation of the coordinated cyclohexadienone **147** from **143**. According to a proposal of Casey, **143** must cyclize to a metallacyclohexadiene. This forms the 7-metallacycloheptadienone **146** by carbonyl insertion, leading to **147** after reductive elimination.^[76] In the opinion of Dötz, ^[77a] the carbonyl ligand is first inserted into **143** to form the alkadienylketene complex **144**, and an electrocyclic ring closure then occurs leading to **147**. In support of the second pathway is the isolation of the alkenylketene complexes **149**^[77] and **150**^[78] which are formed in the reaction of Fischer carbene complexes with alkynes. Moreover, against the first suggestion is the observation that 5-metalla-1,3-cyclohexadienes,

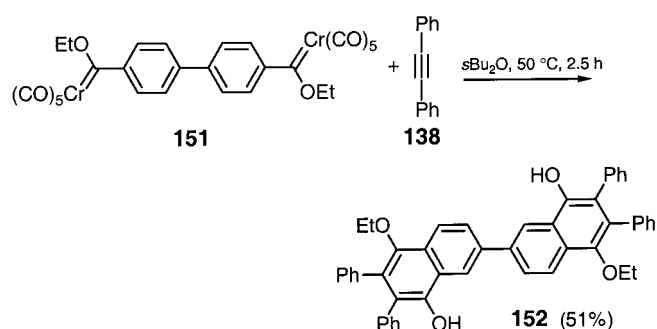


which can form preferentially from 1-metalla-1,3,5-hexatrienes under certain conditions, clearly lose the metal fragment by reductive elimination without insertion of CO to form cyclopentadienes (see below). The complexed cyclohexadienone **147**, which is accordingly formed via **144**, finally tautomerizes to the complexed *para*-alkoxyphenol **148**^[79] if this is not blocked by twofold substitution in the six position.

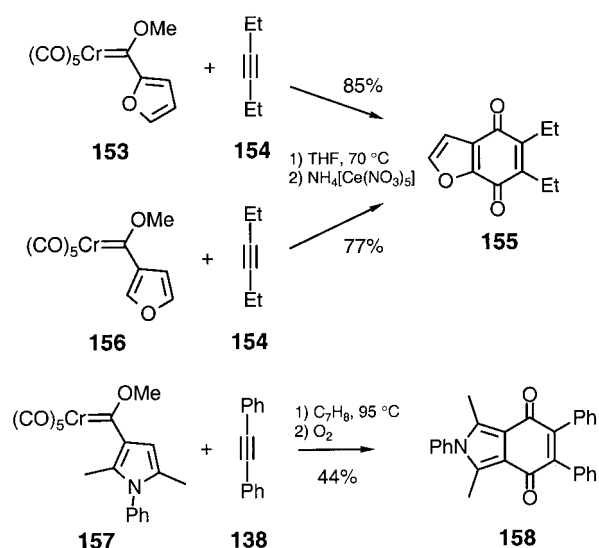
Whereas pentacarbonyl[(*o*-methoxyphenyl)propylthiomethylene]chromium gave the naphthol derivative in only moderate yield,^[80] the Dötz reaction tolerates different substituents on the aryl moiety of alkoxyarylcarbene complexes, as the reactions of a whole series of alkoxy-^[19a, 81] and alkyl-substituted^[72, 81a, 82] phenyl- and naphthylcarbene complexes^[82] with alkynes confirm.

Whereas a phenylcarbenechromium complex with a second carbenechromium group in the *para* position reacts with only one molecule of tolane (**138**) in the usual way,^[83] the binuclear biphenyl complex **151**^[84] gave the binaphthol derivative **152** with **138** in a yield of 51%.^[83]

Heteroarylcarbene complexes with furan,^[74, 82a, 85] thiophene,^[82a, 85a,e] pyrrole,^[85c, 86] or pyrazole residues also undergo



Dötz reactions. The benzo ring is here generally annelated across the *b* side of the heterocycle, regardless of whether the carbene center is bound to the heterocycle at C-2 or C-3. Thus, both **153** and **156** afford the same furo-*p*-benzoquinone **155** in yields of 85 and 77 %, respectively, after addition of 3-hexyne (**154**) and oxidative work-up.^[85a] A different observation was made with carbene complexes with 3-pyrrolyl substituents. Depending upon the position of further substituents on the heterocycle either annelation occurs across the *b* side,^[86b] or an isoindoloquinone is obtained, such as in the formation of **158** from **157** and tolane (**138**).^[87]



The efficiency and scope of the Dötz reaction has been impressively confirmed by numerous examples in earlier reviews^[2] and by more recent work (see Table 2).

Benzoannulation of arylcarbenechromium complexes first reaches a limit when the ferrocenylcarbene complex **159** reacts with tolane (**138**) under standard conditions. Dötz et al. isolated only the furan derivative **160**.^[94] Furans of this type, which are normally formed from iron and cobalt carbene

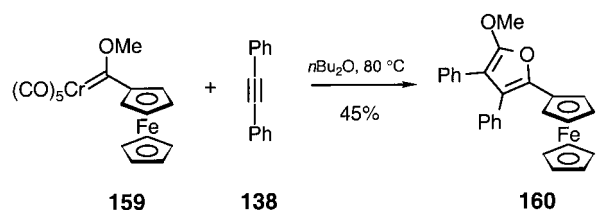


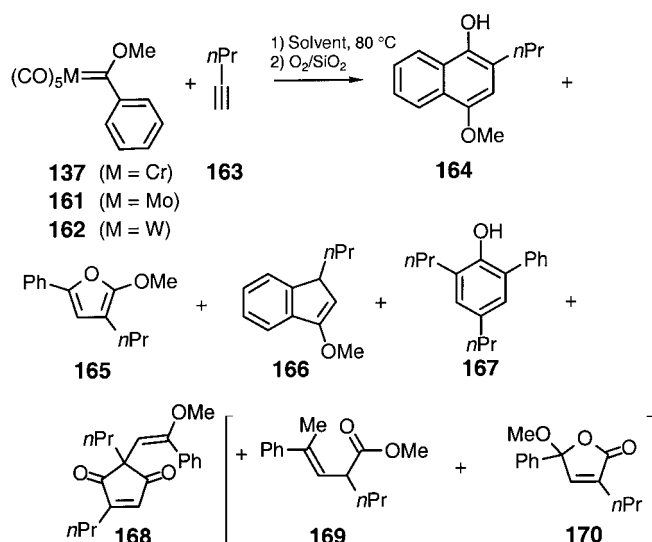
Table 2. Recent examples of the formal cycloaddition of arylcarbenechromium complexes with CO insertion into alkynes.

Carbene complex	Alkyne	Conditions ^[a]	Product and yield	Ref.
		1) Ultrasound, 20 min; 2) CAN	 65%	[85h]
		1) SiO ₂ , without solvent, 3 h; 2) CAN	86 %	[85g,h]
		THF, 60 °C, 12 h	 43% 24%	[88]
 R = H, OMe		1) THF, 55 °C, 2 d; 2) PbO ₂ , CH ₂ Cl ₂	 63-61%	[89]
		300 W Xe lamp, Pyrex, THF, 5.5 h	 88%	[90]
		1) THF, 65 °C, 18 h; 2) O ₂ , 85 °C, PhH, molecular sieve 4 Å	 55%	[85a]
		1) hexane, 50 °C, 24 d; 2) CAN	 95%	[86b]
		1) SiO ₂ , 60 °C, 20 h; 2) SiO ₂	 31%	[91]
		1) <i>t</i> BuOMe, 20 °C, 2 h; 2) <i>t</i> BuMe ₂ SiCl, NEt ₃ , 20 °C, 2 h	 68%	[92]
		BF ₃ · Et ₂ O, C ₇ H ₈ , 65 °C, 3 d	 21%	[80]
		1) THF, 65 °C, 1.5 h; 2) O ₂	 57% 13%	[93]

[a] CAN = cerium(IV) ammonium nitrate.

complexes and alkynes,^[95] have also been observed in part as main products or byproducts in other Dötz reactions.^[4, 19a, 74, 81d, 96] By the use of a carbene complex with a labeled (¹³CO)₅Cr fragment in such transformations Wulff and co-workers were able to show that the alkoxy-substituted C atom in the furan originates from a carbonyl ligand.^[74]

Even though the Dötz reactions normally proceed smoothly, byproducts also occur in a few cases. Of the numerous investigations carried out on this topic, the work of Wulff et al. on the product distribution in the reaction of arylcarbene complexes with alkynes stands out.^[4, 81d,i] Exemplary are the cocyclizations of the three phenylcarbene complexes **137**, **161**, and **162** of chromium, molybdenum, and tungsten, respectively, with pentyne (**163**; Scheme 10).^[4] Under standard

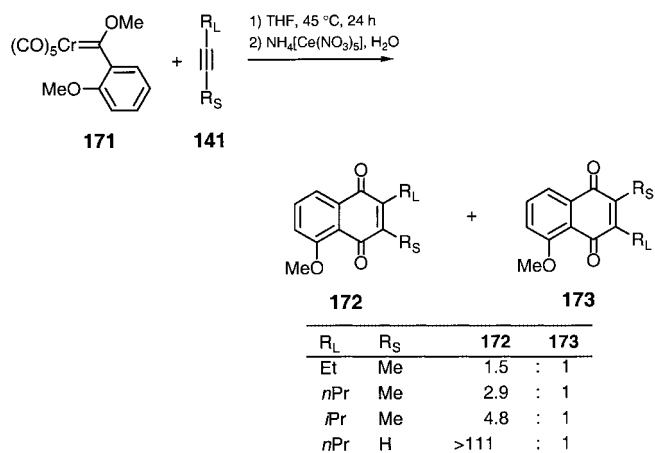


Scheme 10. Solvent = MeCN, C₆H₆, THF. Compounds **169** and **170** are only formed with the tungsten complex **162**.

conditions (THF, 0.1M complex, 24 h) the naphthol **164** is formed from **137** in 84 % (GC, isolated yield 66 %); the type of furan **165**, is known from the reaction of **159** with **138**, is formed in 10 % yield (6 %). The formal [3+2] cycloadduct **166** (2 %) and the phenol **167** (0.5 %) play only minor roles. Lower complex concentrations lead to a higher proportion of **166**; however, in benzene (in this case **168** is also formed in 10 % yield) or acetonitrile as solvent, **164** remains the main product. This high selectivity in the reactions of the chromium complex **137** does not apply to the molybdenum and tungsten complexes **161** and **162**. In THF (0.1M, 27 h) the molybdenum complex **161** yields only 5 % **164**, and instead **165**, **166**, **167**, and **169** are formed each in about 20 %. In benzene **165** becomes the main product (65 %). The tungsten complex **162** requires the longest reaction times (8 days), and gives mixtures of all seven products (**164**–**170**) unselectively. The product distribution was found to be dependent on the solvent, the concentration of the arylcarbenechromium complex used, and the constitution of the alkyne.^[4, 81d,i]

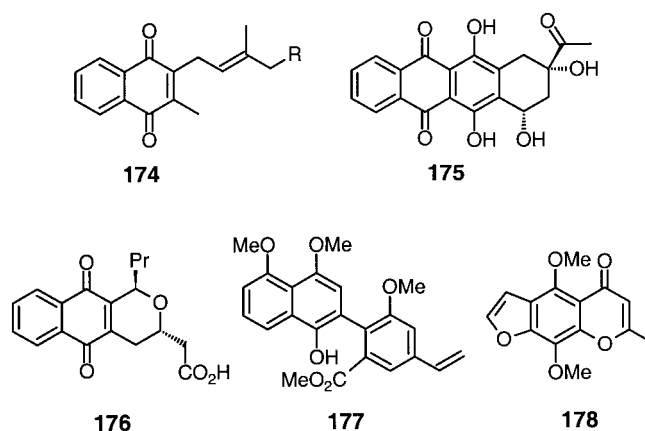
There has also been a series of investigations into the question of regioselectivity (see mechanism in Scheme 9).^[81a, 97] Whereas in the cocyclization with terminal alkynes the regioisomer with the largest residue next to the

C atom of the inserted carbonyl ligand is formed almost exclusively, in reactions with nonterminal alkynes, of the two possible regioisomers one is formed preferentially in each case, though only with moderate selectivity (Scheme 11).^[81]



Scheme 11. Examples of the regioselectivity observed with nonterminal alkynes (R_L = larger, R_S = smaller substituent).

In view of the wide synthetic potential of the Dötz reaction it is not surprising that it finds use in the key steps of numerous natural-product syntheses. Representative examples are the elegant synthesis of vitamins E and K **174**,^[98] daunomycin **175**,^[81f,g, 99] desoxyfrenolycin **176**,^[81b] 1-*O*-methyldefucogilvocarin V **177**,^[100] and khellin **178**.^[101]

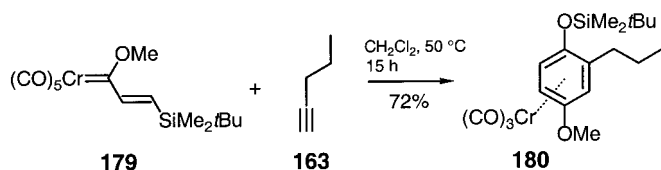


3.2.1.2. Alkenylcarbenechromium Complexes

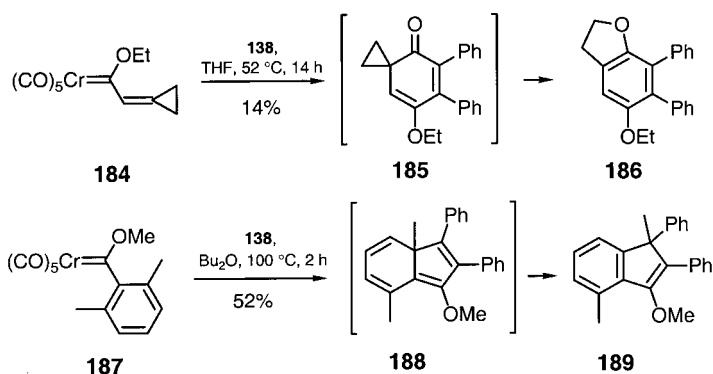
Most alkenylcarbenechromium complexes react with alkynes according to the same principle as arylcarbenechromium complexes, that is they furnish in each case the respective hydroquinone monoethers in a Dötz reaction.^[102]

The simplest representative of the 1-alkenylcarbene complexes, the terminally unsubstituted alkoxyethenylcarbenechromium complex, is unstable and polymerizes as soon as the solvent is distilled off at room temperature.^[17f, 103] As Chamberlin and Wulff have shown, the formal products of the Dötz reaction of the unsubstituted ethenylcarbene complexes may

still be prepared.^[104] In the reaction of (2-trialkylsilyl)ethynylcarbene complexes such as **179** with alkynes, the silyl group migrates to the oxygen atom so that hydroquinone-diethers such as **180** are formed.

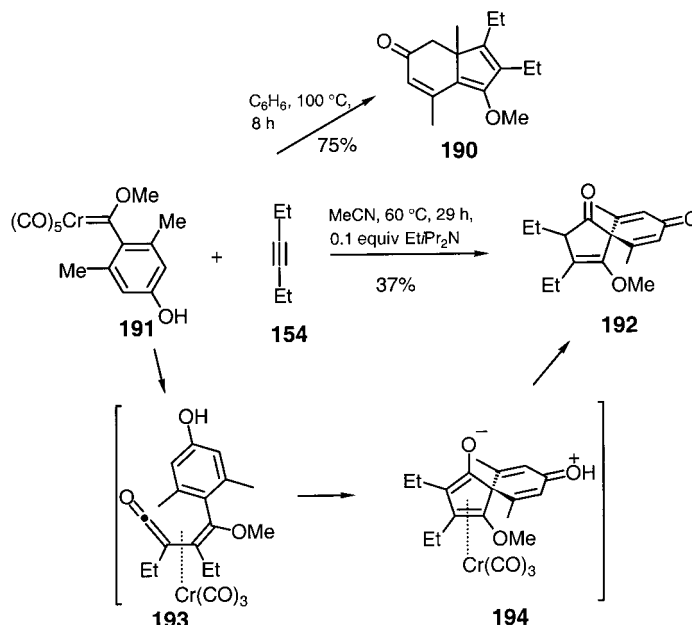


What should be formed with the use of 2',2'-disubstituted ethynylcarbene complexes may be inferred from the mechanism of the Dötz reaction (see Scheme 9), for in the last step of the Dötz reaction the tautomerization of **147** to **148** cannot occur in a 6,6-disubstituted derivative of **147**. In the simplest case, as illustrated with the cycyclization of **181** and alkynes **182**, tautomerization does not occur, and the 6,6-disubstituted cyclohexa-2,4-dienones **183** are isolated.^[77b] The reaction of the cyclopropylidene(methyl)carbene complex **184**, analogous to the isobutenylcarbene complex, with tolane (**138**) gave in contrast the benzodihydrofuran derivative **186** which apparently is readily formed under the influence of the tricarbonylchromium residue from the initially formed complex of spiro[2.5]octa-5,7-dien-4-one **185**, analogous to the tricarbonylchromium complexes of dimethylcyclohexadienones **183**, by heteroanalogous vinylcyclopropane-cyclopentene isomerization.^[20a] Moreover, in the cyclization of (2,6-dimethylphenyl)carbene complex **187** with tolane (**138**)



investigated by Dötz et al., tautomerization of type **147** → **148** cannot occur. However, a benzoannellated cyclohexadienone of type **183** is not formed; instead CO insertion does not occur, and after cyclization to a cyclohexadiene-annellated, methoxymethyldiphenylcyclopentadiene derivative **188**, the indene derivative **189** is formed by sigmatropic 1,5-methyl shift.^[105]

A really unusual reaction course was observed by Wulff and co-workers in the cycyclization of the (4-hydroxy-2,6-dimethylphenyl)carbene complex **191** with 3-hexyne (**154**). In benzene **191** reacts essentially to form the corresponding indene derivative **190** in a formal [3+2] cycloaddition. Only with the use of acetonitrile as solvent was a change in the



course of the reaction observed, with preference for CO insertion. However, the formation of the normal [3+2+1] cycloaddition product (Dötz product) does not occur, instead the intermediate (arylethenyl)ketene complex **193** cyclizes by electrophilic *ipso* attack of the carbonyl group on the electron-rich aryl residue with formation of the spiro[4.5]decatrienone **192**.^[106]

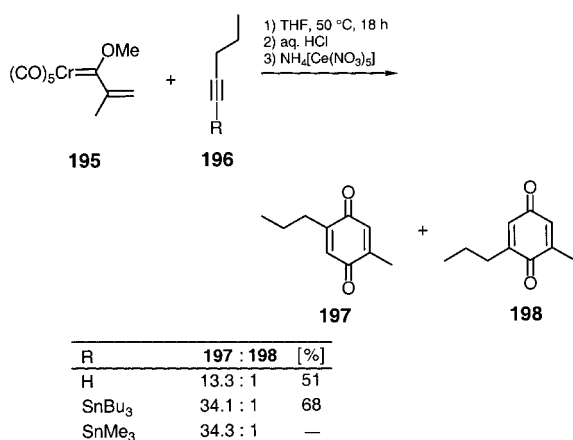
As Wulff and Yang have found, alkynylcarbene complexes react with dienes and alkynes in a one-pot reaction to form dihydronaphthohydroquinone derivatives (see **21–25**).^[18] In actual fact *O*-alkyl-*O'*-trialkylsilyl hydroquinones such as **25** are formed by this route^[18, 19a, 81j] if (2-trialkylsilyl)ethynylcarbenechromium complexes are used. In these reactions (2-alkylethynyl)carbenechromium complexes naturally give the respective bicyclic cyclohexadienone derivatives because the alkyl group does not migrate as readily as the silyl group.^[18, 19a, 20a, 81j]

Further examples of the Dötz reaction of alkenylcarbenechromium complexes are summarized in Table 3.

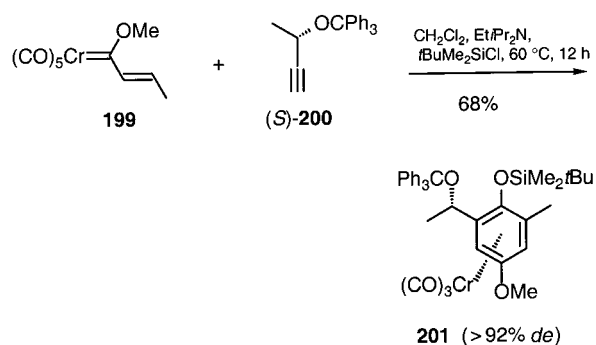
The same rules apply for the regioselectivity of the incorporation of the alkyne as in the Dötz reaction (see above) as long as stannyl or silyl-substituted alkynes are not used.^[112] According to Wulff and co-workers the 2-methylpropenylidene complex **195** reacts with 1-pentyne **196** (R = H)^[111] and also with 1-trialkylstannylpentyne **196** (R = SnBu₃, SnMe₃)^[112] to give, after oxidative work-up, mainly 2-methyl-5-propyl-*p*-benzoquinone (**197**) with the same position of the propyl group (Scheme 12). This means that factors other than exclusively steric effects must play a role here.

Table 3. Recent examples of the formal cycloaddition of alkenylcarbenecromium complexes with CO insertion into alkynes.

Carbene complex	Alkyne	Conditions	Product, Yield	Ref.
		<i>tert</i> -butyl methyl ether, 55 °C, 30 min		[107]
		1) THF, 40 °C, 3 d; 2) pyridine		[108]
		THF, 60 °C, 42 h		[109]
		CH ₂ Cl ₂ , 50 °C, 20 h		[104]
		1) THF, 50 °C; 2) <i>t</i> BuMe ₂ SiOTf, Et ₃ N		[112]
		THF, 50 °C, 20 h		[20]
		THF, 52 °C, 14 h		[20]
		1) THF, 80 °C, 3 d; 2) 1N HCl, THF, H ₂ O, 25 °C, 1 h		[110]

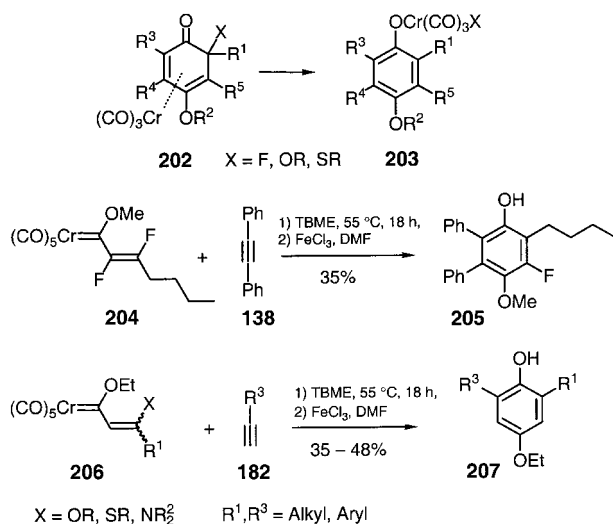

 Scheme 12. The effect of stannylalkynes on regioselectivity; for R = SnMe₃ no yield was reported.

Wulff and et al. have also carried out the first stereo-selective synthesis of an aryltriacarbonylchromium complex with the aid of the Dötz reaction.^[113] By cocyclization of the (*S*)-1-butyne-3-yltriphenylmethyl ether ((*S*)-**200**) with the but-



2-enylidene complex **199**, the complex **201** was formed with a diastereoselectivity of more than 92 %.

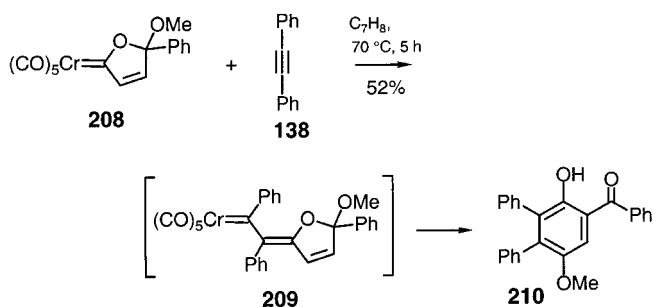
Heteroatom- and in particular donor-substituted alkenyl-carbene complexes frequently deviate extensively from the “normal” Dötz reaction in their reactions with alkynes (see Sections 3.2.2–3.2.7). Even 2-fluoro-, 2-alkoxy-, and 2-alkylthioalkenyl substituted carbene complexes show a remarkably different mode of reaction with alkynes (Scheme 13).



Scheme 13. TBME = *tert*-butyl methyl ether.

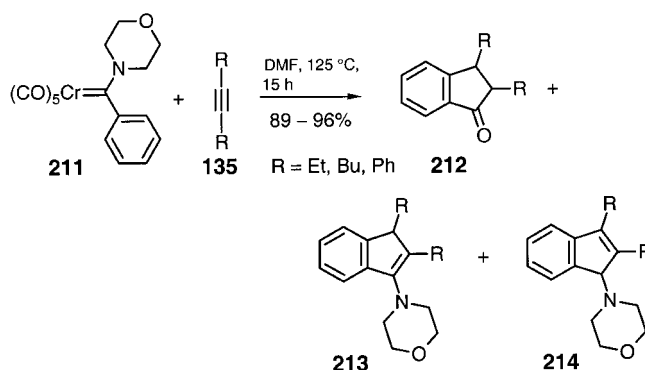
The actually expected cyclohexadienone of type **202** is not found in any instance, rather the respective hydroquinone monoether **203** is formed by loss of the heteroatom group. Thus, for example the 2,3-difluoroheptyliden complex **204** affords the monofluorohydroquinone monoether **205** with tolane (**138**).^[114] The alkenylidene complexes of type **206**, obtained particularly readily by Michael addition of the respective nucleophile to alkynylcarbene complexes (see Section 2.3.2), give hydroquinone monoethers of type **207** with alkynes **182**.^[115]

An interesting result is obtained if an oxacycloalkenylidenechromium complex such as **208** is reacted with an alkyne, for example **138**, for after alkyne insertion the intermediate **209** clearly cannot cyclize. Thanks to the acetal group the ring in intermediate **209** can open so that after CO insertion and renewed cyclization the acylhydroquinone monoether **210** is obtained.^[116] Such a product cannot be readily formed directly by a Dötz reaction because the corresponding starting complex would be very difficult to prepare.

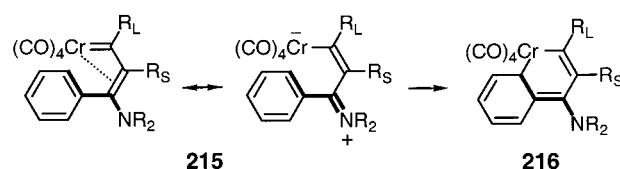


3.2.2. Formal [3+2] Cycloadditions

In one respect 1-aminoarylcabene and 1-aminoalkenylidene complexes behave decisively differently than the previously and exclusively discussed α,β -unsaturated 1-alkoxy-carbene complexes.^[2g] Thus Yamashita et al. found that the morpholinophenylcarbene complex **211** gives exclusively the indene derivatives **213** and **214** in very good yields with symmetrically substituted alkynes **135**, as well as the hydrolysis product **212** of the enamine **213**.^[117a] Dialkylaminofuranylcabene complexes also react in the same way.^[117b]

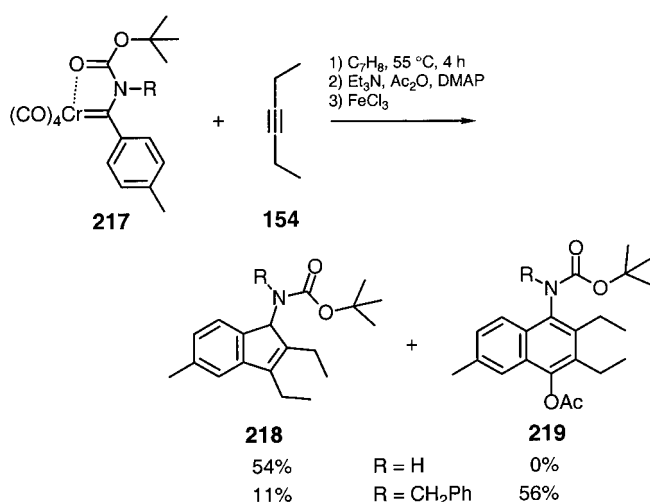


The formation of the indenenes almost certainly takes place by 6π -electrocyclization of the 1-chroma-1,3,5-triene **215**, formed from **211** by alkyne insertion, followed by reductive elimination of the metal fragment from **216** (Scheme 14). According to Wulff and co-workers, amino substituents stabilize intermediate **215**,^[81d] the increased electron density at the chromium atom brought about by the conjugated amino group results in a strengthening of the Cr–CO bond as a result of which the insertion of a *cis*-CO ligand occurs less readily.



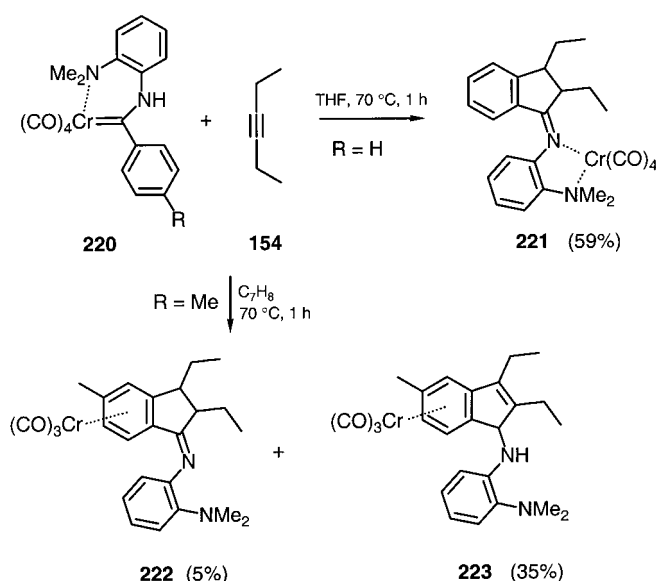
Scheme 14. R_L = larger, R_S = smaller substituent.

That the relative donor ability of the amino substituent in complexes of type **211** or the intermediates of type **215** derived from them is actually responsible for such reaction behavior is demonstrated by the results of Dötz and co-workers (Scheme 15).^[118] This donor ability is clearly reduced by the *tert*-butoxycarbonyl group on the substituents, as in **217**, and, with an additional benzyl substituent on the nitrogen atom mixtures of products with and without CO insertion are formed (**219** and **218**, respectively).^[118] The reaction of **217** is moreover an example of the formal cycloaddition of a chelated carbene chromium complex. Comparative investigations have shown that frequently carbenetetra carbonyl complexes such as **217** are particularly reactive towards alkynes.^[81e, 107, 119]



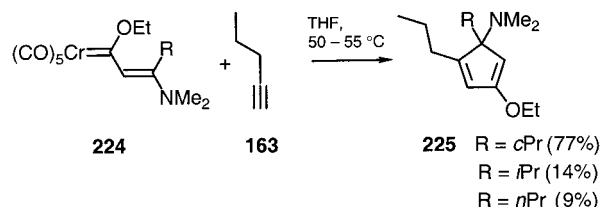
Scheme 15. DMAP = 4-dimethylaminopyridine.

Both aminoindenes and iminoindanes can be formed by the reaction of N-monosubstituted 1-aminocarbenechromium complexes.^[120, 121] Iminoindane- and aminoindenylchromium carbonyl complexes are obtained as products from a 1-(2-aminoaryl)aminocarbenechromium complex with a six-membered chelate ring such as **220**.^[121] Depending upon the solvent, the chromium fragment is bound across the two nitrogen atoms as in **221** (formed in THF) or to the aromatic nucleus as in **222** and **223** (formed in toluene), the R groups do not appear to have any influence.^[121]

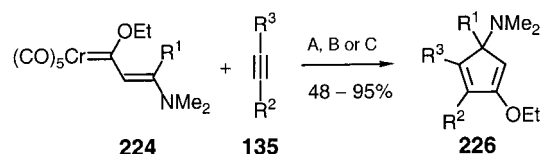


In the case of 1-alkoxycarbene complexes formal [3+2] cycloadducts in significant quantities were, for a long time, observed only starting from *o,o'*-dialkylarylcarbene complexes (see **187** → **189**).^[105] Subsequently de Meijere and co-workers found that with donor substituents in vinyl-analogous α -position to the chromium atom (2-aminoethenyl)carbene complexes such as **224** (readily available from the addition of secondary amines to alkynylcarbene complexes,^[15, 41] see Section 2.3.1) also react under normal reaction conditions

without CO insertion to form 3-alkoxy-5-(dialkylamino)-cyclopentadienes **225**.^[119, 122] The yields however, with tetrahydrofuran as solvent were surprisingly highly dependent upon the nature of the substituent R on the ethenyl terminus of the carbene complex. Only cyclopropyl-substituted complexes gave the corresponding cyclopentadienes in good yields.^[119]



After detailed investigations of all the factors (type of substituent, degree of substitution, solvent, solvent additives) which could influence this reaction, a complete generalization and optimization of this novel five-membered ring synthesis was achieved (Scheme 16).^[21, 123] Cyclopropyl-substituted



Scheme 16. A: hexane, 55 °C; B: pyridine, 55–80 °C; C: MeCN 80 °C, low alkyne concentration (for details see Table 4).

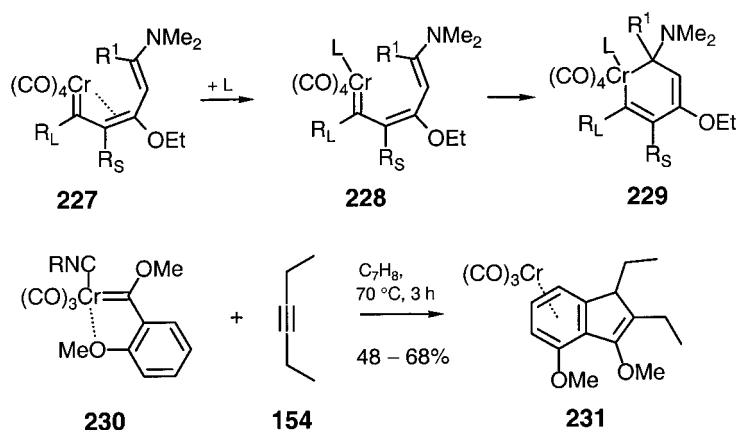
complexes of the type **224** in *n*-hexane as solvent give the correspondingly substituted cyclopentadienes **226** in very good yields (92–95%, see Table 4). Generally good to very good yields are obtained with a wide spectrum of substituents both in the alkenylcarbene complex **224** and in the alkyne component **135** with the use of pyridine as solvent (Table 4).

This is particularly surprising for previously pyridine had been thought of as a “poison” for Fischer carbene complexes, and it was rarely considered as a solvent for their reactions. Clearly, however, pyridine as a good donor ligand stabilizes coordinatively unsaturated intermediate complexes such as **227** and **228**. The increase in the strength of the Cr–CO bond associated with the increased electron density at the chromium atom retards CO insertion and thus favors the direct cyclization of the 1-metalla-1,3,5-triene **227** to a 5-metalla-1,3-cyclohexadiene **229** (Scheme 17).

The cyclopentadiene compounds **226** were also formed in high yield in acetonitrile, although only when a constantly low concentration of the alkyne **135** was maintained by slow addition. At a higher alkyne concentration there is increased CO insertion, although without formation of the Dötz product.^[124] Dötz and Christoffers were able to show that an isocyanide ligand previously introduced into the coordination sphere of the chromium, as in **230**, has exactly the same effect so that indenones **231** are preferentially formed from alkoxy-(aryl)carbenechromium complexes by cyclization to a five-membered ring (Scheme 17).^[125]

Table 4. 5-Dialkylaminocyclopentadienes **226** from β -dialkylamino-substituted carbenechromium complexes **224** and alkynes **135** (see Scheme 16).^[a]

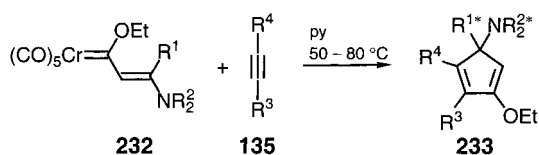
224	R ¹	R ²	135	R ³	Conditions ^[b]	Product	Yield [%]
a		cPr		H	A	226a-cPr	95
b		nPr		H	A	226b-nPr	92
c	Me	Me		Me	B	226c-Me,Me	56
c	Me	CH ₂ OSiMe ₂ tBu		H	B	226c-CH₂OSiMe₂tBu	86
d	nPr	Me		Me	C	226d-Me,Me	95
d	nPr	Ph		Ph	C	226d-Ph,Ph	80
d	nPr	SiMe ₃		H	C	226d-SiMe₃	53
d	nPr			H	B	226d-E,E,Br	69
d	nPr	CH ₂ SiMe ₃		H	C	226d-CH₂SiMe₃	53
d	nPr			H	B	226d-E,E,SiMe₃	81
e		Me		Me	B	226e-Me,Me	91
e				H	B	226e-(CH₂)₃OSiMe₂tBu	77
e				H	B	226e-E,E,Br	51
f		Me		Me	B	226f-Me,Me	94
g		Me		Me	B	226g-Me,Me	69
h		Me		Me	B	226h-Me,Me	46
h				H	B	226h-E,E	50
i		Ph		Ph	B	226i-Ph,Ph	81
i				H	B	226i-E,E,Br	48
j		Me		Me	B	226j-Me,Me	81
k		Me		Me	B	226k-Me,Me	85
l		Me		Me	B	226l-Me,Me	74
m		Me		Me	B	226m-Me,Me	79

[a] E = CO₂Me or CO₂Et. [b] For conditions see Scheme 16.

Scheme 17. L = pyridine, MeCN.

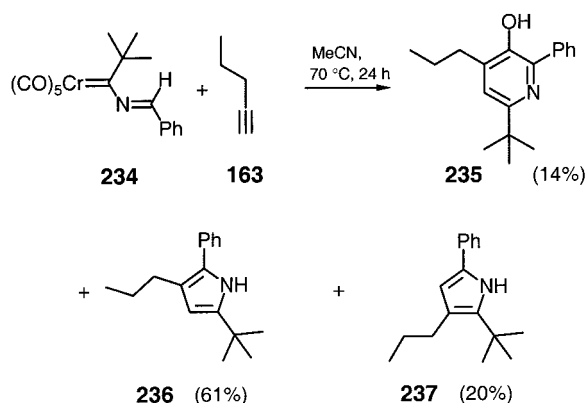
Attempts at the stereoselective control of the formation of cyclopentadienes of type **233** by induction through chiral centers in the carbene ligand (**232**) have been only moderately successful. In cycloaddition with alkynes **135**, the cyclopentadienes **233** were obtained as mixtures of two diastereomers with diastereomeric excesses of up to 80% (Scheme 18).^[126]

Alkylideneaminocarbene complexes such as **234**^[127] are aza analogues of alkenylcarbene complexes and react correspondingly with alkynes. According to the groups of Wulff^[128] and Aumann^[129] these complexes give primarily formal [3+2] cycloadducts analogous to the 1-aminocarbene complexes. The product composition from **234** and 1-pentyne (**163**) is highly solvent dependent.^[128] Although the ratio of the [3+2+1] adduct **235** to [3+2] adducts **236**+**237** changes little in the change from acetonitrile to hexane as solvent, the



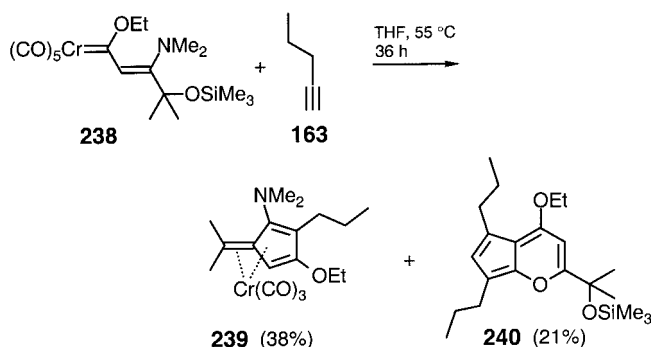
	R ¹	NR ₂ ²	R ³	R ⁴	233 [%]	<i>de</i> [%]
a	<i>n</i> Pr		Me	Me	78	23
b	<i>n</i> Pr		Me	Me	98	31
c	<i>n</i> Pr		Me	Me	22	50
d	<i>n</i> Pr		H	Ph	73	56
e		NMe ₂	H	Ph	48	31
f		NMe ₂	H	<i>t</i> Bu	43	44
g		NMe ₂	H	<i>t</i> Bu	29	75
h		NMe ₂	H	Ph	21	82

Scheme 18. Stereoselective control of the formation of the cyclopentadienes of type **233**.



regiochemistry of the alkyne incorporation, that is, the ratio **236:237** reverses.

The formation of the complexed fulvene **239**^[130] from **238** and 1-pentyne (**163**) also formally follows a [3+2] cycloaddition, even if the mechanism is still obscure. Particularly

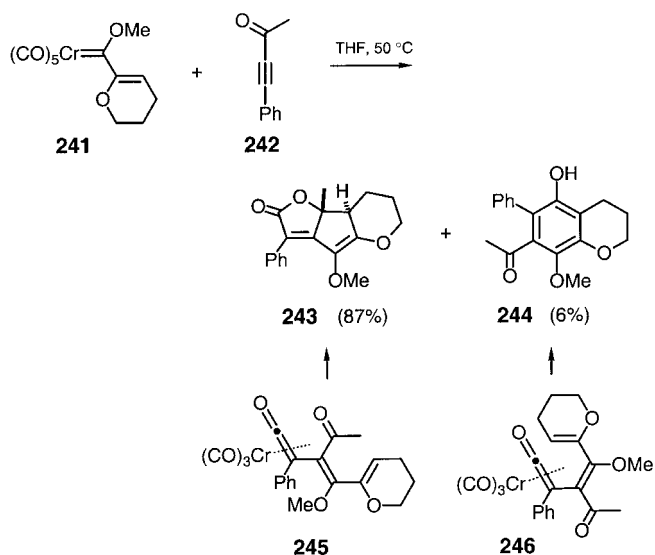


conspicuous here is that the alkyne is inserted contrary to the normal regiochemistry, and in addition, during the reaction the dimethylamino substituent must have migrated. The formation of cyclopenta[*b*]pyrans of type **240** from *Z*-configured complexes such as **238** tends to be the norm (see Section 3.2.3).

3.2.3. [3+4+1] and [3+2+2+1] Cocycloadditions

Both the incorporation of two alkyne molecules each with two C atoms and also that of an alkyne with a total of four C atoms can lead to formal [3+4+*n*] cycloadditions. There are in fact examples of both variants.

Conjugated nonterminal alkynones react with alkenyl-(alkoxy)carbene complexes only to a limited extent to form hydroquinone monoethers in the sense of the normal Dötz reaction. For example, **241** gives only 6% of the Dötz product **244** with 4-phenyl-3-butyne-2-one (**242**).^[111a] The main product is the tricycle **243** (87%) formed as the result of a formal [3+4+1] cocyclization. The insertion of the alkyne to the *E*-configured intermediate **245**, the diastereoisomer of the



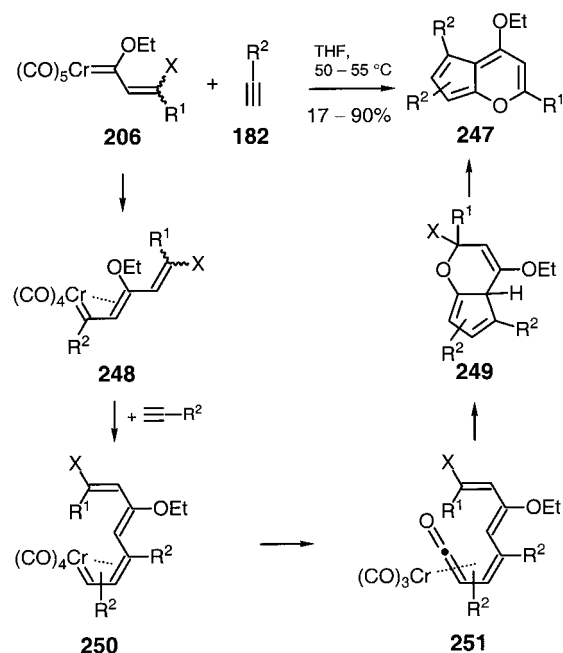
“normal” Dötz intermediate **246**, is held responsible for this unusual reaction course. One special feature is that this acyldienylketene can react further in a Halban–White type cyclization^[131] to form a stable product. Moreover, electronic factors are responsible for the regiochemistry of the insertion, not the size of the substituents on the alkyne, as Wulff et al. were able to demonstrate.^[111a]

In contrast, steric effects may be the reason that in the reaction of 2'-donor-substituted ethynylcarbenochromium complexes **206** with terminal alkynes **182**, cyclopenta[*b*]pyrans **247** are formed, and in yields of up to 90% (Table 5).^[132, 133] An indication for this is primarily that high yields of **247** are obtained particularly when R¹ in **206** is a tertiary or a large secondary substituent. Normally in such cases the Michael adducts of nucleophiles onto alkynylcarbene complexes (type **206**) are not *E*- but *Z*-configured, and

Table 5. Cocyclization of (*E*)- and (*Z*)-(2-dialkylaminoethenyl)carbene-chromium complexes **206** with terminal alkynes **182** (see Scheme 19).

Complex	R ¹	R ²	X	Product	Yield [%]
206a	Ph	Ph	NMe ₂	247a-Ph	24
206a	Ph	Ph	NBn ₂	247a-Ph	48
206a	Ph	Ph	N(<i>CH</i> ₂) ₂) ₂	247a-Ph	43
206a	Ph	Ph	NiPr ₂	247a-Ph	17
206a	Ph	<i>n</i> Pr	NMe ₂	247a-<i>n</i>Pr	19
206b	C(CH ₃) ₂ OEt	Ph	NMe ₂	247b-Ph	51
206b	C(CH ₃) ₂ OEt	Ph	NBn ₂	247b-Ph	68
206b	C(CH ₃) ₂ OEt	Ph	N(<i>CH</i> ₂) ₂) ₂	247b-Ph	42
206b	C(CH ₃) ₂ OEt	<i>n</i> Pr	NMe ₂	247b-<i>n</i>Pr	59
206c	C(CH ₃) ₂ OSiMe ₃	Ph	NMe ₂	247c-Ph	90
206c	C(CH ₃) ₂ OSiMe ₃	Ph	NBn ₂	247c-Ph	78
206c	C(CH ₃) ₂ OSiMe ₃	Ph	NEt ₂	247c-Ph	87
206c	C(CH ₃) ₂ OSiMe ₃	<i>n</i> Pr	NEt ₂	247c-<i>n</i>Pr	25
206d		Ph	NMe ₂	247d-Ph	41
206d		Ph	NBn ₂	247d-Ph	47
206d		<i>n</i> Pr	NMe ₂	247d-<i>n</i>Pr	17
206e		Ph	NMe ₂	247e-Ph	84
206f	Adamantyl	Ph	NMe ₂	247f-Ph	56
206f	Adamantyl	Ph	NBn ₂	247f-Ph	47

the alkyne insertion leads then to a 1-chroma-1,3,5-hexatriene **248** with a *Z*-configured central double bond which is not capable of 6 π -electrocyclization (Scheme 19). Therefore a second molecule of the alkyne and finally CO are inserted, and the resulting hexatrienylketene complex **251** can then

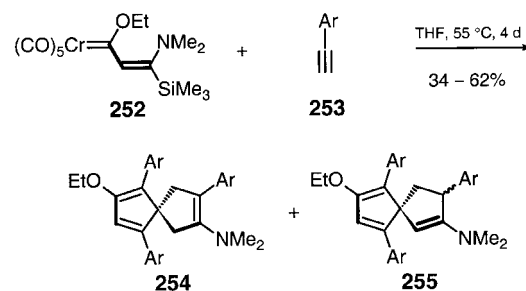
Scheme 19. X = NR₂, OR, SR. For details see Table 5.

participate in an intramolecular [4+2] cycloaddition. The resulting heterobicycle **249** finally furnishes the end product **247** by 1,4-elimination of HX. The yields with better leaving groups (e.g. NBn₂ (Bn = benzyl) compared with NMe₂) are thus generally higher. Because of the lower regioselectivity in

the insertion of the second alkyne molecule the cyclopenta[b]pyrans are formed in part as a mixture of two regioisomers.

3.2.4. [3+2+2+2] Cocyclizations

A quite unusual mode of reaction was displayed by the 2'-trimethylsilyl-substituted (2-dimethylaminoethenyl)carbene-chromium complex **252**. On heating **252** with an excess of phenylethyne (six equivalents) in THF at 55 °C a yellow solid, which fluoresces strongly in solution, was obtained after purification by column chromatography (Scheme 20). ¹H and ¹³C NMR spectra suggested that it was a mixture of three



Scheme 20. For details see Table 6.

isomeric compounds (ratio 3.6:1:1) none of which possessed a trimethylsilyl group. Assuming that the highest positioned peak in the mass spectrum at *m/z* 433 corresponded to the molecular ion, a compound had been formed in a yield of 62% in this reaction from the protodesilylated carbene ligand and three molecules of phenylethyne without carbonyl insertion. However, the three isomers could neither be separated, even by repeated column chromatography, nor was it possible to deduce the correct constitutions from the NMR spectra.^[47a, 115a] Eventually a single crystal of the quaternary ammonium iodide of the main isomer could be obtained and thus the compound could be characterized by X-ray structural analysis as the spiro[4.4]nonatriene **254** (Ar = Ph).^[134]

To probe the scope of this reaction **252** was treated with a series of alkynes. It was thus shown that only arylacetylenes gave spiro[4.4]nonatrienes **254** and **255**. The isomer ratios of the products obtained varied according to the type of substitution of the arylacetylene used (Table 6).^[134b] Terminal alkynes such as 1-pentyne and nonterminal alkynes such as 2-butyne react in the normal manner to form the respectively substituted cyclopentadienes.^[123]

Table 6. Spiro[4.4]nonatrienes **254** and **255** (see Scheme 20) formed by the reaction of three molecules of an arylethyne **253** with the (3-dimethylamino-3-trimethylsilylalkenylidene)chromium complex **252** and subsequent cyclization.

	Ar	Yield [%] 254 + 255	Ratio 254:255
a	C ₆ H ₅	62	1.8:1
b	4-PhC ₆ H ₄	34	4.5:1
c	4-OMeC ₆ H ₄	37	2.5:1
d	3,5-(Me) ₂ C ₆ H ₃	48	1.6:1

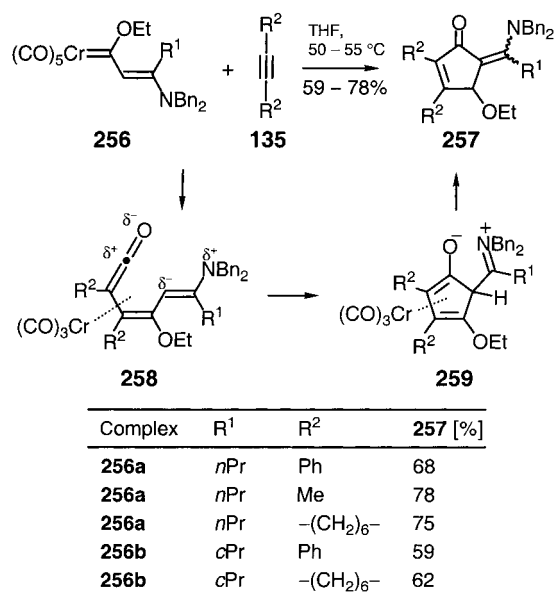
For some of the originally merely speculative concepts concerning the mechanism of formation of these novel cocyclization products of arylethynes with the chromium complex **252** the first experimental evidence has been collected.^[134b] The observation that a ¹³C-labeled carbene carbon atom in the complex **252** ends up as the spirocarbon in the products **254** and **255** consistently demonstrates that the original connectivity of the carbene ligand in **252** is retained in the products **254** and **255** (Scheme 20) just as in all the previously known products from β -dialkylamino-substituted α,β -unsaturated carbenechromium complexes.^[21] This means that at the same time the ethoxy group must have migrated from its original position in **252** to a different carbon atom in the products.^[134b]

3.2.5. [2+2+1] Cocyclizations

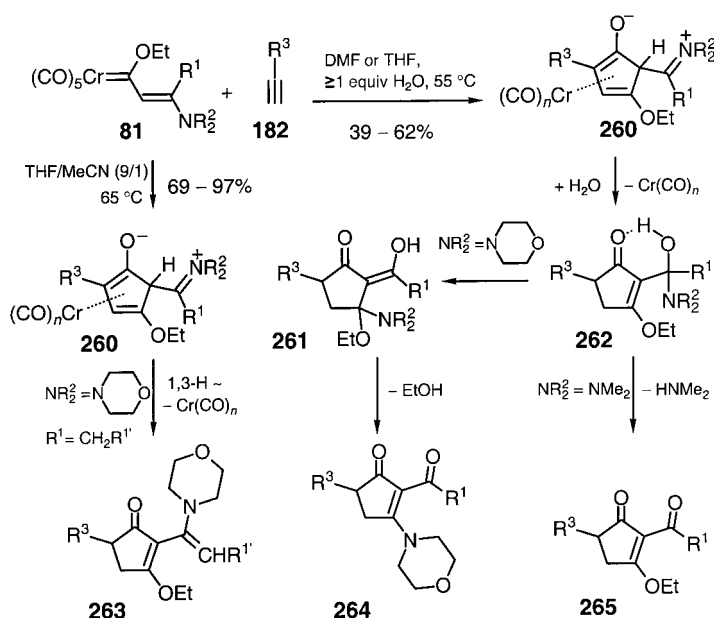
In the classical Dötz reaction (see Section 3.2.1), its analogues, and also the formal [3+2] cycloadditions of (2-dialkylaminoalkenyl)carbene complexes with alkynes, three carbon atoms of the carbene complex reappear in the cyclic system of the product. A further cyclization variant has been described by de Meijere and co-workers in which only the carbene carbon atom and its directly neighboring C atom in the carbene complex are found in the five-membered ring of the product, the amino-substituted β -C atom is in contrast exocyclic. It was first found that nonterminal alkynes do not react with (2-dibenzylaminoalkenyl)carbenechromium complexes of type **256** in tetrahydrofuran to give formal [3+2] cycloadducts of type **226**, but react smoothly with CO insertion to produce 5-(1-dibenzylaminoalkylidene)cyclopent-2-enones **257** (Scheme 21).^[135] Since CO insertion is effectively suppressed with the concurrent presence of two good electron-donor groups in the β position of the complex, or in the presence of a solvent with good donor properties such as pyridine or acetonitrile (see Section 3.2.2), the formation of an intermediate ketenyl complex **258** is under-

standable. Surprisingly however, **258** apparently does not cyclize to form a cyclohexadienone of type **147** as in a Dötz reaction, but clearly cyclizes as a 1,5-dipole to form a five-membered ring. The resulting intermediate **259** can be transformed into the end product **257** by a [1,5] hydrogen shift and loss of the tricarbonylchromium moiety. The (dibenzylaminoalkylidene)cyclopentenones occur as mixtures of *E*- and *Z*-isomers, and the diastereomeric ratio depends upon the nature of the substituent, although the barrier to *E/Z*-isomerization is so low that rapid interconversion of the two isomers occurs at room temperature.

Terminal alkynes **182** also react with complexes of type **256** in this manner under CO insertion to afford [2+2+1] cocyclization products of type **257** if the reaction is carried out in dimethylformamide (DMF).^[21] Two other types of [2+2+1] cocyclization products are obtained, sometimes in very good yields, if carbenechromium complexes **81** with a β -morpholinoethenyl substituent are reacted with alkynes **182**. Here the constitution of the products depends upon the



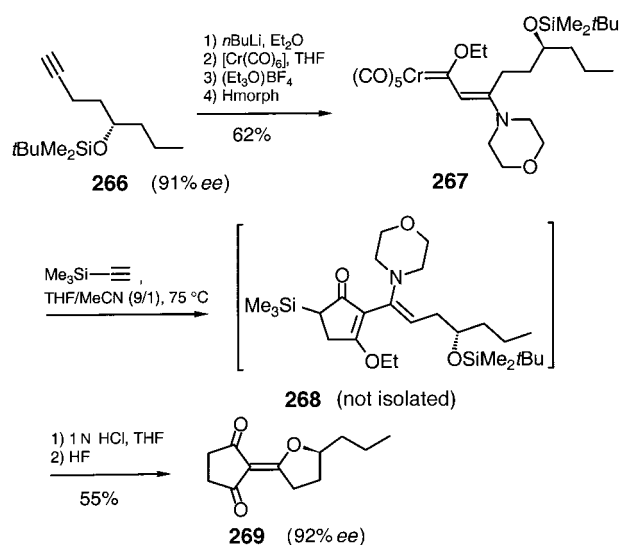
Scheme 21. Substituent effects on the formation of compounds **257**; Bn = benzyl.



reaction conditions. In aqueous DMF or THF 2-acyl-3-morpholinocyclopent-2-enones **264** are formed,^[136] and—in the case of **81** with the dimethylamino substituents (entry 7 in Table 7) in aqueous THF—also the 2-acyl-3-ethoxycyclopent-2-one **265a**.^[137] In contrast, however, in a mixture of THF and acetonitrile (9:1), 2-(1-morpholino-1-alkenyl)cyclopent-2-enones **263** are formed.^[138] Clearly, in a manner similar to the formation of the methylenecyclopentenones **257** an intermediate of type **260** must arise on the way to these cocyclization products. In the case of the alkenylcyclopentenones **263**, a [1,3] hydrogen shift with subsequent tautomerization and loss of the carbonylchromium fragment leads to the cyclopentenone. The acylcyclopentenones **264** and **265** arise through 1,4-addition of water to **260**. On the way to **264** the amino group must migrate from the exocyclic to the endocyclic 3-position, which can occur through elimination–readdition. Subsequent 1,4-elimination of ethanol from **261**

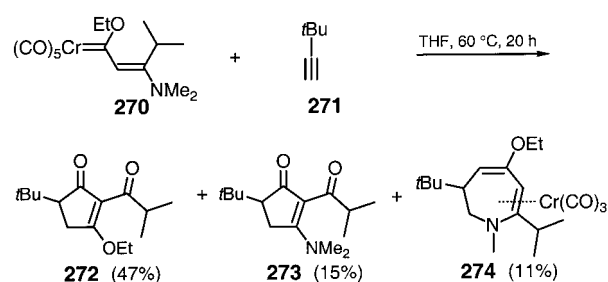
leads to **264**, whereas **265** is formed directly by the elimination of dimethylamine from **262** (Table 7).^[136]

Cyclopentenones of type **263**, **264**, and **265** are protected 2-acylcyclopentane-1,3-diones which should have significant synthetic potential. Since the mild reaction conditions are compatible with a series of functional groups in both starting materials, namely the complexes **81** and the alkynes **182**, the use of this type of reaction in natural-product synthesis is obvious. A simple example was demonstrated by de Meijere and co-workers in the synthesis of (–)-oudenone **269** in essentially two steps from the nonracemic octyn-5-yl silyl ether **266** and trimethylsilylacetylene. Since the stereogenic center in **266** is not affected throughout the whole transformation the enantiomeric purities of the product **269** and of the starting material **266** are essentially identical.^[138]



3.2.6. [5+2] Cocyclizations

In the reaction of the isopropyl-substituted alkenylcarbenechromium complex **270** with sterically demanding substituted terminal alkynes such as 3,3-dimethylbutyne (**271**), the seven-membered heterocycle **274** was obtained as a byproduct in addition to the two acylcyclopentenones **272** and **273**, which are formed by formal [2+2+1] cycloaddition. In the new cocyclization product **274** five atoms of the (dialkylaminoethenyl)carbene complex and the two atoms of the triple bond are involved in the formation of the ring. The

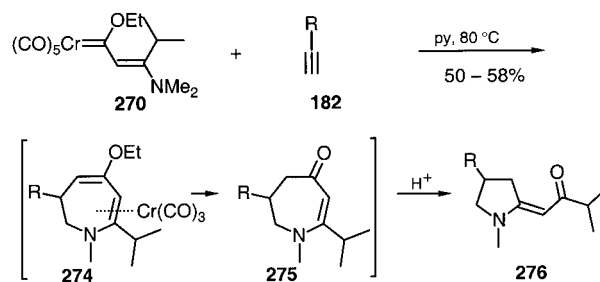


tricarbonylchromium fragment is coordinated η^5 with the aminodienyl unit of the dihydroazepine **274**.

The formation of **274** almost certainly begins in the same way as the Dötz reaction with a regioselective insertion of the alkyne into the chromium–carbon double bond. The result is most readily explained if it is assumed that first the free carbene is formed from the insertion product, possibly facilitated by the bulky *tert*-butyl substituent, and the free carbene then inserts intramolecularly into one of the C–H bonds of the aminomethyl groups.

To favor formation of the dihydroazepine **274** possibly still further two equivalents of triphenylphosphane were added to the reaction mixture, for triphenylphosphane as a donor ligand leads to an increased electron density at the chromium center, which retards CO insertion during cyclization.^[139] Indeed, in this way the yield of **274** could be increased to 23 %.

Even more effective is the use of the donor solvent pyridine, as in the synthesis of the 3-alkoxy-5-dimethylaminocyclopentadienes **226**.^[123] In the reaction of **270** with **182** in pyridine the product ratio was shifted towards the dihydroazepine **274**, and at the same time under these conditions the tricarbonylchromium fragment was cleaved off. After purification by column chromatography the methylenepyrrolidine **276** was formed, not the azepinone **275** (Scheme 22). The former is formed



Scheme 22. R = *t*butyl, adamantyl, CMe₂NMe₂; py = pyridine.

Table 7. Highly-substituted cyclopentenones **263**–**265** from (2-dialkylaminoethenyl)carbenechromium complexes **81** and alkynes **182**.

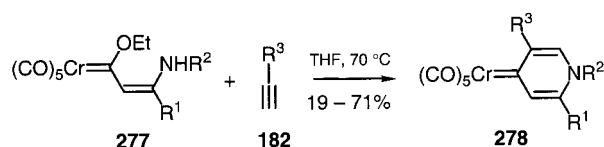
Entry	R ¹	NR ₂ ^[a]	R ³	Conditions ^[b]	Product	[%]	Product	[%]
1	<i>n</i> Pr	morph ^[b]	<i>n</i> Pr	A	264a	54	–	
2	<i>n</i> Pr	morph	<i>t</i> Bu	A	264b	51	–	
3	<i>n</i> Pr	morph	SiMe ₂ <i>t</i> Bu	A	264c	62	–	
4	Ph	morph	<i>n</i> Pr	A	264d	39	–	
5	Ph	morph	<i>t</i> Bu	A	264e	54	–	
6	Ph	morph	SiMe ₂ <i>t</i> Bu	A	264f	48	–	
7	<i>i</i> Pr	NMe ₂	<i>t</i> Bu	A	264g	15	265a	47
8	<i>n</i> Pr	morph	<i>n</i> Pr	B	263a	82	–	
9	<i>n</i> Pr	morph	Ph	B	263b	97	–	
10	Me	morph	SiMe ₂ <i>t</i> Bu	B	263c	72	–	
11	(CH ₂) ₄ OSiMe ₂ <i>t</i> Bu	morph	SiMe ₂ <i>t</i> Bu	B	263d	69	–	

[a] morph = morpholino. [b] A: DMF (1 equiv H₂O) or THF (1 equiv H₂O), 55 °C; B: THF/MeCN 9:1, 65 °C.

most probably by ring-opening hydrolysis of the cyclic enamine **275**, and subsequent ring closure by the addition of the secondary amino group onto the δ -oxo function.^[137] Methylenepyrrolidines **276** are produced in this way only with sterically demanding substituted alkynes **182**, with sterically less congested alkynes only the 3-alkoxy-5-dialkylaminocyclopentadienes of type **226** are formed.^[123]

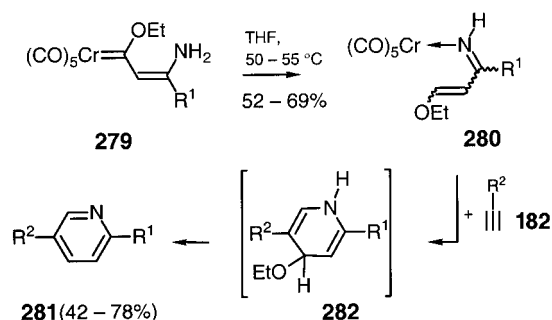
3.2.7. [4+2] Cycloadditions

3-Aminoalkenylidenchromium complexes such as **277** with a secondary amino group, react with alkynes in a totally different way compared to those with tertiary amino groups. When **277** and terminal alkynes **182** are heated in THF, 4-(1*H*)-pyridinyl complexes of type **278** are formed (Scheme 23),^[48a, 111a, 130] the stability of which arises from the nitrogen atom in the ring. Four sequential atoms of the 3-aminoalkenylidene moiety from **277** are thus found in the formal cycloadduct **278**.



Scheme 23. $\text{R}^1 = n\text{Bu}$, Ph ; $\text{R}^2 = \text{Me}$, $i\text{Pr}$, Bn ; $\text{R}^3 = n\text{Bu}$, Ph .

(2-Aminoalkenyl)carbenechromium complexes **279** with a primary amino group behave quite differently (Scheme 24). When heated in THF they rearrange into pentacarbonylchromium-coordinated 1-azabutadienes **280** which may actually be isolated.^[51] In the presence of alkynes, complexes **280** react



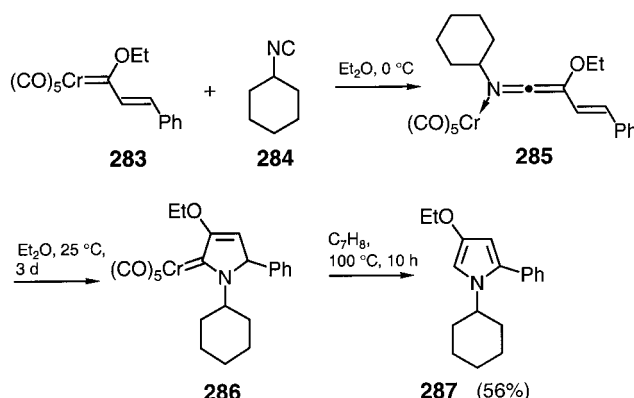
Scheme 24. $\text{R}^1 = n\text{Pr}$, $c\text{Pr}$, Ph ; $\text{R}^2 = \text{Ph}$, $n\text{Pr}$.

in a [4+2] cycloaddition to form dihydropyridines **282**, which give pyridines **281** by 1,4-elimination of ethanol. Thus, the mechanism of the previously observed^[48a] formation of pyridines from (β -aminoethenyl)carbenechromium complexes is also explained, for until then it was not understandable why in the formation of the pyridine almost exclusively that regioisomer is formed which contradicts the usual regioselectivity of an alkyne insertion into an ethenylcarbenechromium complex.

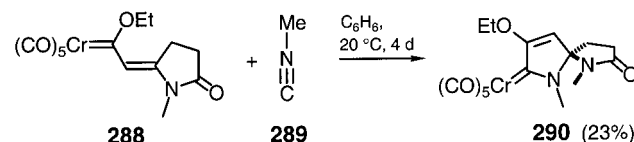
3.2.8. Cocyclizations with Aza- and Phosphaalkynes

3.2.8.1. 2-Aza-1-alkynes (Isocyanides)

Carbene complexes react quite generally with isocyanides to form keteneimine complexes;^[140] this also applies to α,β -unsaturated carbene complexes such as **283** which at 0 °C first form the 3-ethoxy-3-styrylketeneimine complex **285**. On warming to room temperature it produces the 3-ethoxy-2,5-dihydro-2-pyrrylidene complex **286** from which the pyrrole derivative **287** is released on heating to 100 °C.^[141]



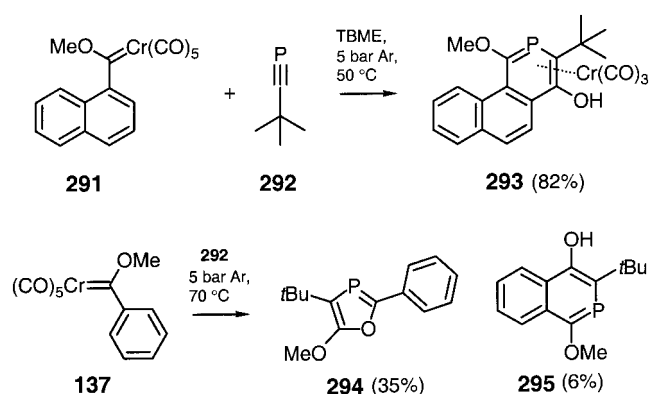
Only a small number of similar cases are known in which the α,β -unsaturated ligand is ultimately included into a cycloadduct by reaction with an isocyanide. One further interesting example of this type of reaction is the formation of the 1,6-diazaspiro[4.4]non-3-en-2-ylidene complex **290** from the β -donor-substituted ethenylcarbenechromium complex **288** and methyl isocyanide (**289**).^[142]



3.2.8.2. Phosphaalkynes

As soon as isolable, kinetically stabilized phosphaalkynes were first known^[143] they were also treated with unsaturated Fischer carbene complexes.^[2f, 144] What effects the presence of the phosphorus atom as a potential electron-donor ligand would have on the course of the reaction was certainly eagerly awaited.

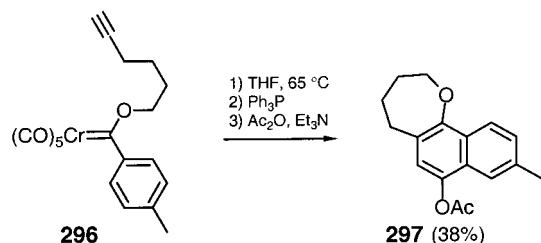
In actual fact, 3,3-dimethyl-1-phospha-1-butyne (**292**) adds to the (1-naphthyl)carbenechromium complex **291**^[144] as in a Dötz reaction and the 3-phosphaphenanthrene derivative **293** is formed in very good yield. In a competition reaction between **292** and 3,3-dimethylbutyne **271** with the complex **291** the phosphaalkyne **292** was found to react six times faster than the alkyne.^[144] A side reaction in which oxaphospholes **294** are formed—corresponding to furan formation during a Dötz reaction—cannot always be suppressed, and frequently even becomes the main reaction, as in the example of the phenylcarbenechromium complex **137**.^[144]



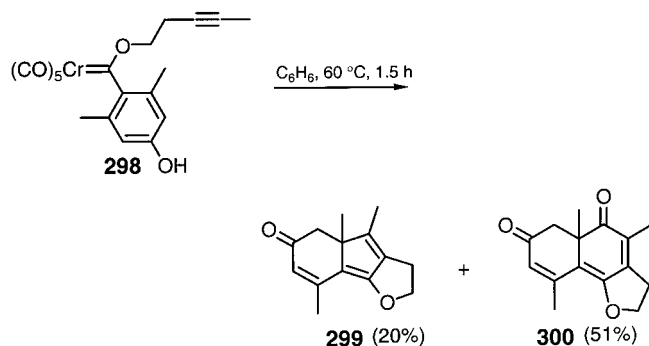
3.2.9. Intramolecular Cocyclization of Carbene Complexes with Alkynyl Groups

There have been only a few investigations of intramolecular variants of the Dötz reaction. In view of the steric demands on alkyne insertion the connection of the alkyne unit only appears useful when through the heteroatom at the carbene center. This principle has been used mainly to control the regioselectivity of ring annelation.^[81b,c, 85a, 145–147]

Annelation follows the classical Dötz reaction when a THF solution of the complex **296** with a 5-hexynyl chain on the oxygen atom is heated under reflux.^[146] Neither added 1-hexyne nor diphenylacetylene (tolane (**138**)) could suppress the intramolecular reaction or influence it in any way. However, the product **297** was isolated in only 38% yield

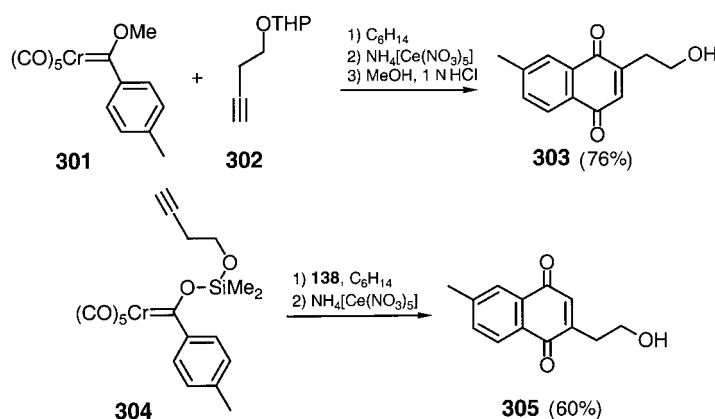


following acetylation of the phenol hydroxyl group. A significantly better yield was obtained in the intramolecular cycloaddition of the complex **298**, in which in addition to the formal Dötz product **300** the indene derivative **299**, arising from a formal [3+2] cycloaddition, was also obtained.^[106]



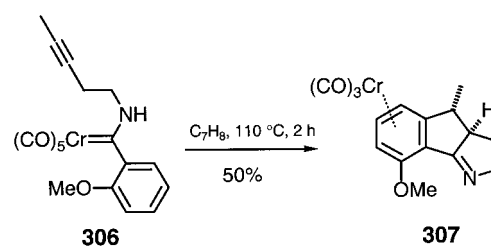
With this principle Gross and Finn developed a method for the preparation of quinones with a regiochemistry of alkyne insertion contrary to that of the normal Dötz reaction

(Scheme 25).^[146] The handle with the O–Si–O group in **304** can be readily cleaved after successful annelation so that after oxidation with cerium(IV) ammonium nitrate the (hydroxyethyl)naphthoquinone **305** is formed. This is a regioisomer of the normal oxidized Dötz product **303** from **301** and protected 3-butynol **302**. Unlike the intramolecular reaction of **296** the addition of tolane (**138**) dramatically favors the intramolecular cycloaddition of silyloxycarbenechromium complexes such as **304**.^[146]



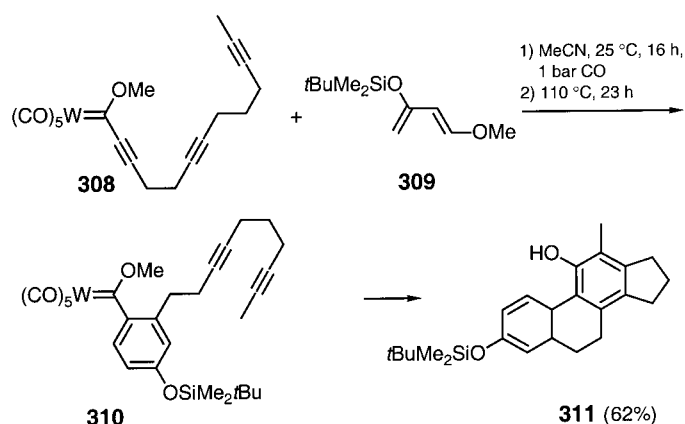
Scheme 25. Reversal of the regioselectivity in the Dötz reaction by intramolecularization.

The intramolecular formal [3+2] cycloaddition of 1-amino-substituted carbene complexes to alkynes has also been carried out. One example is the transformation of the 1-(alkynylamino)carbene complex **306** into the benzo-tetrahydroazapentalene derivative **307** by heating to 110 °C. As in



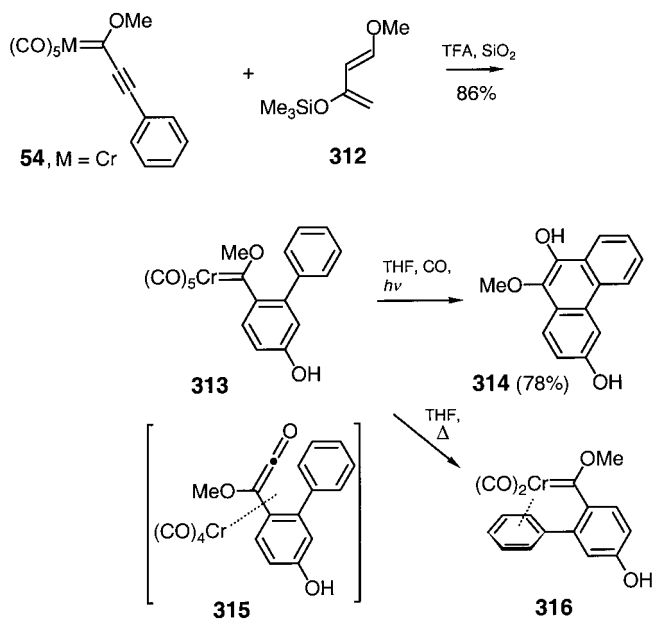
the intermolecular cycloaddition of 1-aminocarbene complexes, CO insertion is suppressed.^[147, 148] The *cis* arrangement of the tricarbonylchromium fragment and methyl substituent in the five-membered ring was established by X-ray structural analysis,^[147] thus the double ring annelation takes place diastereoselectively.

Particularly impressive is the cascade reaction of the tungsten alkynylcarbene complex **308** described by Wulff and co-workers which leads to a one-step construction of the steroid framework **311** in 62% yield. The sequence begins with a [4+2] cycloaddition of the diene **309** to the alkynylcarbene complex **308** with subsequent aromatization and generation of the arylcarbene complex **310**. Similar to the formation of the cyclopenta[*b*]pyrans^[132] (Section 3.2.3.), a double alkyne insertion into the metal–carbene carbon bond, CO insertion, and electrocyclic ring closure then follow.^[149]



3.3. [5+1] and [4+1] Cocyclizations with Carbon Monoxide and Isocyanides

In the normal Dötz reaction alkyne insertion generally leads to a butadienylcarbene complex of type **143** which inserts a *cis*-positioned carbonyl ligand to form a butadienylketene complex **144** (see Scheme 9). Merlic et al. have developed a procedure for the synthesis of *o*-alkoxyphenols and *o*-quinones^[150] in which external CO is inserted into a stable dienylcarbene complex analogous to **143**. This transformation begins (Scheme 26) with the [4+2] cycloaddition of

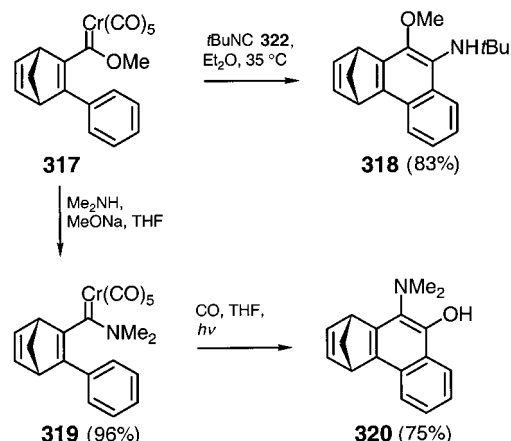


Scheme 26. TFA = trifluoroacetic acid.

1-methoxy-3-trimethylsilyloxy-1,3-butadiene (**312**) to the (phenylethynyl)carbenechromium complex **54** (M = Cr) in which the stable biphenylcarbene complex **313**, formally a dienylcarbenechromium complex analogous to **143**, is formed (see Section 2.2). The *o*-methoxyphenol **314** (78% yield) is produced smoothly by irradiation of **313** in a CO atmosphere.^[150a] It can be safely assumed that—as in the Dötz reaction—a dienylketene complex occurs as an intermediate, in this case it contains a methoxyketenyl moiety **315**.

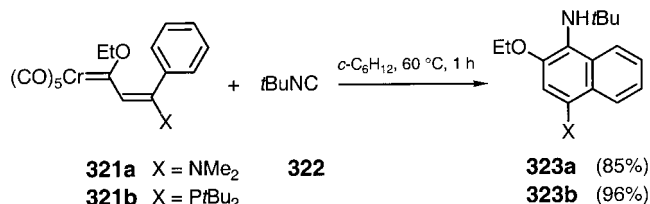
Derivatives of **54** with a *meta*-substituted aryl residue lead to products of type **314** with astonishingly high regioselectivities.^[150b] The reaction proceeds surprisingly differently if the solution of **313** in THF is not irradiated but is heated under reflux,^[151] then a carbenedicarbonylchromium complex **316**, chelated to a phenyl ring, is formed (Scheme 26).

In contrast, the [5+1] cocyclizations of complexes such as **313** with isocyanides also occur purely thermally.^[54, 152, 153] On heating complex **317**, analogous to **313** and formed by cycloaddition of cyclopentadiene to **54** (M = Cr), with *tert*-butylisocyanide, the naphthonorbornadiene derivative **318** is formed.^[152a] Merlic et al. showed impressively that by simple



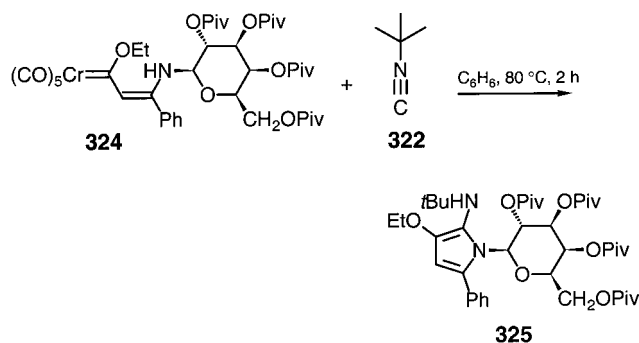
manipulations a product of type **318** with reversed positioning of the oxygen and the nitrogen substituent (**320**) can be obtained.^[152a] Thus, if **317** is first treated with dimethylamine and the resulting 1-dimethylaminocarbene complex is irradiated in a carbon monoxide atmosphere, the naphthonorbornadiene **320** is formed smoothly.

Whereas (3-dialkylaminoalkenylidene)chromium complexes differ in their reactions with alkynes from other α,β -unsaturated carbene complexes, this does not apply to their reactions with isocyanides. Aumann et al. have shown that alkoxydiaminonaphthalenes such as **323a** are accessible from (3-dialkylamino-3-phenylpropenylidene)carbene complexes of the type **321a** and the isocyanide **322**.^[50, 153] β -Phosphanyl-substituted ethenylcarbene complexes such as **321b** also react with isocyanides in the same manner to yield alkoxyamino-phosphanylnaphthalenes such as **323b**.^[54]



Stable butadienylcarbenechromium complexes can not only form *o*-alkoxy- or *o*-aminophenol derivatives by [5+1] cocyclization with CO or isocyanides, but react in the manner of [4+1] cocyclizations. In the reaction of (β -aminoethenyl)-

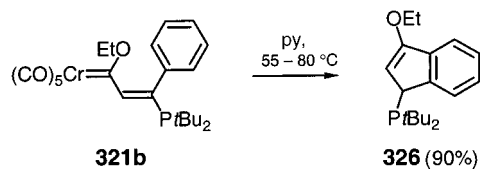
carbene complexes with secondary amino groups such as **324** with an isocyanide such as **322**, a keteneimine complex analogous to **285** is first formed, which has two possibilities for cyclization.^[49] Of these the one with inclusion of the phenyl substituent would give a formal [5+1] cocyclization product. However, the other variant is preferred, in which clearly the secondary amino group attacks the keteneimino group as a nucleophile so that the pyrrole **325** is formed.^[49b]



3.4. Intramolecular Cyclizations and Rearrangements of the Carbene Ligand

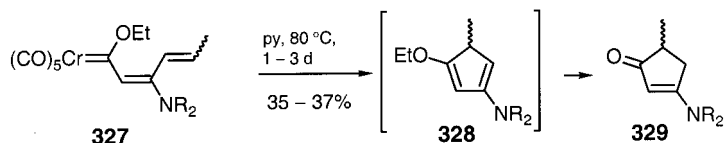
In addition to the intermolecular reactions of α,β -unsaturated carbenechromium complexes described above, in which—apart from the intramolecular variants of the Dötz reaction—one, two, or even three external partner molecules are involved, there are also reactions with ethenyl- and phenylcarbenechromium complexes in which only the carbene ligand cyclizes or rearranges.

With the [5+1] cocyclizations (see Section 3.3) of the stable butadienylcarbene complexes a kind of side entry into the Dötz reaction occurs which—as is usual after the insertion of carbon monoxide—concludes with the cyclization of a dienylketene complex. The formal [3+2] cycloadditions to indene and cyclopentadiene derivatives are examples of how butadienylcarbenechromium complexes, formed as intermediates by alkyne insertion, can also cyclize directly (see Section 3.2.2). That stable butadienylcarbenechromium complexes also cyclize to five-membered rings was eventually demonstrated by Aumann et al.^[54] If the 3-di-*tert*-butylphosphanyl-3-phenylpropenylidene complex **321b** is not heated with *tert*-butylisocyanide in cyclohexane, but simply warmed in pyridine, the phosphanylindene **326** is formed. What special



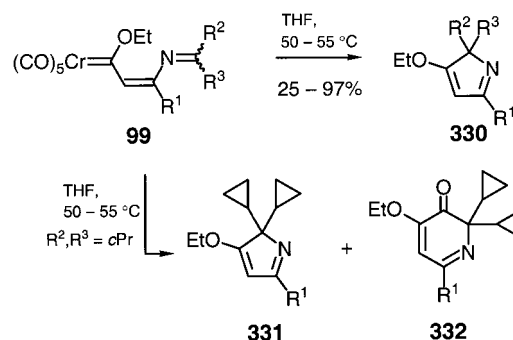
role pyridine as solvent plays has already been demonstrated by de Meijere and co-workers with the broadly applicable synthesis of 5-dialkylaminocyclopentadienes (see Section 3.2.2).^[123] Thus, the relatively unstable 4-aminochroma-hexatrienes of type **327** also react intramolecularly to the

corresponding cyclopentadienes **328**, which upon aqueous work-up hydrolyze to 3-dialkylaminocyclopentenones **329** (Scheme 27).^[154]



Scheme 27. R = Me, Et.

1-Chroma-1,3,5-hexatrienes evidently have a fundamental reaction pathway which leads to formation of a five-membered ring. In this case it does not matter how the 1-chroma-1,3,5-hexatriene is assembled, or whether it contains further heteroatoms, as the example of 5-aza-1-chroma-1,3,5-hexatrienes **99** shows. These complexes, which are readily obtained by the addition of imines to alkynylcarbene complexes (see Scheme 5), cyclize on warming in THF—even in the presence of alkynes without their incorporation—to 2*H*-pyrroles **330**, mostly in good yields. With two cyclopropyl substituents in **99**, the pyridone **332** might be formed as a byproduct along with the 2*H*-pyrroles **331** on heating (Scheme 28 and Table 8).^[52, 155]

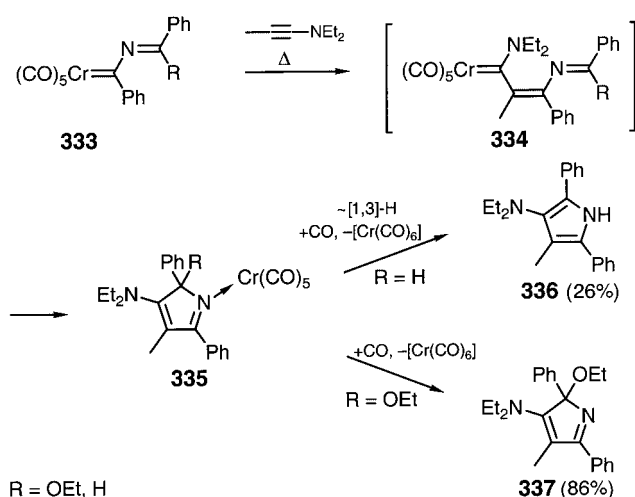


Scheme 28. For details see Table 8.

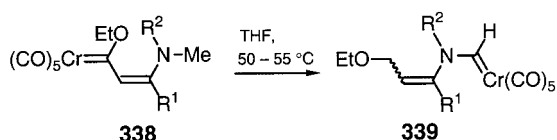
Table 8. Reactions of 5-aza-1-chroma-1,3,5-hexatrienes **99** according to Scheme 28.

Complex	R ¹	R ²	R ³	Product	[%]	Product	Yield [%]
99a (41)	Ph	<i>p</i> -MeOC ₆ H ₄	<i>p</i> -MeOC ₆ H ₄	330a	25	—	—
99b (63)	<i>n</i> Pr	<i>p</i> -MeOC ₆ H ₄	<i>p</i> -MeOC ₆ H ₄	330b	57	—	—
99c (64)	<i>c</i> Pr	<i>p</i> -MeOC ₆ H ₄	<i>p</i> -MeOC ₆ H ₄	330c	65	—	—
99d (59)	<i>t</i> Bu	<i>p</i> -MeOC ₆ H ₄	<i>p</i> -MeOC ₆ H ₄	330d	78	—	—
99e (72)	Ph	Ph	<i>c</i> Pr	330e	62	—	—
99f (88)	<i>n</i> Pr	Ph	<i>c</i> Pr	330f	88	—	—
99g (98)	<i>c</i> Pr	Ph	<i>c</i> Pr	330g	97	—	—
99h (85)	<i>t</i> Bu	Ph	<i>c</i> Pr	330h	85	—	—
99i (63)	Ph	<i>c</i> Pr	<i>c</i> Pr	331a	45	332a	21
99j (81)	<i>n</i> Pr	<i>c</i> Pr	<i>c</i> Pr	331b	63	332b	22
99k (84)	<i>c</i> Pr	<i>c</i> Pr	<i>c</i> Pr	331c	81	332c	0
99l (86)	<i>t</i> Bu	<i>c</i> Pr	<i>c</i> Pr	331d	92	332d	0

The 2-dialkylamino-5-aza-1-metalla-1,3,5-hexatrienes **334**, which arise from alkyne insertion into 1-(methyleneamino)-alkydenemetal complexes **333**, cyclize similarly and give pyrroles of type **336** and **337**.^[35b, 129]



The rearrangement to the pentacarbonyl(ethenylamino)-methylenchromium complexes **339**, observed with (β -aminoethenyl)carbene complexes of the type **338** with a tertiary substituent R^1 in the β position, appears particularly bizarre (Scheme 29 and Table 9).^[156] The mechanism of this transformation, which in view of the β -lactam synthesis developed by Hegedus et al. with aminocarbene complexes^[157] could achieve preparative significance, is still open to speculation.



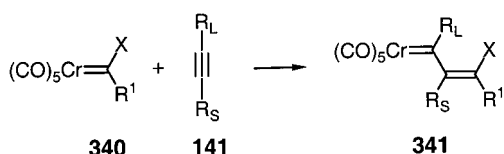
Scheme 29. For details see Table 9.

Table 9. Rearrangement of β -aminoethenylcarbene complexes **338** (see Scheme 29).

Complex	R^1	R^2	Product	[%]	Ratio E:Z
338 a	<i>t</i> Bu	Me	339 a	59	13:1
338 b	CMe_2OEt	Me	339 b	54	5:1
338 c	SiMe_3	Me	339 c	20	9:1
338 d	<i>t</i> Bu	<i>c</i> Hex	339 d	74	19:1

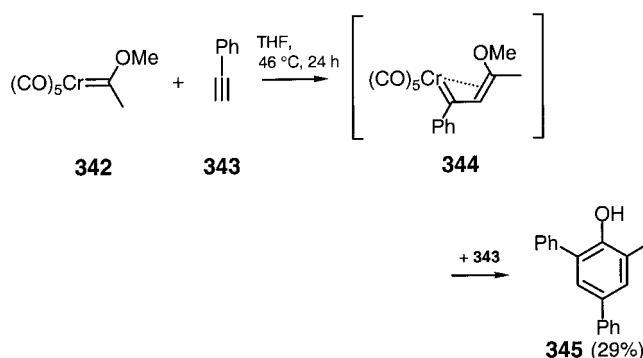
4. Reactions of α,β -Unsaturated Carbenchromium Complexes Prepared in situ from Alkynes and Alkylcarbene Complexes

The insertion of alkynes into a chromium–carbon double bond is not restricted to α,β -unsaturated carbene complexes. Simple alkylcarbene complexes **340** also react with alkynes primarily to form unstabilized alkenylcarbene complexes **341** (Scheme 30),^[158] which can react further in a number of ways, both intermolecularly as well as intramolecularly.

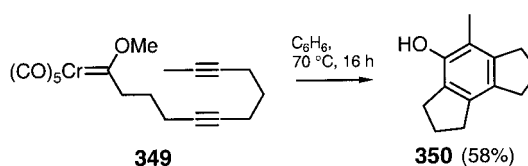
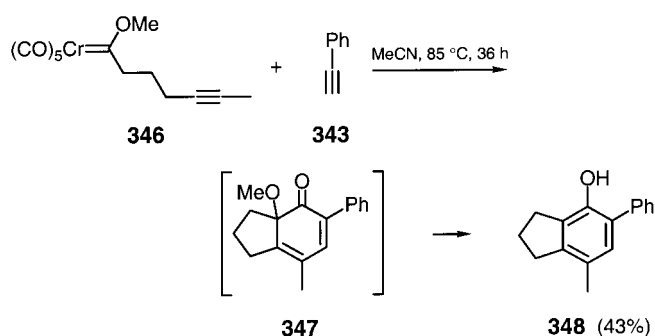


Scheme 30. R_L = larger, R_S = smaller substituent; X = OR, NR_2 .

In the simplest case the carbene complex **344**, formed initially from **342** with phenylethyne (**343**), can react with a further molecule of the added alkyne by insertion. In this way the substituted phenol **345** is produced after CO insertion and cyclization, in what is a classical Dötz reaction.^[159]



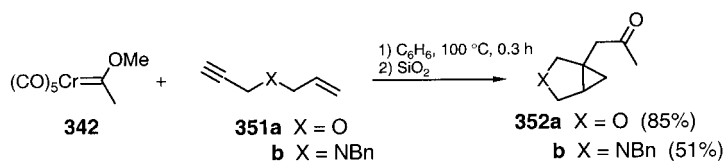
The sequence of an intramolecular and a subsequent intermolecular insertion of an alkyne into an alkylcarbene complex is even more clever, although a wide spectrum of products often ensues.^[160] However, the indane derivative **348** is formed in moderate yield (43%) by heating the (4-hexynyl)carbene complex **346** with phenylethyne (**343**) in acetonitrile.^[160a] That the obviously formed intermediate **347** cannot be isolated is in agreement with the behavior of the similarly substituted intermediate **202** with a heteroatom leaving group (see Scheme 13).



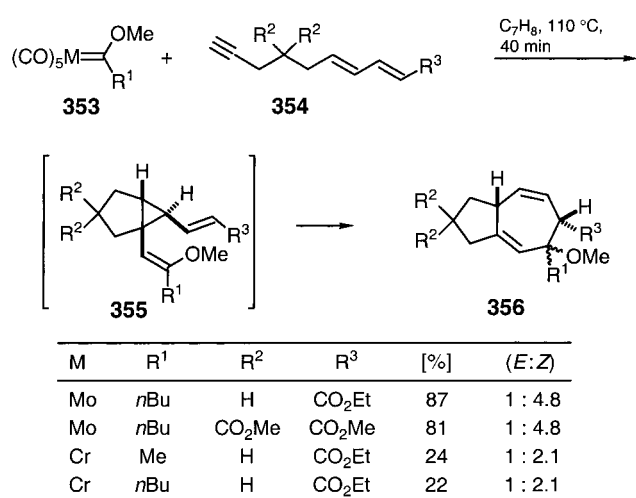
The reaction cascade which is initiated by heating the diynylcarbene complex **349** in benzene is also impressive. The methylphenol derivative **350** with two annulated five-membered rings is formed by two sequential intramolecular alkyne insertions and subsequent elimination of methanol (see earlier).^[161]

A combination of alkyne insertion and [2+1] cycloaddition with cyclopropane formation has also been reported. Harvey et al.^[162] and Katz and Yang^[163] have shown that the allylpropargyl ether **351a** and the allylpropargylamine **351b** react

with the complex **342** to furnish the 1-(2-oxopropyl)-3-oxabicyclo[3.1.0]hexane **352a** and 1-(2-oxopropyl)-3-azabicyclo[3.1.0]hexane **352b**.

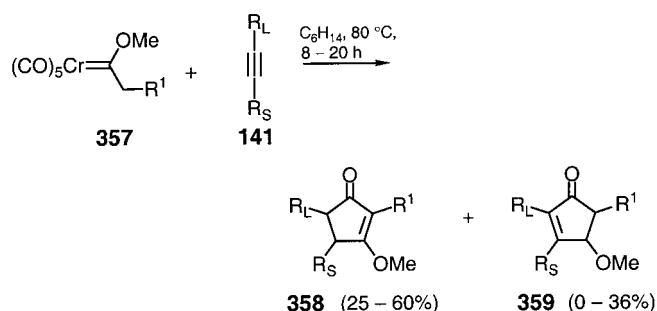


Dienynes of the type **354** react analogously with complexes **353** by alkyne insertion with subsequent intramolecular cyclopropanation to 1,6-dialkenylbicyclo[3.1.0]hexanes **355**, which then immediately undergo a [3,3] sigmatropic rearrangement under the conditions employed (110 °C) to form hexahydroazulenes **356** (Scheme 31).^[164]



Scheme 31. The formation of hexahydroazulenes from a variety of dienynes and complexes **353**.

Disubstituted alkynes of type **141** insert a second time, only slowly, into the intermediates from complexes of the type **353**; instead, other reaction pathways are followed. The cyclopentenones **358** and **359** are formed, for example, from **357** by insertion of a carbonyl ligand in the second step, and subsequent cyclization (Scheme 32 and Table 10).^[165] This reaction resembles the cyclopentenone formation by [2+2+1] cocyclization of (2-aminoalkenyl)carbenechromium com-



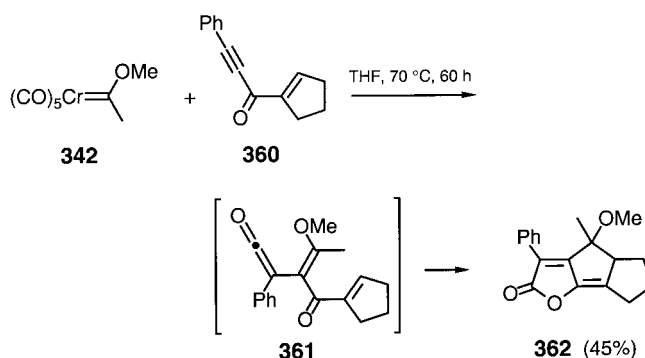
Scheme 32. For details see Table 10.

Table 10. Cyclopentenones **358** and **359** by cocyclization of **357** with alkynes **141** (Scheme 32).

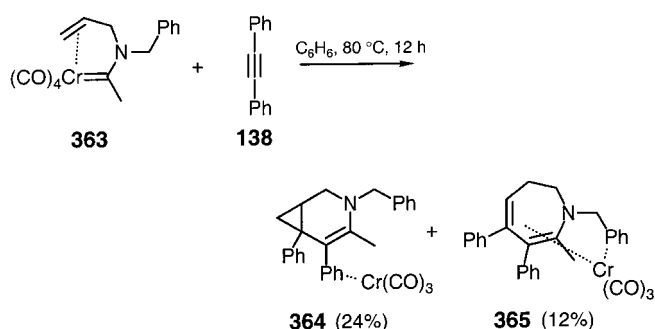
Complex	R ¹	R _L	R _S	Product	[%]	Product	[%]
357a	Ph	<i>n</i> Pr	H	358a	28	359a	0
357b	<i>n</i> Pr	Et	Et	358b	42	359b	18
357c	<i>c</i> Pr	<i>n</i> Pr	<i>n</i> Pr	358c	57	359c	33
357d	<i>t</i> Bu	Ph	Ph	358d	36	359d	36
357a	Ph	<i>n</i> Pr	H	358e	25	359e	8
357b	<i>n</i> Pr	Ph	Ph	358f	38	359f	24
357c	<i>c</i> Pr	<i>t</i> Bu	H	358g	28	359g	0
357d	<i>t</i> Bu	<i>t</i> Bu	H	358h	34	359h	0
357a	Ph	Ph	Ph	358i	60	359i	20

plexes **256** with nonterminal alkynes under CO insertion discovered by de Meijere et al.^[135] (see Scheme 21). The difference lies only in that R¹ in **357** corresponds to a substituted methylene group, the product of type **257** corresponds accordingly to **359**.

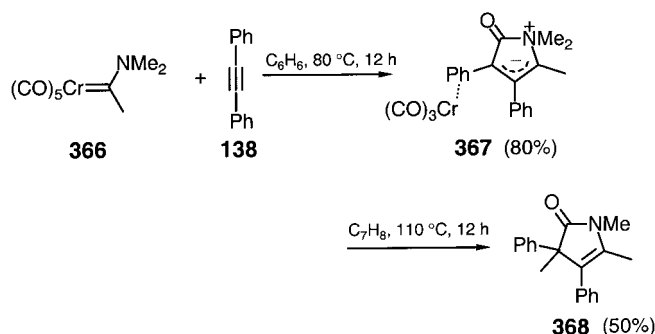
A particularly interesting reaction product is obtained on heating the methoxymethylcarbene complex **342** with the cyclopentenyl(phenylethynyl)ketone (**360**).^[111b] The cross-conjugated ketene **361**, formed by alkyne and carbonyl insertion, cyclizes in a criss-cross fashion with the inclusion of five atoms from **360** to give the tricycle **362** in a yield of 45%.



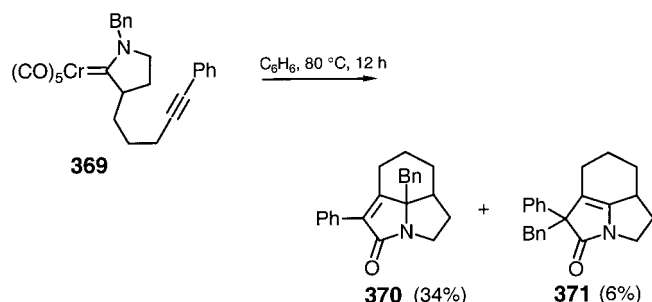
After alkyne insertion, (allylamino)carbenechromium complexes such as **363** react by intramolecular carbene transfer with cyclopropane formation.^[111b, 166, 167] However, as well as the bicyclic cyclopropane derivative **364** the dihydroazepine derivative **365** (which must have arisen through opening of the cyclopropane ring in **364**^[168]) and other ring-expansion products are also found.^[169]



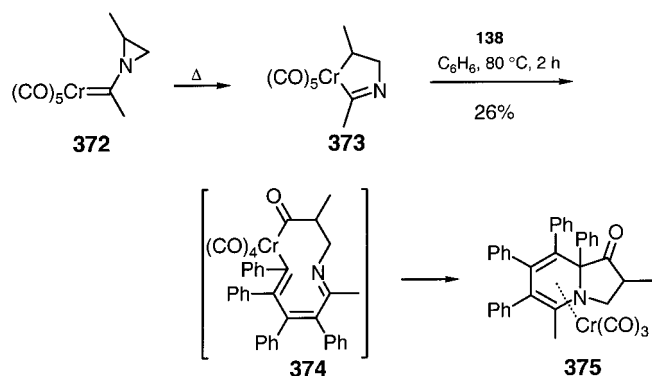
If, however, after alkyne insertion of, for example, tolane (**138**) into the complex **366**, there is no double bond for further reaction, a carbonyl ligand is inserted and the vinylketene complex thus formed cyclizes to a zwitterion of the type **367**,^[170–172] which on heating to 110 °C is stabilized as the γ -lactam **368** after methyl migration.^[171]



By proper selection of substituents in the starting material this type of reaction can be carried to extremes. Thus, the pyrrolidinylidene complex **369**, with the phenylpentynyl side chain, affords the tricyclic lactams **370** and **371** on heating in benzene.^[172] Clearly in this reaction a [1,3] benzyl migration is more favorable than a [1,5] benzyl migration, as the relative amounts of **370** and **371** confirm.



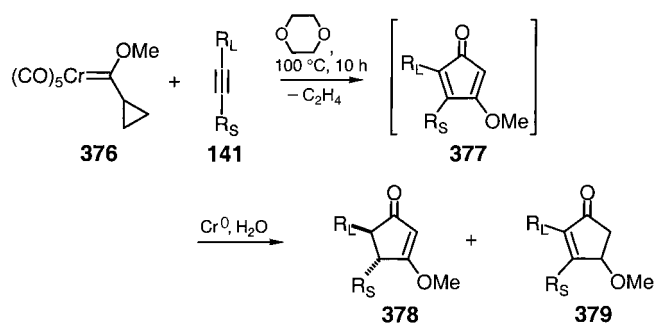
The (1-aziridinylethylidene)chromium complex **372** shows a different reaction behavior. Initially, a chromapyrroline **373** is formed by a thermally induced sigmatropic rearrangement, and this is probably followed by double alkyne insertion into the metal–carbene carbon bond and subsequent CO insertion with formation of a 10-membered ring intermediate **374**, from



which the indolizidinonechromium complex **375** is produced by electrocyclization and final reductive elimination.^[173]

The chemistry of the cyclopropylcarbenechromium complexes has been described in an independent review,^[174] however, the reactions of these homologues of vinylcarbene complexes with alkynes should also be mentioned briefly here.

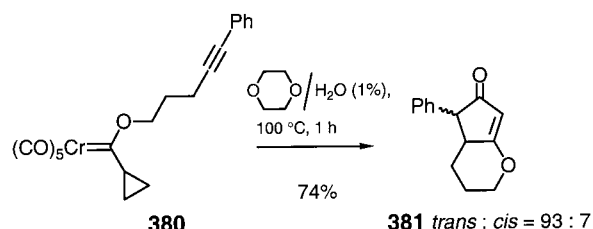
The simple cyclopropyl(methoxy)carbenechromium complex **376** reacts with alkynes **141** with release of ethene to form the intermediate cyclopentadienone **377**,^[175] which in the presence of residual amounts of chromium(0) derivatives and water in the reaction mixture reacts to form the same cyclopentenones^[174, 176] (Scheme 33) as the alkylmethoxycarbene complexes **357** (see Scheme 32).



	R_L	R_S	378 [%]	379 [%]
a	Ph	Ph	79	4
b	Ph	H	62	0
c	Ph	Me	73	12
d	<i>n</i> Pr	<i>n</i> Pr	55	0
e	<i>n</i> Pr	H	68	0
f	$(\text{CH}_2)_4\text{OH}$	H	54	0

Scheme 33. Reactions of cyclopropyl(methoxy)carbenechromium complex **376** with a variety of alkynes.

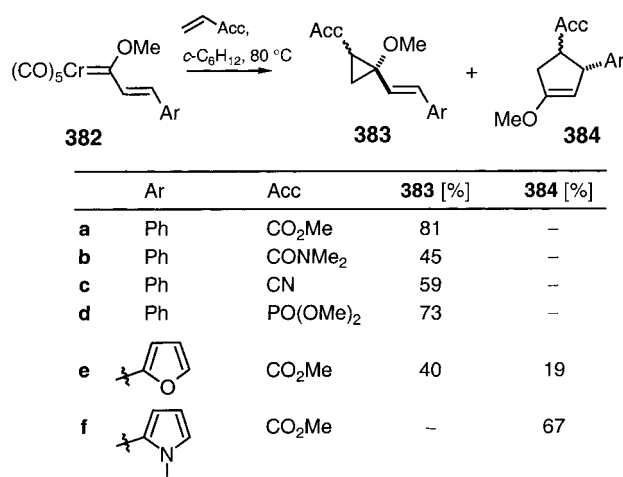
An intramolecular variant of this cyclopentenone synthesis has been realized. On heating complex **380**, with a phenylpentynyl moiety connected through the alkoxy substituent, in aqueous dioxan intramolecular insertion and cyclization takes place with high diastereoselectivity to furnish the phenyl-substituted ring-annulated cyclopentenone **381**.^[174, 177]



5. Reactions of Alkenylcarbene Complexes with Alkenes and Butadienes

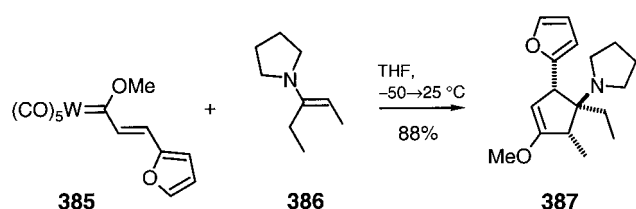
Fischer carbene complexes react mainly with acceptor-substituted alkenes, preferentially in a formal [2+1] cycloaddition reaction, to afford cyclopropane derivatives.^[2g, 178]

Two particularly impressive applications of this type of reaction in domino reactions have been described by Harvey et al.^[162, 164] and Katz and Yang^[163] (see **342** + **351** → **352** and Scheme 31). As Reissig and co-workers have found, alkenylcarbene complexes react principally in the same way, but the alkenylcyclopropanes **383** thus formed can rearrange to the cyclopentenes **384** (Scheme 34).^[179] Clearly, this occurs particularly readily with the alkenylcyclopropane **383 f** formed from the 2-pyrrolyl-substituted complex **382 f** and methyl acrylate, for in this case only the cyclopentene derivative **384 f** was found by Reissig and co-workers.^[179]



Scheme 34. Rearrangement of alkenylcyclopropanes **383** to the cyclopentenes **384**.

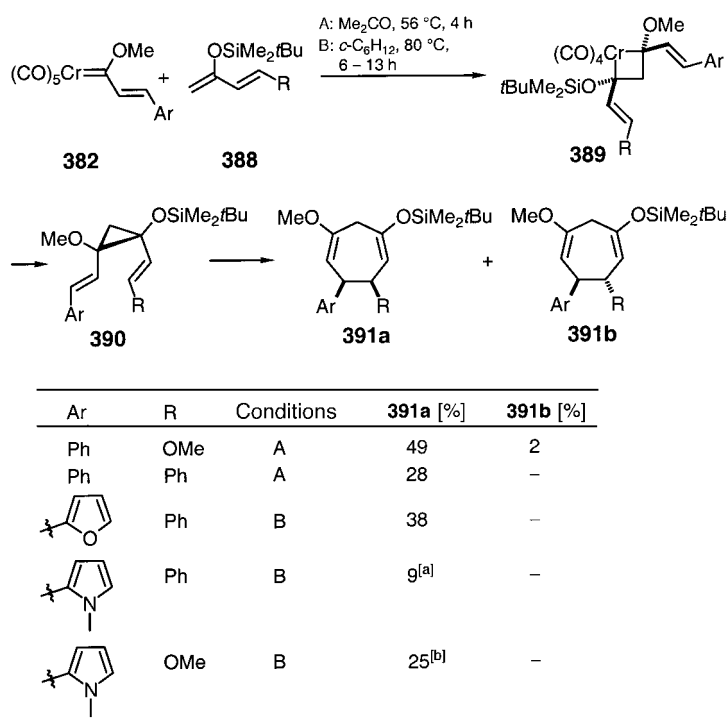
The 2-furyl-substituted (alkenylcarbene)tungsten complex **385** similarly reacts diastereoselectively with 2-pyrrolidino-2-pentene (**386**), even at relatively low temperatures, to afford the correspondingly highly substituted cyclopentene **387**.^[180]



Electron-rich dienes of the type **388** (R = OMe, Ph) also react with alkenylcarbene complexes in a formal [2+1] cycloaddition. The *cis*-dialkenylcyclopropane **390** formed by a formal carbene transfer from **382** to the dienes **388**—by [2+2] cycloaddition of the Cr=C bond to the most electron-rich double bond in **388** with subsequent reductive elimination—give, however, cycloheptadienes of type **391** through immediately succeeding [3,3] sigmatropic rearrangements (Scheme 35).

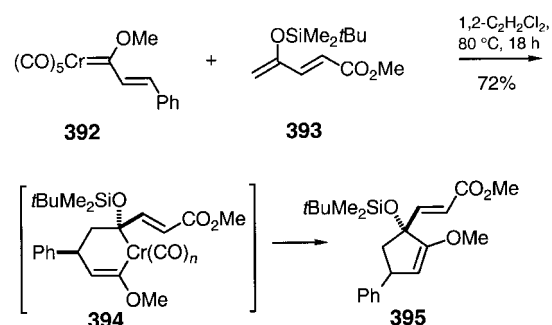
Of note here is the high stereoselectivity of the formal [2+1] cycloaddition; normally such cyclopropanations with Fischer carbene complexes occur with only low diastereoselectivity.^[178, 179, 181]

Dienes with a donor–acceptor substitution pattern, such as **393**, cocyclize with alkenylcarbene complexes to produce

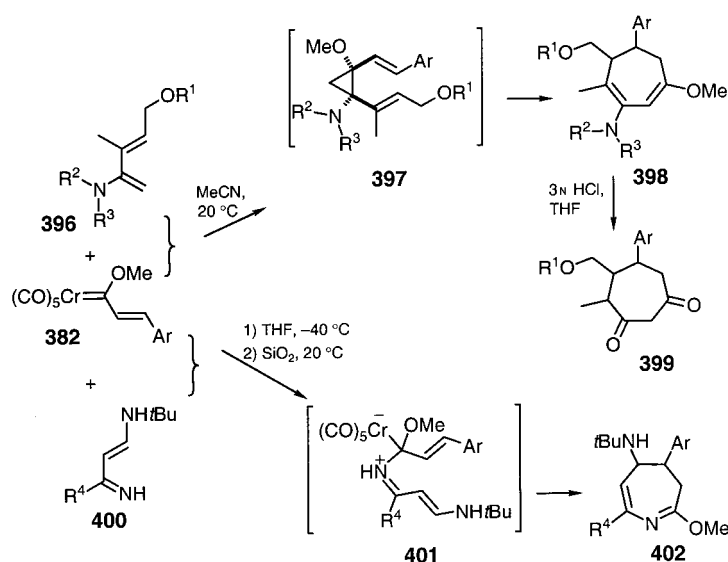


Scheme 35. [a] 1 % of an alkenylcyclopentene was isolated. [b] 16 % of an alkenylcyclopentene was isolated.

cyclopentenes. This transformation probably begins with a [4+2] cycloaddition of the electron-poor 1-chroma-1,3-butadiene **392** to the more electron-rich double bond—in a hetero-Diels–Alder reaction with inverse electron demand—to form the chromacyclohexene **394**, which affords the cyclopentene **395** by reductive elimination of the metal complex fragment.^[181]



The reactions of $\alpha\beta$ -unsaturated Fischer carbene complexes with aminobutadienes and aza-1,3-dienes represent a special situation. Barluenga and co-workers^[182] have shown that aminobutadienes **396** and azadienes **400** react with the alkenylcarbene complexes **382** mainly to form the amino-cycloheptadienes **398** (which are then hydrolyzed to the cycloheptanediones **399**) and the 2-azacyclohepta-1,3-dienes **402**, respectively (Scheme 36 and Table 11). The formations of these seven-membered ring derivatives probably each begin with a [2+1] cycloaddition to a dialkenylcyclopropane **397** or stabilized ylid **401**. A [3,3] sigmatropic rearrangement and



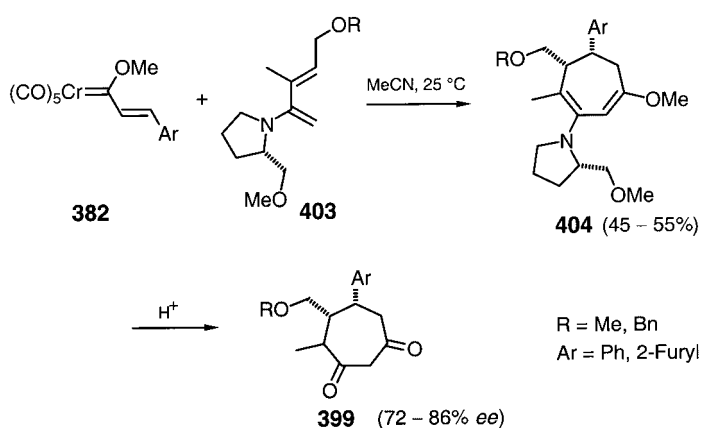
Scheme 36. For details see Table 11

Table 11. Carbo- and heterocyclic seven-ring derivatives from α,β -unsaturated Fischer carbene complexes **382** and 2-dialkylaminobuta-1,3-dienes **396** or 1-aza-1,3-butadienes **400**.

	Ar	R ¹	R ²	R ³	R ⁴	399 [%]	402 [%]
a	2-Furyl	Me	Me	Ph	–	82	–
b	2-Furyl	Me	(CH ₂) ₂ -O-(CH ₂) ₂	–	–	58	–
c	Ph	Me	Me	Ph	–	71	–
d	2-Furyl	Bn	Me	Ph	–	78	–
e	Ph	Bn	Me	Ph	–	76	–
f	Ph	–	–	–	cPr	–	90
g	2-Furyl	–	–	–	cPr	–	80
h	Ph	–	–	–	Et	–	91
i	2-Furyl	–	–	–	4-MeC ₆ H ₄	–	62
j	Ph	–	–	–	Ph	–	70
k	Ph	–	–	–	4-ClC ₆ H ₄	–	52

subsequent [1,3] proton shift then give in each case the respective cycloheptadiene **398**^[182b,e] or dihydroazepine **402**.^[182f,g]

These formal [3+4] cycloadditions occur even at relatively low temperatures. It has also been shown convincingly that this synthesis is suitable for the stereoselective construction of highly substituted cycloheptanediones of type **399**.^[182b,e]



6. Summary and Outlook

The results summarized here show clearly that since their pioneering days of almost three decades ago, α,β -unsaturated Fischer carbene complexes have secured a permanent place in organic synthesis. Thanks to the unbelievable reaction versatility with which these metal-stabilized carbenes are endowed, a plethora of synthetically interesting carbo- and heterocyclic compounds are readily available in convincing yields. The selectivity with which these products are formed, can be excellently controlled by targeted fine tuning of all the factors which influence the reactions. The proper combination of cycloadditions such as Diels–Alder reactions and 6 π -electrocyclization leads to domino reactions with which several new bonds can be connected selectively at the same time in a single operational step. The increase in molecular complexity, which is used as a suitable measure of the efficiency of a synthetic step, is particularly great in such reactions.

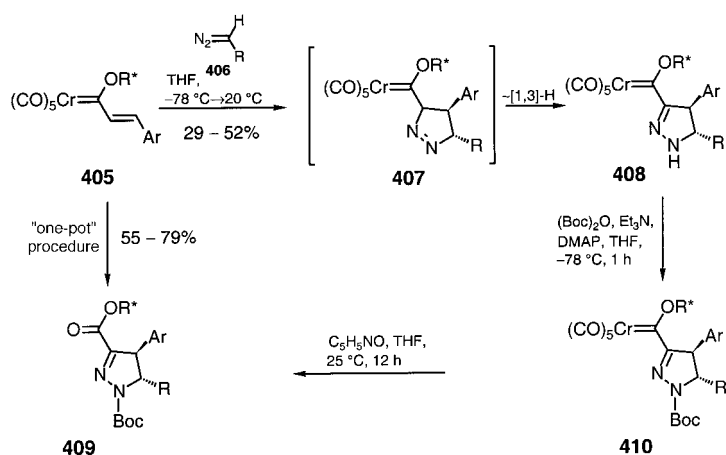
Even if concrete ideas on the mechanism of formation have in the meantime been deduced for most of the numerous cycloaddition and cocyclization products of α,β -unsaturated Fischer carbene complexes, the study of the bonding relationships in these organometallic reagents and their chemistry must be pushed forward continuously to further pave the way for their use in key steps of demanding syntheses.

In spite of the widespread repertoire of cycloadditions and cocyclizations no end is yet in sight for the discovery of new reactions of these complexes. The numerous possibilities of the domino-like coupling of carbene complex cocyclizations with other processes are far from exhausted, in spite of the many successes, and the imagination of chemists will not quickly reach the limits of this area. Even though positive beginnings have already been made in some areas, for example in asymmetric synthesis, a huge potential still lies slumbering. The ever increasing interest in the chemical industry for new lead structures for highly active pharmaceuticals or plant protection agents could secure new possibilities for the use of Fischer carbene complexes. It remains to be seen whether the high expectations can be justified in all these points.

Addendum

Since the preparation of the final version of this review further publications have appeared which will have an impact to further establish the reactions of α,β -unsaturated carbene-pentacarbonylmetal complexes in organic synthesis.

Thus, Barluenga et al. have reported recently on [3+2] cycloadditions of alkenylcarbenechromium complexes with 1,3-dipoles.^[183] In analogy to the highly reactive alkynylcarbenechromium complexes (see Section 2.2) alkenylcarbenechromium complexes also react with diazoalkanes in [3+2] cycloadditions, as was demonstrated impressively in an enantioselective synthesis of esters of 1*H*-pyrazolin-3-carboxylic acids **409** from (–)-8-phenylmenthol or (±)-menthol-substituted (1-alkenyl-1-oxycarbene)pentacarbonylchromium complexes **405** (Scheme 37). Regio- and diastereoselective addi-



Scheme 37. Ar = Ph, 2-furyl; R* = (±)-menthyl, (–)-8-phenylmenthyl; R = Ph, SiMe₃, CH₂=CH; DMAP = 4-dimethylaminopyridine.

tion of the diazomethane derivative **406** to the complexes **405** and a subsequent [1,3] hydrogen shift led to the (4,5-dihydro-1*H*-pyrazolyl)carbenechromium complexes **408**. Protection of the secondary nitrogen with a *tert*-butoxycarbonyl (Boc) group and oxidation with pyridine-*N*-oxide gave the final products **409**. When all three steps were carried out in a single operational step without isolation of the intermediates **408** and **410** the yields of **409** were increased significantly.^[183]

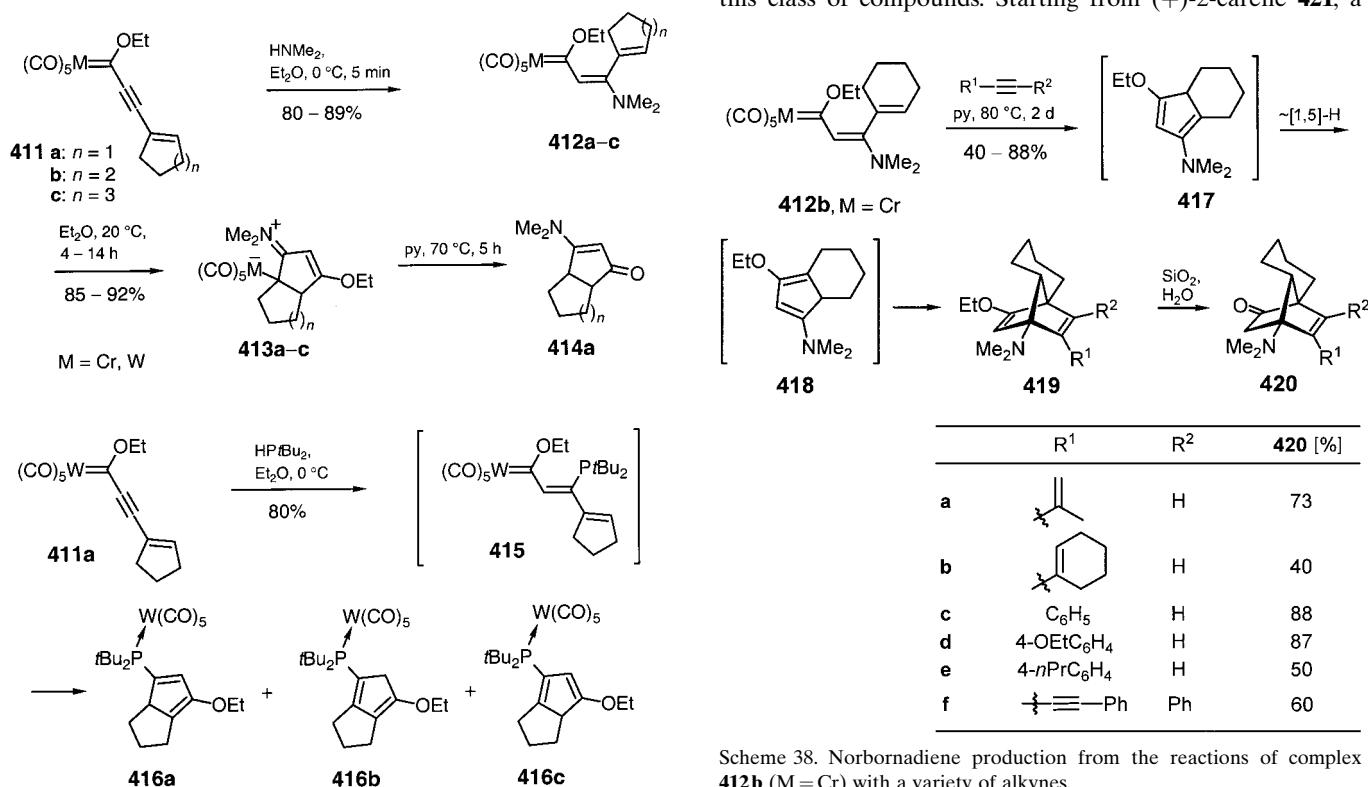
A new five-membered-ring formation which must involve intramolecular insertion of a cycloalkene double bond into the metal–carbon double bond of the 1-metalla-1,3,5-hexatriene **412**, prepared from the corresponding alkenynylcarbene complex **411** by 1,4-addition of a secondary amine, has been reported by Aumann et al.^[184] The complexes **412** cyclize

on standing in diethyl ether even at room temperature within 4–14 h to give the zwitterionic η^1 -cyclopentenyl complexes **413** from which the carbonylchromium fragment is eliminated by treatment with pyridine. Subsequent hydrolysis of the enol ether function then gives the bicyclo[3.3.0]octenones **414** in excellent yields. The addition of di-*tert*-butylphosphane to the (cyclopentenylpropynylidene)tungsten complex **411a** affords directly a mixture of the three isomeric phosphanyl-cyclopentadiene complexes **416a–c**.^[184]

Although β -cycloalkenyl- β -dimethylamino-substituted ethynylcarbene complexes of the type **412** undergo further intramolecular reactions even at room temperature,^[184] the cyclohexenyl-substituted complex **412b** (M = Cr) could be isolated in 94% yield from hexacarbonylchromium and 1-ethynylcyclohexene by working in THF and applying the one-pot process.^[21, 123, 185] In pyridine at 80 °C complex **412b** reacted with a series of alkenyl- and arylsubstituted terminal alkynes to afford the tricyclic dienes **419**, which were hydrolyzed to the ketones **420**, by traces of water during chromatographic purification.^[185]

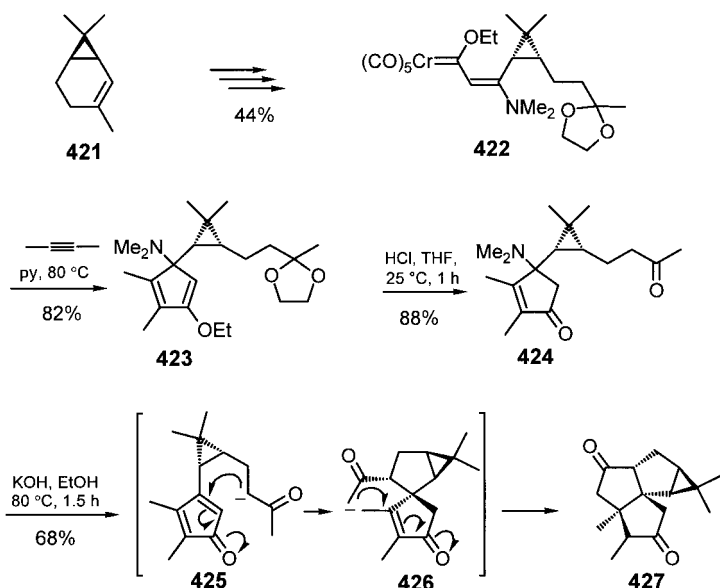
The formation of **419** most probably begins with the rapid cyclization of **412b** (M = Cr) as already observed by Aumann et al.^[184] which in pyridine, however, would directly lead to the metal free six-ring annelated cyclopentadiene derivative **417**. By [1,5] hydride shift this may yield **418**, which reacts with the added alkyne in a [4 + 2] cycloaddition to give the tricyclic compound **419**. It is remarkable though, that even donor-substituted aryethynes apparently are sufficiently reactive dienophiles for the substrate **418** (Scheme 38).

A new use of the alkoxy-cyclopentadienes,^[123] readily prepared from (β -aminoalkenyl)carbenechromium complexes and alkynes in pyridine by the method of de Meijere et al., convincingly demonstrates the synthetic potential of this class of compounds. Starting from (+)-2-carene **421**, a



Scheme 38. Norbornadiene production from the reactions of complex **412b** (M = Cr) with a variety of alkynes.

terpene from the "chiral pool", the enantiomerically pure alkenylcarbenochromium complex **422** was prepared in four steps in an overall yield of 44%. The formal [3+2] cycloaddition of **422** to 2-butyne afforded the corresponding



ethoxycyclopentadiene **423** (82%) which was hydrolyzed to the cyclopentenone **424** under acidic conditions. Treatment of the diketone **424** with ethanolic potassium hydroxide apparently leads to elimination of dimethylamine to give the cyclopentadienone **425**, which reacts immediately in a cascade of two sequential Michael additions to form the angular triquinane **427**.^[186] This almost totally diastereoselective sequence of elimination and twofold Michael addition in which four new stereocenters are formed affords the highly substituted, enantiomerically pure angular triquinane **427** in an overall yield of 26% from (+)-2-carene **421**.

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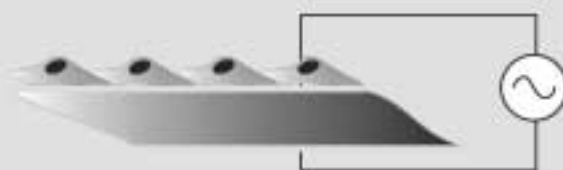
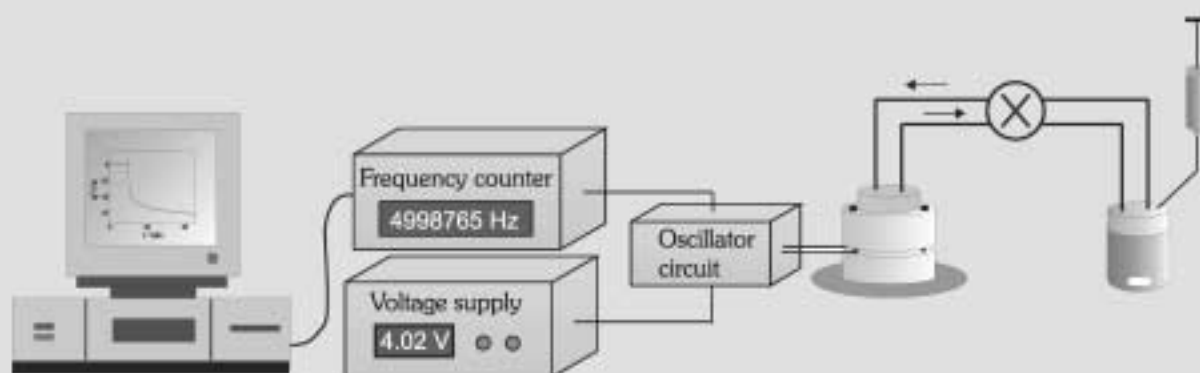



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THE QUARZ-CRYSTAL MICROBALANCE IN LIFE SCIENCE



Piezoelectric Mass-Sensing Devices as Biosensors—An Alternative to Optical Biosensors?

Andreas Janshoff, Hans-Joachim Galla, and Claudia Steinem*

Dedicated to Professor Erich Sackmann on the occasion of his 65th birthday

In the early days of electronic communication—as a result of the limited number of quartz resonators available—frequency adjustment was accomplished by a pencil mark depositing a foreign mass layer on the crystal. In 1959, Sauerbrey showed that the shift in resonance frequency of thickness-shear-mode resonators is proportional to the deposited mass. This was the starting point for the development of a new generation of piezoelectric mass-sensitive devices. However, it was the development of new powerful oscillator circuits that were capable of operating thickness shear mode resonators in fluids that enabled this technique to be introduced into bioanalytic applications. In the last decade adsorption of biomolecules on functionalized

surfaces turned in to one of the paramount applications of piezoelectric transducers. These applications include the study of the interaction of DNA and RNA with complementary strands, specific recognition of protein ligands by immobilized receptors, the detection of virus capsids, bacteria, mammalian cells, and last but not least the development of complete immunosensors. Piezoelectric transducers allow a label-free detection of molecules; they are more than mere mass sensors since the sensor response is also influenced by interfacial phenomena, viscoelastic properties of the adhered biomaterial, surface charges of adsorbed molecules, and surface roughness. These new insights have recently been used to investigate the adhesion of cells, lip-

osomes, and proteins onto surfaces, thus allowing the determination of the morphological changes of cells as a response to pharmacological substances and changes in the water content of biopolymers without employing labor-intensive techniques. However, the future will show whether the quartz-crystal microbalance will assert itself against established label-free sensor devices such as surface plasmon resonance spectroscopy and interferometry.

Keywords: analytical methods • biosensors • molecular recognition • quartz-crystal microbalance • surface chemistry

1. Introduction

As a consequence of their extraordinary properties quartz resonators can be found in all kinds of electronic devices, such as watches and computers to give an accurate time base, and as signal generators or reference systems in electronic devices. Quartz resonators did not become of interest commercially until immediately prior to World War II when there was an enormous requirement for communication devices. More than 30 million resonators were necessary to cover the demand. Quartz crystals attained significance as an analytical device after the discovery that there is linear relationship

between deposited mass and the frequency response,^[1, 2] as demonstrated by Sauerbrey in 1959.^[3] He showed that this proportionality only holds if the ideal layer of foreign mass is strongly coupled to the resonator. This is the reason for calling such a device a “quartz-crystal microbalance” (QCM). The mass sensitivity of a 5 MHz quartz crystal is approximately $0.057 \text{ Hz cm}^2 \text{ ng}^{-1}$, which is approximately 100 times higher than that of an electronic fine-balance with a sensitivity of $0.1 \mu\text{g}$. In the 1960s and 1970s the QCM technique gained importance as devices for monitoring thicknesses in vacuum and air, and is still used in today's laboratories to determine the thicknesses of layers. The lack of suitable oscillator circuits that enabled the shear-wave resonator to be operated in fluids prevented, however, the extension of this technique to bioanalytical areas. In 1982, Nomura and Okuhara^[4] were the first to report on a circuitry capable of breaking this barrier, thus giving the starting point for the development of a new class of bioanalytic tools.

[*] Dr. C. Steinem, Dr. A. Janshoff, Prof. Dr. H.-J. Galla
Institut für Biochemie
Westfälische Wilhelms-Universität
Wilhelm-Klemm-Strasse 2, 48149 Münster (Germany)
Fax: (+49) 251-83-33-206
E-mail: steinec@uni-muenster.de

Nowadays quartz resonators can be regarded as typical mechanical transducers for chemical and biological sensor devices that transform the mass or thickness of a foreign layer (physical quantity) of an analyte into an electrical signal. A mass adsorbed on a shear-wave resonator experiences an acceleration of more than 10^6 g. The amplitude of vibration is usually 10–20 nm in air and is reduced in water to a mere 1–2 nm.^[5]

This article is intended to show that specific interactions between biomolecules can be quantified in terms of thermodynamic and kinetic parameters by means of QCM. Furthermore, the objective is to put emphasis on the fact that the QCM is more than just a mass sensor. A new application of this classical technique is the determination of viscoelastic properties of cellular systems. The strength of piezoelectric transducers is that they can couple mechanical and electrical variables, which allows the formulation of equivalent circuits that describe the mechanical properties of biopolymers and

complex multilayers, such as confluent cell monolayers. Thus, it is possible to determine molecular recognition events apart from interfacial phenomena, surface energy, viscoelasticity, roughness, surface charge density, and the water content of biomolecules.

2. Basic Piezoelectric Resonators

2.1. Acoustic Waves in Solids

Acoustic waves cover a frequency range of 14 orders of magnitude—from 10^{-2} Hz (seismic waves) and extending to 10^{12} Hz (thermoelastic excited phonons; Figure 1). The acoustic resonators such as those mentioned in this article oscillate in a narrow frequency range of 10^6 – 10^9 Hz.

A brief introduction to acoustic wave theory and in particular piezoelectric excited vibrations is given and then

Andreas Janshoff was born in 1966. He attended the Westfälische Wilhelms-University (WWU) Münster, where he received a B.S. in biology and chemistry. He received his M.S. in 1994 and his Ph.D. in 1997 for studies on the biofunctionalization of gold surfaces and its applications in bioanalytics, which he carried out at the Institute for Biochemistry at the university of Münster. As a postdoctoral fellow, he attended the group of M. Reza Ghadiri at the Scripps Research Institute, La Jolla (CA), USA



A. Janshoff



H.-J. Galla



C. Steinem

for 18 months. During this time he worked on the imaging of lipid membranes by scanning force microscopy. In 1999 he returned to Germany, joining the group of Harald Fuchs at the WWU Münster to start his habilitation in close collaboration with the Institute for Biochemistry (WWU). His research fields include the development of new sensor systems based on micropatterned surfaces and the application of acoustic resonators as a tool to investigate biological relevant questions.

Hans-Joachim Galla, born in 1948, attended the university of Göttingen. After receiving his B.S. in chemistry in 1973, he received his Ph.D. in 1975 in Göttingen under the tutelage of Erich Sackmann for work on phase-separation phenomena and lipid–protein interactions carried out at the Max-Planck Institute for Biophysical Chemistry. In 1976 he changed to the Department of Biophysics at the university of Ulm as a research associate, where he finished his habilitation in 1979. At the same time (1979/1980) he was a research associate at Stanford university before he returned to the university of Ulm as a Heisenberg fellow. In 1983 he became professor of biophysical chemistry at the Institute for Biochemistry of the technical university of Darmstadt. In 1990 he took a professorship of biochemistry at the university of Münster. His main research fields include the differentiation of cerebral endothelia of the blood–brain barrier, monomolecular lipid–peptide layers as model systems for the alveolar lung surfactant, and biofunctionalization of solid-supported lipid membranes for applications in bioanalytics.

Claudia Steinem, born in 1967, attended the Westfälische Wilhelms-University in Münster, where she received a B.S. in biology and chemistry and a M.S. in chemistry. In 1997 she received her Ph.D. under the tutelage of Hans-Joachim Galla on studying the ion transport in solid-supported lipid bilayers. After a postdoctoral stay at the Scripps Research Institute in La Jolla, CA (USA) for 18 months in the group of M. Reza Ghadiri she returned as Lise-Meitner fellow at the Institute for Biochemistry of the Westfälische Wilhelms-University in 1999. She is currently organizing her own research group in the course of her habilitation. Her main research interests include the study of the interaction of artificial peptides, annexins, and proteins of the signal transduction pathway with lipids, and the development of new lipid membrane systems based on porous surfaces.

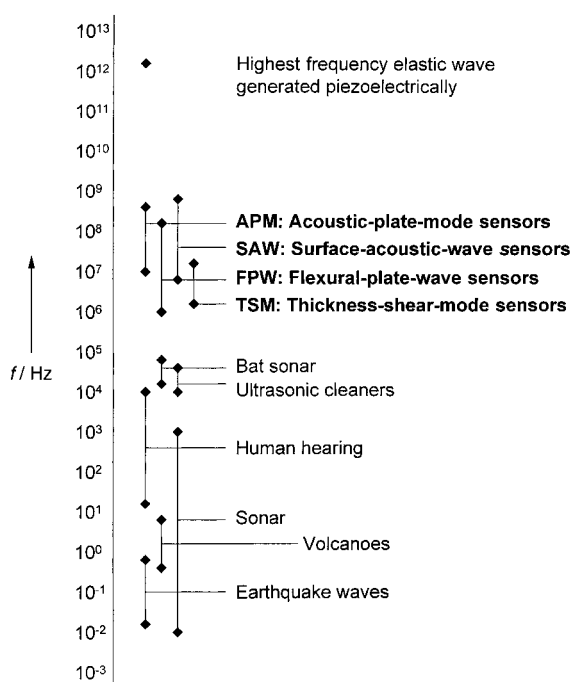


Figure 1. The spectrum of acoustic waves covers roughly 14 orders of magnitude. The frequency range of the four resonators which are described in this article range in operation between 1 and 1000 MHz.^[7]

this is followed by a more detailed description of different types of piezoelectric transducers.

The application of a periodic perturbation (stress) to a solid results in elastic deformations (strain), which travel as waves through the solid. The type of wave—transversal or longitudinal—and the phase velocity both depend on the crystal structure. The wave equation that describes acoustic waves in solids [Eq. (1)] can be derived using the equation of motion, the definition of strain, and the constitutive equations.^[6]

$$\nabla \cdot \mathbf{c} : \nabla_s \mathbf{u} = \rho \frac{\partial^2 \mathbf{u}}{\partial t^2} \quad (1)$$

$\mathbf{u}$ is the displacement of the particle, ρ the density of the material, and t denotes time. The matrix $\mathbf{c}$ describes the elasticity moduli of the solid. Table 1 summarizes the relevant expressions for one and three dimensions.

Table 1. The dynamic equations of motion for one and three dimensions. $\mathbf{c}$ is the tensor of elasticity. Stress $\mathbf{T}$ and strain $\mathbf{S}$ are symmetric tensors with nine elements of which six are independent because of symmetry. $\mathbf{u}$ is the particle displacement, z indicates the direction where strain occurs, and ρ is the density of the material.^[6]

	One-dimensional system	Three-dimensional system
Newton's law	$\frac{\partial T}{\partial z} = \rho \frac{\partial^2 u}{\partial t^2}$	$\nabla \cdot \mathbf{T} = \rho \frac{\partial^2 \mathbf{u}}{\partial t^2}$
definition of stress	$S = \frac{\partial u}{\partial z}$	$\mathbf{S} = \nabla_s \mathbf{u}$
<i>constitutive relations</i>		
Hooke's law	$T = CS$	$\mathbf{T} = \mathbf{c} : \mathbf{S}$
particle velocity	$v = \frac{\partial u}{\partial t}$	$\mathbf{v} = \frac{\partial \mathbf{u}}{\partial t}$
mechanical impedance	$Z = \sqrt{\rho C}$	
phase rate	$v_p = \sqrt{C/\rho}$	

2.2. Piezoelectric Excited Acoustic Waves in Solids

Piezoelectricity was first reported in 1880 by the Curie brothers and describes the generation of electrical charges on the surface of a solid caused by pulling, pressure, or torsion. In contrast, the occurrence of a mechanical deformation arising from an external electric field is called the converse piezoelectric effect. Figure 2 shows schematically the general relationships between mechanical and electrical variables.

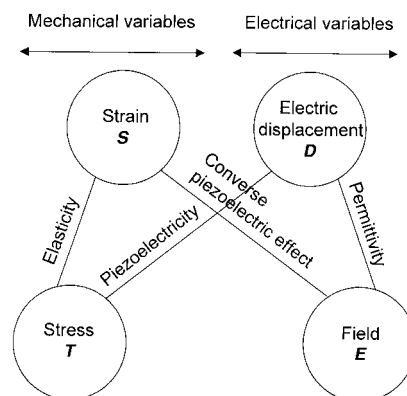


Figure 2. Relationship between mechanical and electrical variables. The direct piezoelectric effect is the production of electric displacement by applying a mechanical stress. The converse piezoelectric effect results in a strain in the crystal when an electrical field is applied. The relation between stress and strain is determined by the elasticity of the solid.^[7]

Prerequisite for the occurrence of piezoelectricity in crystals is an inversion center. In total 21 point groups fulfill this requirement but only 20 classes have nonzero piezoelectric constants. Although a large number of crystals show piezoelectricity only quartz provides the unique combination of mechanical, electrical, chemical, and thermal properties, which has led to its commercial significance. Equation 1 needs to be expanded by the term $\mathbf{e} \nabla^2 \Phi$ in order to introduce piezoelectricity in the general wave equation, in which Φ is the electrical potential and $\mathbf{e}$ the piezomodulus composed of piezoelectric constants as matrix elements.^[7] The additional expression is responsible for the electrical excitation of elastic waves in a piezoelectric solid. The resulting motion causes a change in Φ , which can in turn be detected electrically. Since the gradient of the displacement current is zero, that is, no free charge density occurs, integration of the extended Equation (1) results in a comprehensive expression that describes acoustic waves in a piezoelectric medium. The following section deals with its physical consequences.

2.3. Piezoelectric Resonators

The main emphasis of this article is placed on bulk-acoustic-wave (BAW) or thickness-shear-mode (TSM) resonators, which are also known as quartz-crystal microbalances (QCMs). Although TSM resonators are considerably less sensitive than flexural-plate-wave (FPW) or surface-acoustic-wave (SAW) sensors they are widely used as a result of their robust nature, availability, and affordable electronics. Figure 3 depicts schematically various resonator types.

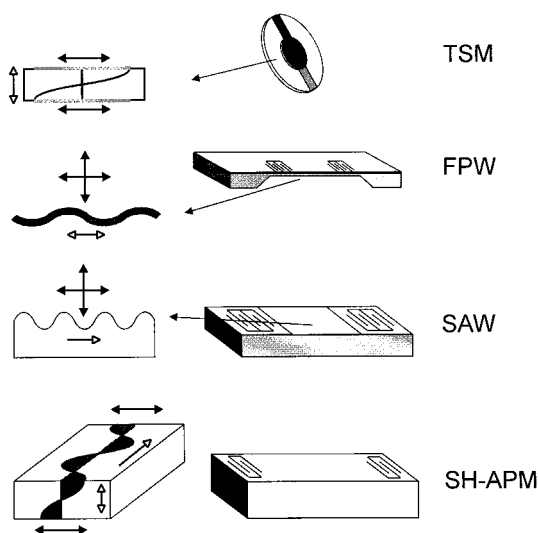


Figure 3. Schematic sketches of the four common types of acoustic resonators and their wave propagation modes. The particle displacement is indicated by a black arrow, and the direction of the wave propagation by an open arrow. TSM: thickness-shear-mode resonator, also known as the quartz-crystal microbalance technique; FPW: flexural-plate-wave resonator; SAW: surface-acoustic-wave resonator (two port delay line) and SH-APM: shear-horizontal-acoustic-plate-mode resonator.

2.3.1. Thickness-Shear-Mode (TSM) Resonators (Quartz-Crystal Microbalances)

Depending on the cut-angle a large number of different resonator types such as thickness-shear-mode, plate, and flexural resonators can be obtained from a mother crystal (Figure 4A) with eigenfrequencies ranging from 5×10^2 – 3×10^8 Hz. Generally, AT-cut crystals are used for QCM purposes, being cut with an angle of 35.25° to the z -axis (Figure 4B). AT-cut quartz crystals exhibit a high frequency stability of $\Delta f/f \approx 10^{-8}$, which makes them well-suited for many electronic devices.^[8, 9]

Since AT-cut quartz crystals have a temperature coefficient that is almost zero between 0 – 50°C , this particular cut is the most suitable one for QCM sensors.^[10] The following parameters are always related to AT-cut quartz.

A convenient way to describe acoustic waves traveling in a circular, lossless, and stress-free AT-cut crystal with thickness d_q is to express the wave equation in cylindrical coordinates (Figure 5). The propagation velocity of the

acoustic wave in a piezoelectric thickness-shear-mode resonator is $(\bar{c}_{66}/\rho_q)^{1/2}$ (ρ_q = density of the quartz).^[11] The boundary conditions require that the amplitude of the shear displacement is zero at the electrode edges, while it is at a maximum at $r=0$ and exhibits a node at $z=0$. θ follows periodic boundary conditions. The wave equation can be solved by a usual separation process.^[12] The solution yields important properties of the shear vibration. The eigenfrequencies are found in general from Equation (2).^[9]

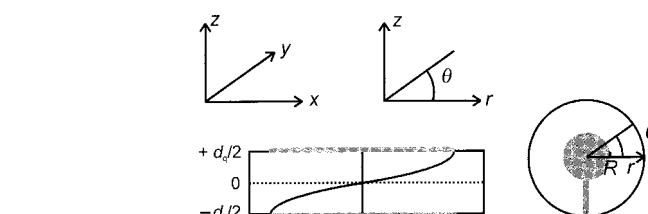


Figure 5. Definition of the coordinates of a circular-shaped quartz crystal. The gray areas depict the metal electrodes.

acoustic wave in a piezoelectric thickness-shear-mode resonator is $(\bar{c}_{66}/\rho_q)^{1/2}$ (ρ_q = density of the quartz).^[11] The boundary conditions require that the amplitude of the shear displacement is zero at the electrode edges, while it is at a maximum at $r=0$ and exhibits a node at $z=0$. θ follows periodic boundary conditions. The wave equation can be solved by a usual separation process.^[12] The solution yields important properties of the shear vibration. The eigenfrequencies are found in general from Equation (2).^[9]

$$f_{mnk} = \frac{v_p}{2\pi} \sqrt{\frac{n^2 \pi^2}{d_q^2} + \frac{\lambda_{mk}^2}{R^2}} \approx \frac{v_p}{2\pi} \sqrt{\frac{n^2 \pi^2}{d_q^2}} = \frac{v_p n}{2 d_q} = \frac{n K_R}{d_q} \quad (2)$$

From the solution of the wave equation, it is clear that only odd overtones n can be excited.^[13] The displacement at $r=0$ and $z = \pm d_q/2$ is at a maximum for the fundamental frequency, while the amplitude vanishes at the electrode edges where $r=R$. Thus, the transversal wave exhibits a node at $z=0$ and maximum amplitude at $z = \pm d_q/2$.^[14]

If the lateral dimensions of the quartz plate greatly exceeds the thickness of the crystal the problem can be treated one-dimensionally. The function $u_x(z,t)$ describes two transversal waves traveling in opposite directions, which thus form a standing wave within the crystal. Assuming stress-free surfaces ($\partial u/\partial z=0$), the shear vibration can be described as a simple cosine function. The use of constructive interference $d_q = n\lambda/2$ and $R \gg d_q$ simplifies Equation (2).^[15] K_R denotes the so-called frequency constant of AT-cut quartz with $K_R = 1664 \text{ ms}^{-1}$.^[9] It can be deduced from Equation (2) that the resonance frequency of an AT-cut quartz increases with decreasing thickness of the crystal. For instance, a 5 MHz quartz exhibits a thickness of 0.33 mm, while a 30 MHz crystal is only 55 μm thick.

TSM resonators provide numerous material-specific parameters. In order to fully exploit the capability of acoustic resonators it is essential to understand the conversion between mechanical and electrical parameters since the last ones are those which are readily accessible to the user. Mechanical models can readily be transformed into equivalent electrical circuits which permit a complete description of the oscillation in the presence of an adsorbent. A general one-dimensional acoustic wideband model has been suggested by Mason^[16] (Figure 6A) and provides a basis for the theoretical description of complex composite resonators as they occur in life science. In particular cases, such as low load, the Mason model can be easily transformed near resonance into an equivalent circuit with lumped elements—the so termed Butterworth–van-Dyke (BVD) circuit (Figure 6B). A comprehensive treatment of this issue has been given by Rosenbaum.^[6]

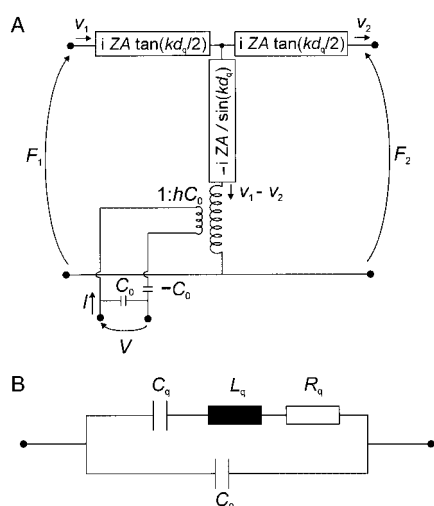


Figure 6. A) Equivalent circuit (wideband model) of the three-port Mason model of a finite thickness piezoelectric layer. The equivalent circuit consists of two mechanical ports and one electrical port. Z is the characteristic impedance of the quartz, k the complex wave number, A the area, and d_q the thickness of the resonator. hC_0 is the turns ratio of the transformer and is defined as $(e_{26}/\varepsilon_{22})C_0$. B) Butterworth–van-Dyke-equivalent circuit. Near resonance the three-port Mason model can be transformed into an equivalent circuit composed of discrete impedance elements. The capacitance C_q represents the mechanical elasticity of the quartz, the inductance L_q the initial mass, and the resistance R_q the energy losses arising from viscous effects, internal friction, and damping induced by the crystal holder. The static capacitance C_0 determines the admittance away from resonance, while the motional components dominate near resonance.^[6]

The BVD circuit combines a parallel and series resonance circuit (motional branch). The motional branch consists of L_q , C_q , and R_q . The electrodes on both sides of the crystal plate provide an additional parallel capacitance C_0 , which gives rise to a parallel circuit. Table 2 summarizes the expressions for L_q , C_q , R_q , and C_0 as they occur at the fundamental vibration.

The quartz material between the two electrodes (Figure 5) serves as a typical dielectric material, thus the resonator behaves as a plate condenser at high frequencies. Figure 7 displays parameter curves of impedance spectra of a 5 MHz quartz crystal on varying L_q , C_q , R_q , and C_0 . The motional resistance R_q contains intrinsic viscosities of the quartz and is responsible for energy dissipation. The phase maximum is a measure of the damping of a quartz resonator, as pointed out by Martin et al.^[17]

If damping is negligible ($R_q \rightarrow 0$) the quartz resonator shows two resonance frequencies corresponding to a phase shift $\varphi = 0$ at minimum and maximum magnitudes of the impedance $|Z|$. The corresponding resonant frequencies are referred to as resonant frequency f_R and antiresonant frequency f_A .^[9, 18, 19] The separation between the frequencies rises with an increase in the electroacoustic coupling constant K . Four different resonant frequencies

Table 2. Parameters of the BVD-equivalent circuit and their relationship to the physical characteristic numbers of an AT-cut quartz together with the different resonance frequencies. A is the electrode area, η_q the viscosity, d_q the thickness of the quartz, ε_{22} the dielectric constant of the quartz material, and e_{26} the piezoelectric constant dependent on the cutting angle, which can be obtained from the matrix elements e_{11} and e_{14} of the piezoelectric polarization modulus $\mathbf{e}$ and the cutting angle. The material constants shown in this Table are only valid for AT-cut quartz.^[9]

Parameter	Expression
C_0	$\frac{\varepsilon_{22}A}{d_q}$
C_q	$\frac{8Ae_{26}^2}{\pi^2 d_q \varepsilon_{22}}$
L_q	$\frac{d_q^3 \rho_q}{8Ae_{26}^2}$
R_q	$\frac{d_q \eta_q \pi^2}{8Ae_{26}^2}$
f_s	$\frac{1}{2\pi} \sqrt{\frac{1}{L_q C_q}} \left(1 + \frac{C_0 R_q^2}{2L_q}\right)$
f_p	$\frac{1}{2\pi} \sqrt{\frac{1}{L_q C_q}} \left(1 + \frac{C_q}{2C_0} - \frac{C_0 R_q^2}{2L_q}\right)$
f_{Zmin}	$\frac{1}{2\pi} \sqrt{\frac{1}{L_q C_q}} \left(1 - \frac{C_0 R_q^2}{2L_q}\right)$
f_{Zmax}	$\frac{1}{2\pi} \sqrt{\frac{1}{L_q C_q}} \left(1 + \frac{C_q}{2C_0} + \frac{C_0 R_q^2}{2L_q}\right)$

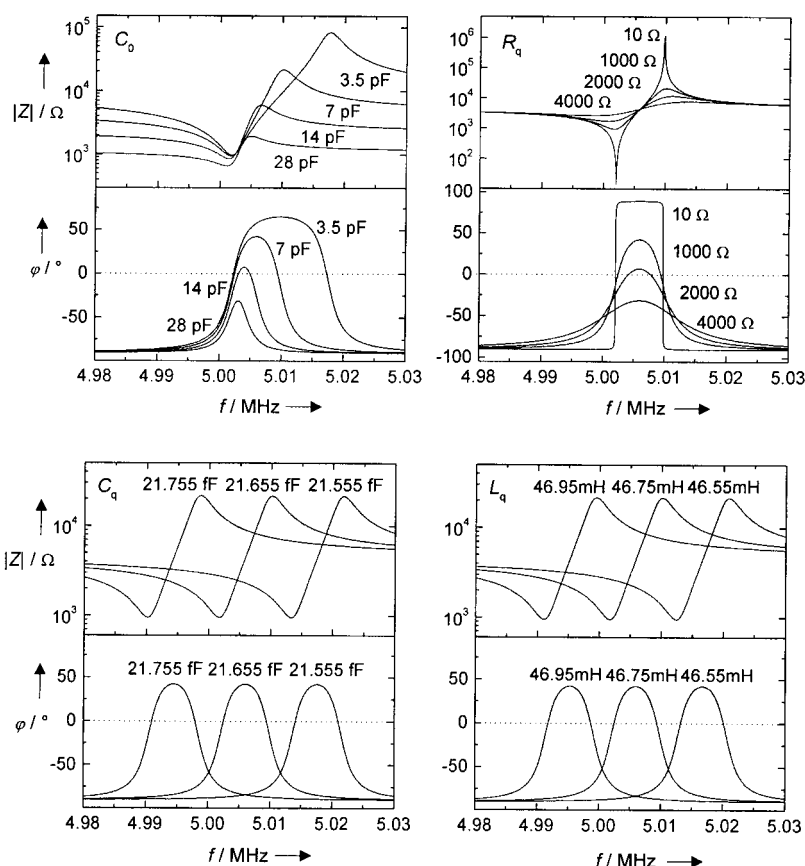


Figure 7. Simulated impedance spectra from the BVD-equivalent circuit with discrete variations of the impedance element values C_0 , C_q , L_q , and R_q .

are discernable if damping occurs ($R_q > 0$; Figure 8A). f_R separates into f_{Zmin} , the frequency at minimal impedance ($|Z|$), and f_s , the frequency at zero phase φ at the low frequency branch. In turn f_A separates into f_p , the frequency at zero phase at the high frequency branch, and f_{Zmax} , the frequency at maximum impedance. The expressions for f_s , f_p ,

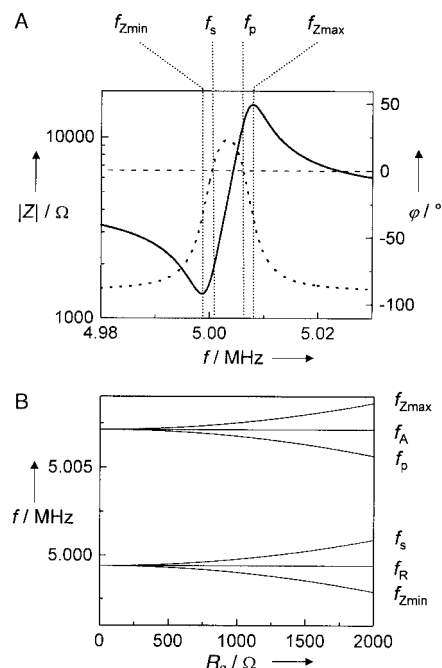


Figure 8. A) Simulated impedance spectrum from the BVD-equivalent circuit with marked resonance frequencies f_{Zmin} , f_s , f_p , and f_{Zmax} for the case of $R_q > 0$. B) Dependence of the resonance frequencies f_{Zmin} , f_s , f_p , and f_{Zmax} on the motional resistance R_q calculated according to the approximation of Bottom.^[9] Accurate calculations show that f_s and f_p converge at $R_q = 2280 \Omega$.

f_{Zmin} , and f_{Zmax} assuming low R_q and their dependencies on the electrical parameters of the simple BVD equivalent circuit are given in Table 2.

The dependence of the resonance frequency on the damping resistance R_q are shown in Figure 8B. f_{Zmax} and f_s rise with increasing damping, while f_{Zmin} and f_p decrease. The resonant frequencies f_s and f_p converge at $\varphi_{max} = 0$.

Active oscillation of shear resonators is restricted to $\varphi_{max} > 0$. At larger resistances leading to $\varphi_{max} \leq 0$ impedance analysis by a network analyzer is necessary to further study the behavior of the resonator.

2.3.1.1. Mass Loading

Sauerbrey has provided the first treatment of this issue.^[3] By analytically solving the one-dimensional equation of motion he showed that an ideal layer of foreign mass results in a frequency decrease Δf that is proportional to the deposited mass Δm if the resonator is operated in air or vacuum. If the density of the

mass layer is equal to that of the quartz crystal, Equation (3) applies.

$$\Delta f = -\frac{2f_0^2}{A\sqrt{c_{66}\rho_q}} \Delta m = -S_t \Delta m \quad (3)$$

Equation (3) describes the frequency response of a resonator on deposition of thin, rigid, and uniform films. The integral mass sensitivity or Sauerbrey constant S_t depends on the square of the fundamental frequency [Eq. (3)], and increases proportionally to the overtone number.

The differential mass sensitivity, however, is maximum in the center of the quartz resonator and decreases towards the borders of the electrodes (Figure 9).

The concept of energy trapping explains the differential mass sensitivity observed.^[6] The conditions for resonance and hence the resonant frequencies of the quartz crystal in the electrode-free region are different from those at the electrodes as a result of the additional mass of the electrodes. Analogous to the total reflection of light in an optical waveguide, in which the incoming light cannot penetrate the optically more dense material, standing acoustic waves generated at the electrodes are confined to this region, and is referred to as energy trapping. The lateral components of the acoustic wave, which travel tangential to the surface of the crystal are almost completely reflected at the interface between the electrode and electrode-free region. Energy trapping suppresses spurious modes and the quality factor of the resonator increases.^[20] However, if the thickness of the electrodes is too thin (usually less than 500 Å) the resonant frequencies of regions with and without electrodes are very similar, so that the acoustic wave is not confined to the electrode-covered region, and as a consequence the quality factor Q of the resonator decreases. Very thick electrodes, however, cause a decrease in the Q -factor as a result of the

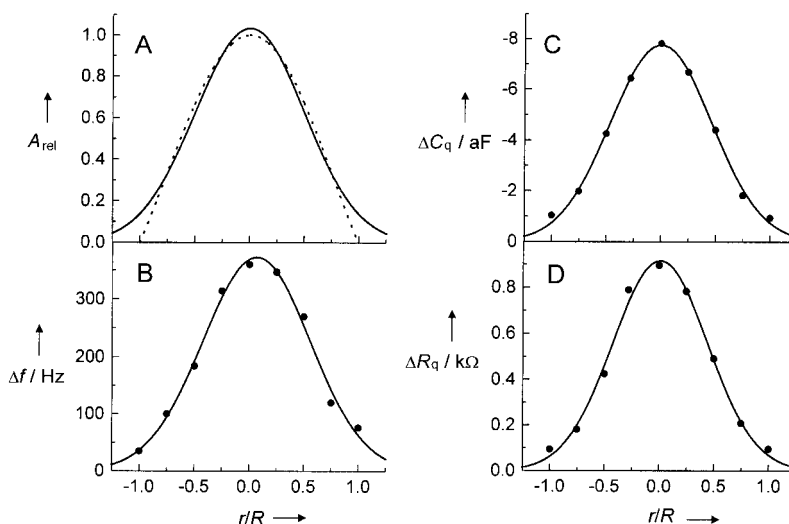


Figure 9. A) Radial distribution of the relative oscillation amplitude at the quartz surface according to a Bessel distribution (dotted line) and a Gaussian distribution (solid line) with $a = 2$. The Bessel function drops to zero at the electrode edges where $r/R = 1$, whereas the Gaussian function includes motion near the electrode edges. B) The increase in frequency of a 5 MHz AT-cut quartz while pressing a polyethylene tip on the quartz surface immersed in water. The change in resonance frequency is defined as $\Delta f = f(r) - f_0$. C) Corresponding capacitance change c_q of a 5 MHz AT-cut quartz. D) Change in motional resistance R_q . The solid lines are the results of the fitting parameters of a Gaussian distribution to the data.

presence of a dead dielectric, which is particularly pronounced by using gold electrodes and high frequency resonators.^[21] Electrodes made of aluminum instead of gold improve the situation because of the lower acoustic adsorption coefficient of aluminum. Further improvement can be achieved by using larger electrode areas.

The radial mass sensitivity s_r is proportional to the square of the radial displacement ($s_r(r) \propto |u(r)|^2$),^[22] which is described by a Bessel function J_0 of the first kind and zeroth order (Figure 9 A).^[12] The highest sensitivity is located at the center of the crystal at $r=0$ and vanishes at the electrode edges. However, Martin and Hager^[23] were the first to show that the amplitude of vibration is nonzero beyond the electrode edges ($r=R$) as a result of field fringing (inhomogeneous electric field, Figure 16), which is not considered by the energy-trapping concept. Field fringing is enhanced in an environment of higher permittivity such as water. The amplitude of the shear vibration depends on energy dissipation and therefore on the kind of load on the quartz. Liquid loading usually results in considerable damping and increased field fringing leading to a broadening of the curve and a decreased amplitude. The radial distribution of the shear amplitude can empirically be described by a Gaussian function [Eq. (4)].^[23]

$$u(r) = u_{\max} \exp\left(-a \frac{r^2}{R^2}\right) \quad (4)$$

$u_{\max}$ denotes the maximum displacement at $r=0$ and a is the characteristic width of the distribution, with typical values of $a \approx 2$ for a plane-parallel 5 MHz quartz in water. Figure 9 A displays a comparison of the two distributions. The spatial distribution of the differential mass sensitivity can be experimentally probed by depositing small defined masses or attaching a sharp probe at certain locations of the crystal surface. The latter approach has been used to map the sensitivity distribution of a 5 MHz quartz resonator as shown in Figure 9 B–D. The maximum change of Δf , ΔR_q , and ΔC_q occurs in the center of the plate. The distribution is well described by an empiric Gaussian function. By including field fringing, Hillier and Ward^[12] provided a solution of the wave equation that shows proportionality between frequency shift and deposited mass only if the material is homogeneously distributed over the crystal. Employing electrolysis to calibrate the QCM, however, does not always result in the formation of homogeneous metal films but often leads to an increased amount of deposited material at the electrode edges, which thus results in a systematic underestimation of the integral mass sensitivity.

The Sauerbrey Equation does not apply for thick films, viscous liquids, elastic solids, and viscoelastic bodies. In order to describe these various types of loading, theoretical models are needed to account for the observed frequency shifts and impedance spectra.^[24–29] Furthermore, the development of special oscillator circuits was necessary to cope with the high damping with liquid loads. Since the theoretical description of composite loading is common in life science problems and biosensor applications are of paramount interest, the theoretical framework developed by Bandey et al.^[25] has been chosen in this Review to explain some of the most relevant load situations in bioanalytics.

Starting with the three-port-Mason model (Figure 6 A) the piezoelectric resonator loaded on one side can be characterized by its mechanical impedance Z_s [Eq. 5] (T_{xz} = mechanical strain in the xz direction; V_x = potential in the x direction).

$$Z_s = \frac{T_{xz}}{V_x} \bigg|_{z=0} \quad (5)$$

The electrical impedance of the system Z_m is composed of Z_m^0 [Eq. (6a)], the impedance of the unperturbed resonator, and a term Z_m^1 [Eq. (6b)] representing the load (ω = angle frequency).

$$Z_m^0 = \frac{1}{i\omega C_0} \left(\frac{k d_q / K^2}{2 \tan(k d_q / 2)} - 1 \right) \approx R_q + i\omega L_q + \frac{1}{i\omega C_q} \quad (6a)$$

$$Z_m^1 = \frac{k d_q (Z_s / Z_q)}{4 K^2 \omega C_0} \left(1 - \frac{i(Z_s / Z_q)}{2 \tan(k d_q / 2)} \right)^{-1} \approx \frac{n\pi}{4 K^2 \omega C_0 Z_q} Z_s \quad (6b)$$

K is the electroacoustic coupling factor. The approximations on the right hand side are only valid for low-load conditions of the quartz ($Z_s / Z_q < 2 \tan(k d_q / 2)$). If $Z_s / Z_q \leq 0.1$ the impedance of the loaded resonator can be expressed as a lumped element equivalent circuit with Z_m^1 (Figure 10) as an additional element in series to the motional branch of the BVD-equivalent circuit (Figure 6 B). The mechanical impedance Z_s is a complex number in which the real part represents mechanical energy losses whereas the imaginary part stands for mechanical energy storage at the surface. Table 3 shows the impedance elements of Z_s for various surface coatings.

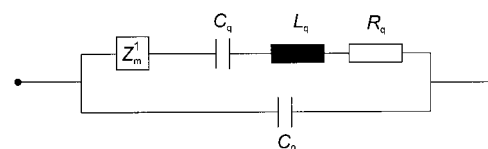


Figure 10. Modified BVD-equivalent circuit with an additional complex impedance Z_m^1 representing the load on the surface.

Table 3. Mathematical expressions of motional inductance L_L and resistance R_L for particular loads on AT-cut quartz resonators.^[25]

Load	L_L	R_L
rigid mass	$\frac{n\pi}{4 K^2 \omega C_0 Z_q} \frac{\rho_s}{\omega}$	0
Newtonian liquid	$\frac{n\pi}{4 K^2 \omega C_0 Z_q} \sqrt{\frac{\rho_L \eta_L}{2\omega}}$	$\frac{n\pi}{4 K^2 \omega C_0 Z_q} \sqrt{\frac{\omega \rho_L \eta_L}{2}}$
semi-infinite viscoelastic layer	$\frac{n\pi}{4 K^2 \omega C_0 Z_q} \left[\frac{\rho_v (G - G')}{2} \right]^{1/2}$	$\frac{n\pi}{4 K^2 \omega C_0 Z_q} \left[\frac{\rho_v (G + G')}{2} \right]^{1/2}$

Equations (6a) and (6b) enables one to describe typical load situations such as thin rigid films, viscous liquids, and viscoelastic polymers.^[25, 30, 31] Multilayers consisting of different kinds of surface layers can be described as a linear combination if interaction terms occurring between the different materials can be neglected. Nonlinearities will be discussed in more detail later. Three relevant cases, which are particularly interesting for applications in life science, are

thoroughly discussed in the following section: a) thin rigid films covered by a Newtonian liquid, b) semi-infinite viscoelastic solids, c) thin viscoelastic solids covered by a Newtonian liquid.

2.3.1.1.1. With a Newtonian Liquid Covering Thin Rigid Films

Covering a sufficiently thin film^[32] with a semi-infinite Newtonian liquid permits a linear combination of the mechanical impedances of the rigid mass and the liquid (Figure 11).

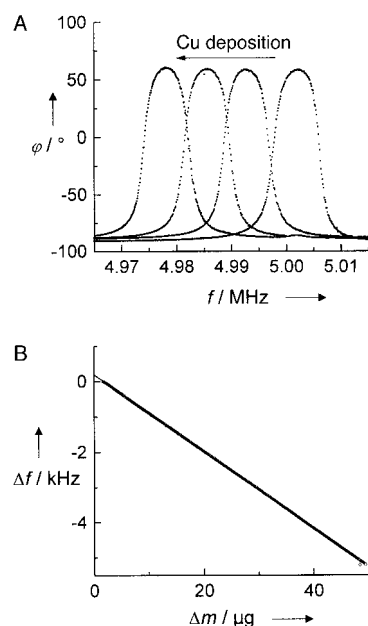


Figure 11. A) Impedance spectra of a 5 MHz AT-cut quartz before and after sequential deposition of 79, 158, and 237 μg of copper. The measurement was performed with one side of the quartz in contact with water. B) Change in the resonance frequency with increased amount of deposited copper.

The mechanical impedance of a rigid film of mass per unit area ρ_s can be expressed by $Z_s = i\omega\rho_s$, Z_s is purely imaginary, which means that the mass vibrates in phase with the resonator surface. The mechanical impedance Z_s of a liquid with viscosity η_L and density ρ_L can be determined from the velocity profile of a laminar flow parallel to the surface of the crystal [Eq. (7)], where $\delta = \sqrt{2\eta_L/(\omega\rho_L)}$ is the decay length of the damped shear wave within the fluid.

$$v_x(z,t) = v_{x,0} e^{-z/\delta} \cos(z/\delta) e^{i\omega t} \quad (7)$$

The shear wave propagates deeper into the medium with increasing kinematic viscosity (η_L/ρ_L). For instance, the decay length of a 5 MHz quartz is 250 nm in water. Assuming a mass load of a Newtonian liquid with a mass of $\Delta m = A\rho_L\delta$, Equation (3) results in the well-known expression found by Kanazawa and Gordon^[29] [Eq. (8)], who related the product of the density and viscosity of the liquid to the frequency shift of the shear wave resonator.

$$\Delta f = -f_0^{3/2} \sqrt{\frac{\eta_L \rho_L}{\pi \rho_q c_{66}}} \quad (8)$$

Kanazawa and Gordon did not consider the damping of the resonator from viscous loading. However, the electromechanical model of Martin and co-workers^[17, 25] shows that the resistance R_L and the inductance L_L both exhibit a proportionality to the square root of the density-viscosity product of the corresponding liquid (Figure 12 A and B).

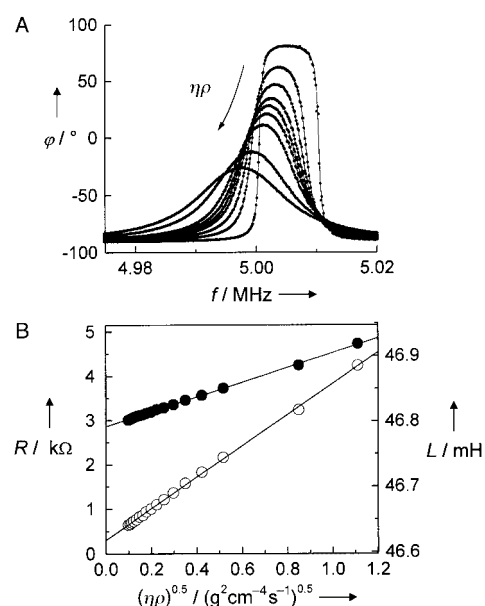


Figure 12. A) Influence of various glycerol–water mixtures on the phase shift. The solid lines are the results of fitting the parameters of the BVD-equivalent circuit. The measurements were taken in air (spectrum with the largest phase maximum), in water, as well as with glycerol–water mixtures of 40, 55, 60, 65, 70, 80, 85 % glycerol (the arrow indicates an increasing amount of glycerol in solution).^[167] B) Dependence of the resistance R ($\circ$) and inductance L ($\bullet$) on the square root of the density/viscosity product of various glycerol–water mixtures. The parameters were fitted using the generalized BVD-equivalent circuit composed of L , C , R , and C_0 . The capacitance C (21.655 fF) can be calculated from the intrinsic properties of the unloaded quartz (5 MHz).

A thin layer of a rigid mass covered by a semi-infinite layer of Newtonian liquid yields a linear combination, that is, addition of both impedances. The corresponding elements R_L and L_L can be obtained from a linear combination of the expressions shown in Table 3. Impedance spectra illustrating the situation of sequentially deposited copper films on a quartz crystal vibrating in aqueous solution are shown in Figure 11. Mass deposition of thin rigid films in a Newtonian liquid at constant density and viscosity can be described by Sauerbrey's equation without changes in R_L (Figure 11B).

2.3.1.1.2. Semi-Infinite Viscoelastic Solids

The semi-infinite viscoelastic solid is characterized by the absence of reflections at the air–solid interface of the shear wave traveling within the viscoelastic body. The decay length is smaller than the thickness of the solid. Therefore, wave propagation is unidirectional within the viscoelastic solid of density ρ_v , which leads to a simple expression for the mechanical impedance Z_s [Eq. (9)].^[25]

$$Z_s = \sqrt{G\rho_v} \quad (9)$$

G is the complex shear modulus, in which $\text{Re}(G)$ is the storage modulus G' and $\text{Im}(G)$ the loss modulus G'' . Both G' and G'' can be obtained from the expression of R_L and L_L . Using a specific model for viscoelastic solids, such as the Kelvin or Voight model, one can calculate viscosity and elasticity, respectively. Figure 13 shows the frequency response and the shear displacement for different viscoelastic materials.

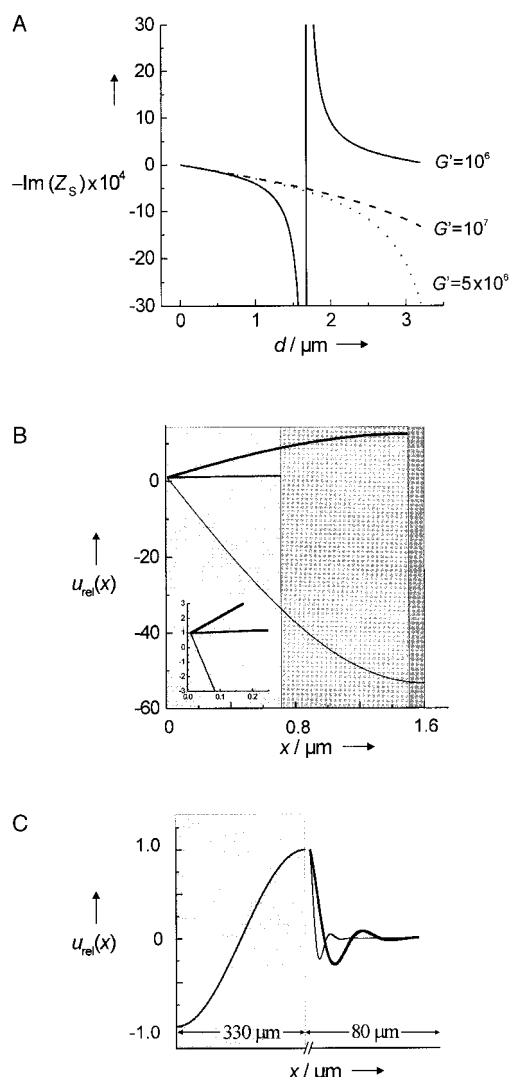


Figure 13. A) Numerical simulations of the imaginary part of the mechanical impedance (proportional to the frequency change) of a lossless viscoelastic film with different film thicknesses. An increase of film softness, namely, the shear modulus G becomes smaller, and leads to a considerable deviation from the Sauerbrey Equation even at small film thicknesses. For very soft films, even the resonance of the film is visible in the observed film thickness range, which starts at $\phi = \pi/2$ and is characterized by a sudden increase in the resonance frequency. B) Shear vibration amplitude within the film. Within the linear region of $\text{Im}(Z_s)$ versus d the polymer surface oscillates in phase with the quartz and the damping is almost zero (middle curve). With larger thicknesses an overshoot of the displacement at the film/air interface occurs; the surface of the elastic film does move synchronously with the quartz oscillation but with a higher amplitude (top curve). Immediately after resonance the oscillation of the film is 90° behind the oscillation of the vibrating quartz (bottom curve). C) Within an infinite extended viscoelastic solid the shear oscillation propagates with different wave and decay lengths. The situation depends on the complex shear modulus G and the density of the solid.

2.3.1.1.3. With a Newtonian Liquid Covering Thin Viscoelastic Solids

The combination of a sufficiently thin viscoelastic film covered by a semi-infinite layer of a Newtonian liquid cannot be treated as a simple linear combination of the individual mechanical impedances as was valid for the two previous examples. Instead, interaction terms between the two different materials have to be taken into account. Granstaff and Martin^[26] derived a general recursion formula [Eq. (10)] for a layer system with n different viscoelastic materials.

$$Z_s = Z_s^{(n)} \frac{Z_s^{(n+1)} \cosh(\gamma^{(n)} d^{(n)}) + Z_s^{(n)} \sinh(\gamma^{(n)} d^{(n)})}{Z_s^{(n)} \cosh(\gamma^{(n)} d^{(n)}) + Z_s^{(n+1)} \sinh(\gamma^{(n)} d^{(n)})} \quad (10)$$

γ denotes the complex wavenumber ik and $Z_s^{(n)}$ is the characteristic impedance of the n th viscoelastic layer. The procedure for evaluating the mechanical impedance of single layers is to start with the knowledge of the characteristic impedance of the top layer (stress free). Then, one has to work backward toward calculating the surface mechanical impedance at the resonator–film interface. This process allows the influence of many viscoelastic layers on the surface of the TSM resonator to be estimated, although extracting physical properties becomes cumbersome with increasing amount of layers. One example is a thin viscoelastic film of thickness d covered by a liquid. A description with discrete impedance elements is permitted because of the phase lag of the shear oscillation at the surface of the viscoelastic film with respect to the resonator–film interface. Resonance of the viscoelastic solid is reached at an acoustic phase shift $\phi = \omega d \text{Re} \sqrt{\rho_v / G}$ of $\pi/2$ accompanied by a sudden increase in the resonance frequency and damping of the system (Figure 13). At $\phi > \pi/2$ the surface of the film oscillates at 90° to the surface of the resonator, and exhibits higher amplitude (overshoot) than the quartz material, which is almost critically damped.

2.3.1.1.4. Conclusion

The previous examples demonstrate that the operation of TSM resonators in liquids raises theoretical and experimental problems. Besides the difficult evaluation of multilayers composed of different viscoelastic solids or fluids, contributions from the electrolyte, roughness of the surfaces, surface energy of the outermost layer, and influence of compressional waves have to be considered. Some of the most important aspects are discussed in more detail below.

2.3.1.2. Longitudinal waves

Longitudinal or compressional waves can readily be observed by filling a measuring chamber equipped with a TSM resonator at the bottom and opened to air with a volatile liquid. Periodical changes in the resonant frequency can be recorded (Figure 14) as a result of the evaporation of the liquid. Closing the chamber, however, abolishes the observed instability instantaneously.

This observation can be explained in terms of compressional wave generation. The evaporation of the liquid with time results in the height of the liquid varying, and therefore constructive and destructive interference of the compres-

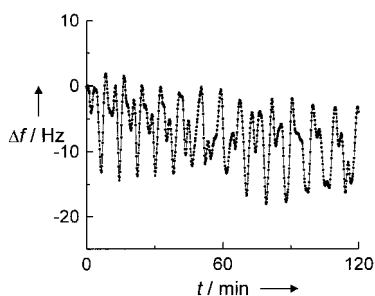


Figure 14. Periodic changes in the resonance frequency of a 5 MHz quartz immersed in ethanol. As ethanol evaporates over time, the resonance condition of the longitudinal wave, which is reflected at the ethanol–air interface, alters continuously.

sional waves reflected at the air–liquid interface occurs. This process results in a typical standing wave pattern. The origin of longitudinal waves can be reasoned by the occurrence of longitudinal or flexural mode or as shown by Martin and Hager^[23] by a nonzero velocity gradient of the shear oscillation along the x -axis (Figure 15).

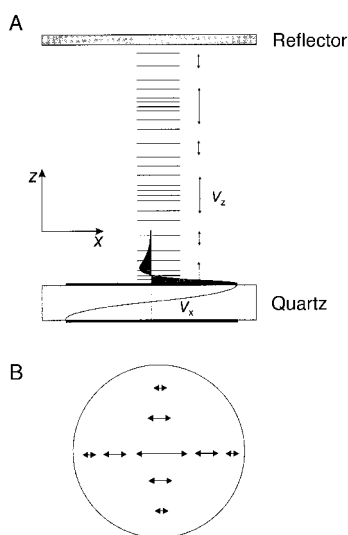


Figure 15. A) Representation of compressional waves generated in the fluid above the quartz crystal induced by a velocity gradient of the particle displacement, which are reflected at the glass surface and form a standing wave. B) Velocity profile of the particle movement on the quartz surface.^[34]

Lin and Ward^[33] as well as Schneider and Martin^[34] demonstrated by a simple experiment and theoretical treatment of the problem that indeed compressional waves are generated from the quartz surface and can be reflected at an interface. They mounted a reflector (glass plate), adjustable to the desired distance from the quartz plate (z -direction), several 100 μm away from the resonator. Both, the resonance frequency and the damping resistance R varied periodically with the distance of the reflector from the shear resonator. The periodicity was $\lambda_c/2$, in which λ_c represents the wavelength of the longitudinal wave in solution.

2.3.1.3. Conductance and Permittivity of the Solution

Other important parameters that influence the resonant frequency of the quartz crystal in solution are the ionic

strength and dielectric constant of the electrolyte. Supposing that the operator of the QCM has to change the buffer conditions for a particular experiment an undesired parasitic frequency shift may occur, which can be more or less of the same magnitude as the measuring signal itself. This effect strongly depends on the shape of the electrodes and conductance of the solution. The series resonance shows only a minor response to changes in conductance and permittivity,^[35] since all effects parallel to the motional branch add on the parallel resonant frequency of the crystal.^[36] The parallel resonant frequency decreases with increasing conductance of the solution. An expanded BVD-equivalent circuit which explained the observations was first introduced by Shana and Josse.^[37]

An additional parallel RC circuit (Figure 16B) has to be introduced to account for the influence of the conductance and dielectric constant of the medium. R_G can be neglected at high ionic strength, thus C_G and $C_{0,A}$ can be lumped into one capacitance to give the well-known BVD circuit with a modified capacitance C_0 . Rodahl et al.^[38] investigated the

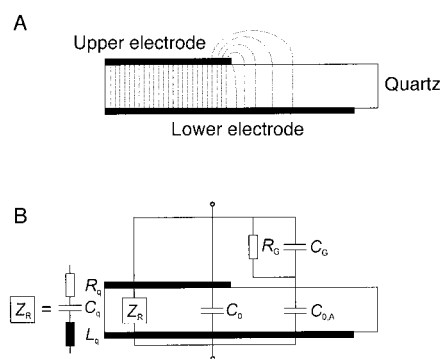


Figure 16. A) Course of the field lines at the electrode edges of a quartz oscillator. The origin of the changes in the quartz resonance frequency upon changing the ion conductance of the solution are field-fringing effects. B) These edge effects of the electric field can be represented by a RC element (R_G and C_G). As a result of the overlap of the electric field the effective area of the quartz is larger, hence the capacitance C_0 of the quartz is also increased by $C_{0,A}$.^[37, 38]

influence of the conductance on the series and parallel resonant frequency as well as the damping of the resonator using differently shaped electrodes.^[39] They found that field fringing is the predominant reason for changes in the resonant frequencies (Figure 16A). The extent of field fringing strongly depends on the shape of the electrodes. The strongest dependence of the parallel resonant frequency on the conductance of the electrolyte solution was seen for ring-shaped working electrodes in which the center of the quartz resonator is not layered, for instance, while completely covered quartz plates do not display significant frequency shifts with changing conductivity.

2.3.1.4 Surface Roughness

Interpretation of adsorption phenomena is strongly influenced by the surface roughness of quartz resonators. In particular an alteration in the hydrophilicity upon adsorption can lead to tremendous changes in the resonant frequency. Rough and hydrophilic surfaces entrap liquids in small

cavities thus contributing to the overall mass detected by the device.^[40, 41] Hydrophobic cavities, however, are often not wetted by the liquid and result in the inclusion of air or vacuum (Figure 17),^[41] thus leading to smaller energy losses on hydrophobic surfaces than hydrophilic ones. This observation implies that the resonant frequency shifts to smaller values when changing from a hydrophobic to a hydrophilic surface. Hence, smooth surfaces are required when operating in fluids so as to avoid frequency shifts arising from changes in surface energies.

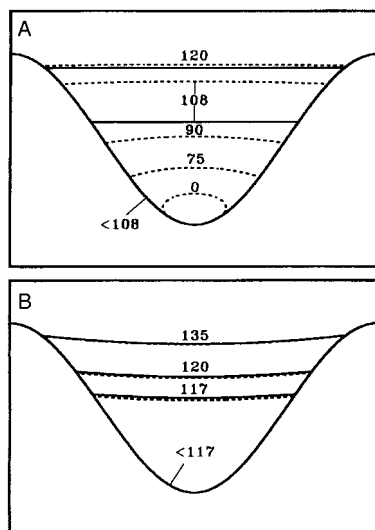


Figure 17. Cross-section of different liquid menisci formed in a sinusoidally textured surface illustrating contact angle dependent trapping (A) with trapped air and (B) without trapped air. The dashed lines indicate the initial penetration of liquid for the indicated microscopic contact angle. The solid lines indicate the equilibrium penetration, which becomes complete at $<108^\circ$ (A) and $<117^\circ$ (B).^[7]

2.3.1.5. Electrochemical Double Layer

Tsionsky et al.^[42] presented a consistent treatment of the influence of the electrochemical double layer on the resonant frequency. Considering the electrochemical double layer as a rigid mass on the basis of its extension of a few nanometers in the z -direction leads to an additional frequency change Δf_{DS} . The thickness of the double layer can be estimated to be about 1 nm for a 1:1 electrolyte of 0.1M. By taking into account that the extension of the shear wave of a 5 MHz quartz is 250 nm in water and therefore exceeds by far the thickness of the double layer this approximation holds for most cases. The influence of a thin rigid mass oscillating in phase with the resonator surface can be expressed by Sauerbrey's Equation. A change of the electrochemical double layer as a result of additional charges adsorbing to the surface leads to the following change in resonant frequency [Eq. (11 a) and (11 b)].

$$\Delta f_{DS} = - \frac{2f_0^2}{A\sqrt{c_{66}\rho_q}} \Delta m_{DS} \quad (11a)$$

$$\Delta m_{DS} = \frac{q_+}{F}(M_A - M_w n_A) - \frac{q_-}{F}(M_K - M_w n_K) \quad (11b)$$

M_A and M_K are the molecular masses of the corresponding anions and cations, M_w the molecular weight of water, n_A and

n_K denote the number of substituted water molecules, and q_- and q_+ are the excess charges on the surface. Adsorption of positively charged particles on the resonator surface leads to an increase in adsorbed mass if it is assumed that sufficiently large counter ions are near the quartz. Therefore, charged particles can cause larger frequency shifts than uncharged ones. The influence of charges on the hydrodynamic properties of the adjacent water layer has not been considered yet.

2.3.1.6. Conclusion

In conclusion, interpretation of QCM data is not unambiguous and can lead to controversial results. The factors mentioned in Sections 2.3.1.2–2.3.1.5 have to be taken into account if conditions such as hydrophilicity, surface charges, meniscus, temperature, viscosity of the liquid, and buffer composition cannot be preserved. The QCM in fluids is not a simple mass-sensor but provides valuable information about interfacial reactions. Besides elasticity and viscosity, information about surface charges of biomolecules can be obtained. The following section gives an overview of other acoustic resonators.

2.3.2. Surface-Acoustic-Wave (SAW) Sensors

Lord Rayleigh was the first to discovered this acoustic mode known as surface acoustic waves (SAW). The stress-free boundary of a solid gives rise to these surface-confined waves that propagate as coupled longitudinal and transversal waves. The frequencies of SAW sensors are usually between 50 MHz and a few GHz.^[7] The displacement components decay exponentially within the solid. The surface acts as an acoustic waveguide. Surface acoustic waves can be excited and detected by patterned interdigital transducers (IDT, see Figure 3) on the surface of single piezoelectric crystals such as quartz, lithium niobate, or lithium tantalate. Each “finger” is the origin of a surface acoustic wave. The transducer works most efficiently if the periodicity matches the wavelength of the surface wave occurring at $f = v_p/d$, in which v_p denotes the propagation velocity and d the distance between the interdigital fingers, that is, the periodicity. Generally, resonators can be distinguished as two port delay lines and one-port resonators.^[43] Two port delay lines work with one IDT as a transmitter and one as a receiver. The separation between them determines the delay between the transmission and receiving of the surface wave. One-port resonators consist of one IDT structure in between two reflectors thus producing a standing wave in both directions. The resonant frequency is given by $f_n = n v_p/2l$, in which l is the distance between the reflectors. The quality factor Q of SAW devices is between 6000 and 12000 and is considerably lower than that of TSM resonators operating in air (20000–50000 for a 10 MHz fundamental frequency).

2.3.2.1. Mass Loading

If the deposited mass is a thin rigid film the kinetic energy of the synchronously vibrating system is increased without any energy loss arising from viscous damping. This then leads to a

decrease in the propagation velocity. The influence on the resonant frequency is given by Equation (12).^[7]

$$\frac{\Delta f}{f} = \frac{\Delta v}{v_p} = -c_t^{\text{SAW}} f \rho_s \quad (12)$$

This equation resembles Equation (3). Again the mass sensitivity increases with the square of the fundamental frequency, which is considerably higher (> 100 MHz) than that of TSM resonators, and renders the sensitivity of a 100 MHz SAW sensor 200 times higher than the mass sensitivity of a 5 MHz quartz crystal. However, bioanalytic applications require operation in water, thus SAW sensors are less suited than TSM resonators as a result of the high energy loss occurring in an aqueous environment. Damping is a consequence of compressional waves generated by displacement components parallel to the surface normal and the viscous coupling of displacement components parallel to the surface.

2.3.3. Acoustic-Plate-Mode (APM) Sensors

APM sensors are cut from single-crystalline quartz to serve as acoustic waveguides that are particularly suited for operation in liquids. The acoustic wave is confined between the upper and lower surfaces of the plate propagating between input and output transducers, in contrast to SAW sensors in which they are restricted to one surface.^[44] Shear-horizontal (SH) APM resonators do not exhibit displacement components parallel to the surface normal but predominantly display displacement parallel to the surface and the direction of wave propagation. Therefore, loss of acoustic energy from interaction with the environment is drastically reduced. The particle displacement has only one component.^[45] SH modes can be considered as a superposition of plane waves with an in-plane (shear horizontal) displacement reflected at a particular angle between the upper and lower face of the quartz resonator. Similar to SAW sensors, acoustic waves can be excited and detected by lithographically patterned IDT metal structures (Figure 3). The input transducer with periodicity b and thickness d generates approximately the eigenfrequency calculated by Equation (13).^[45]

$$f_n = \frac{v_p}{b} \left[1 + \left(\frac{nb}{2d} \right)^2 \right]^{1/2} \quad (13)$$

2.3.3.1. Mass Loading

When the mass is strongly bound (nonslip boundary condition) to the surface the layer moves synchronously with the quartz surface. As a consequence the kinetic energy increases and the propagation velocity decreases as discussed previously for TSM and SAW resonators [Eq. (14)].^[7]

$$\frac{\Delta f}{f} = \frac{\Delta v}{v_p} = -c_t^{\text{APM}} \rho_s \quad (14)$$

c_t^{APM} denotes the mass sensitivity, and ρ_s the surface mass density (mass/area on face) of the foreign mass layer.

2.3.4. Flexural-Plate-Wave (FPW) Resonators

FPW resonators are thin, rectangular membranes made of tension-free silicon nitride embedded in a frame of silicon that are manufactured photolithographically. Oscillations of these plates, which are only a few micrometers thick, can be excited piezoelectrically by IDT, electrostatically, or by using magnetic transducers. FPW resonators are characterized by a high quality factor and low energy loss in fluids at a low resonant frequency. Although the mechanical amplitude is rather high (100 nm) energy dissipation is low since the phase velocity of the acoustic wave is lower than the velocity of sound in most liquids (900–1500 m s⁻¹).^[7] The low resonant frequencies (1–10 MHz) permit the use of low-cost electronics, thus providing an attractive alternative to the less sensitive TSM resonators. FPW devices can also be used as actuators for granular solids as a result of their high amplitude.

The simplest case of an oscillating isotropic plate includes an infinite set of waves known as Lamb waves.^[46, 47] Two sets of waves can be distinguished: symmetric waves (S), with particle displacements symmetric about the neutral plane, and antisymmetric waves (A), whose displacements have odd symmetry. Only two waves, A_0 and S_0 (both of lowest order), occur in sufficiently thin plates. Notably, the phase velocity of the two waves differs tremendously. Thinner membranes exhibit lower phase velocities for the A_0 mode, in which the plate undergoes flexing as the wave propagates, while the phase velocity reaches its maximum value for the S_0 mode. In the case of the A_0 mode the eigenfrequency decreases with decreasing thickness of the membrane at a given wavelength λ [Eq. (15)].^[7]

$$f = \frac{1\sqrt{B}}{\lambda M} \quad (15)$$

B denotes the bending stiffness of the membrane^[48] and M the specific mass of the membrane per unit area.

2.3.4.1. Mass Loading

Increasing the mass of the isotropic plate by a thin rigid layer of foreign mass results in a decreased phase velocity of the A_0 Lamb wave [Eq. (16)].^[7]

$$\frac{\Delta f}{f} = \frac{\Delta v}{v_p} = -\frac{\rho_s}{2M} \quad (16)$$

The integral mass sensitivity $-1/2M$ can be increased by using thinner plates, thus reducing the phase velocity and hence the resonant frequency. A comparison of the different acoustic resonators is given in Table 4.

3. Adsorption of Biomolecules and Cells

3.1. Quartz-Crystal Microbalances

The core component of a quartz-crystal microbalance is the AT-cut quartz plate with fundamental resonance frequencies predominately in the range of 5–30 MHz. Since quartz plates with high fundamental frequencies are very thin and therefore difficult to handle, most quartz plates in use have fundamental

Table 4. Comparison of the different acoustic resonators. d = plate thickness; λ is the wavelength of the acoustic wave.

Resonator	d	Medium	f [Hz]	Example	Temperature stability	Mass sensitivity $S_m^{[a]}$ [(Hz/MHz)(ng/cm ²) ⁻¹]
TSM	$\lambda/2$	g, fl	4–30	quartz 6 MHz, $d = 277 \mu\text{m}$ $v_p = 3330 \text{ m s}^{-1}$, $\lambda = 554 \mu\text{m}$	high	0.014 (6 MHz)
SAW	$\gg \lambda$	g	30–500	quartz 158 MHz, $d = 760 \mu\text{m}$ $v_p = 3160 \text{ m s}^{-1}$, $\lambda = 20 \mu\text{m}$	high/medium	0.20 (158 MHz)
SH/APM	$3-10\lambda$	g, fl	25–200	quartz 101 MHz, $d = 203 \mu\text{m}$ $v_p = 5060 \text{ m s}^{-1}$, $\lambda = 50 \mu\text{m}$	high	0.019 (101 MHz)
FPW	$\ll \lambda/2$	g, fl	2–7	ZnO 5.5 MHz, $d = 3.5 \mu\text{m}$ $v_p = 550 \text{ m s}^{-1}$, $\lambda = 100 \mu\text{m}$	medium	0.38 (5.5 MHz)

[a] $S_m^{\Delta m=0} = (\Delta f/f)1/\rho_s$.

resonance frequencies in the range of 4–10 MHz. Overtones are excited in order to obtain resonance frequencies larger than 15 MHz. Despite mechanical problems, very sensitive quartz crystals with fundamental resonance frequencies of about 30 MHz are also used.^[49]

For the analysis of biomolecules and cells, measurements in fluids are required—particularly in aqueous solutions. By developing appropriate oscillator circuits capable of exciting AT-cut quartz crystals to their resonance frequencies under liquid load,^[50, 51] the quartz crystal microbalance could be introduced as a powerful tool in life science. Up to now several quartz crystal microbalance setups have been realized:

- A flow system comprising a quartz plate that is usually clamped between two O-rings. Since the radial mass sensitivity, described by a Gaussian function, decays towards the edges of the quartz plate it is advisable to minimize the contact area of the O-ring with the resonator and to place it as far outside the quartz as possible, so that damping is minimized and thus the quality factor is at a maximum. The measurement chamber has to be sealed to air to avoid air bubbles and alteration of the liquid meniscus in order to avoid reflection of induced longitudinal waves at the air–liquid interface which influence the resonance frequency of the quartz. An outlet and inlet of the quartz holder allows the addition of analytes at any given time while monitoring the resonance frequency. Apart from thermodynamic equilibrium values, kinetic data can also be obtained with this setup. The time resolution of the quartz crystal is limited by its quality factor and is in the range of milliseconds. Using a flow system ensures a proper mixing of the solution, so that rate-controlled kinetics might be assumed. Moreover, most setups can be fully automated for sensor applications.
- A different approach to excite the quartz which has only one side in contact with liquids is to seal one side of the quartz with a rubber casing. Only one side is exposed to the fluid when the quartz is completely immersed into solution, with the other one kept in air. Addition of the analyte can be performed using a syringe while stirring the solution continuously.
- Further techniques to immerse quartz crystals with only one side in aqueous solution are based on moving a quartz plate horizontally at the air–water interface; beforehand,

a film is spread at the air–water interface and compressed to the desired film pressure. In this way, the quartz plate is in close contact with the monolayer. Upon addition of the analyte into the subphase, the interaction can be monitored.

- The simplest approach is to monitor frequency shifts in air after adsorption of the biomolecules in fluids and drying of the surface. This technique allows only the determination of final frequency values with large errors arising from the continuous change between air and liquid. The resonance frequencies obtained vary considerably compared to those gathered from measurements in liquids, since the water content of the biomolecules influences the response of the quartz significantly. The only advantage of this technique is given that the Sauerbrey Equation might be valid under these conditions.
- Inspired by the finding that the vibration of the quartz is considerably influenced by a liquid, the so-called reference crystal method has been developed. With this setup, two quartz crystals are excited in parallel. One of the surfaces of the two quartz crystals is functionalized while both of them are immersed in the same medium. This results in a net detection of the biomolecules. However, this method is not frequently used since other disadvantages occur from the different surfaces of the two crystals which lead to temperature and resonance frequency instabilities.

There are basically two different modes of operation for TSM resonators. One is based on the quartz crystal being the frequency-determining element of the oscillator circuit. The surface coverage of the quartz can be obtained by monitoring the resonance frequency. The read out of the resonance frequency is routinely performed using an oscillator circuit connected to a frequency counter. This mode of operation is termed active, since the crystal is excited to its resonance frequency and the oscillator circuit compensates for the energy loss. One should keep in mind that the exact resonance frequency that is excited by the oscillator circuit is not necessarily known so that experimental results might not be comparable using different oscillator circuits. Especially, if energy dissipation as a result of viscoelastic load occurs then frequency responses can be quite different. The equations depicted in Table 2 outline the various resonance frequencies of a moderately damped system to demonstrate this problem.

Therefore, it is important to know whether the serial or parallel resonance is supported by the oscillator circuit. However, the feedback of the oscillator circuit often introduces inevitable phase shifts, which result in considerable deviations from the serial or parallel resonance so that an accurate determination of the excited resonance frequency remains difficult.

The other mode of operation, the so termed passive method, uses a network analyzer/frequency generator to excite the crystal to a constraint vibration near resonance while monitoring the complex electrical impedance or admittance dependent on the applied frequency. By fitting the parameters of the BVD-equivalent circuit to the spectra (Figure 6B) both the mass load and energy dissipation can be determined separately.

A connecting link between simple frequency determination and complex network analysis is the so-called QCM-D technique developed and commercialized by Kasemo and co-workers,^[52] which allows the resonance frequency and dissipation factor to be monitored simultaneously. In this technique a quartz plate is excited with a frequency generator followed by switching off the source and recording the free decay of the quartz oscillation. This procedure is repeated each second. The dissipation factor together with the resonance frequency is obtained by a curve fit. Damping can also be recorded using an amplitude-controlled oscillator circuit that monitors the amplitude separately from the resonance frequency.^[53]

3.2. In Situ Hybridization of DNA/RNA on Quartz Surfaces

The analysis of genetic material, such as that essential for the diagnosis of hereditary and infectious diseases, for the classification of an organism, and in the field of forensic chemistry, has attained enormous importance and has led to numerous techniques to quantify nucleic acids in a sensitive, selective, and fast manner, that is, high-throughput screening. In the course of these developments mass-sensing devices were included in the repertoire of signal transducers that are capable of detecting oligonucleotides label-free and online. The first evidence of a direct measure of nucleic acids using an acoustic resonator was given by Fawcett et al.,^[54] however, the experiments were performed in air. The first in situ experiment was accomplished in fluids using an acoustic-plate-mode resonator^[44] and the second one with a thickness-shear-mode resonator, which was functionalized with a single-stranded DNA coupled through a self organized 11-sulfanylundecanol monolayer chemisorbed on gold.^[55]

The crucial step in developing a mass-sensitive nucleic acid detecting device is the immobilization of a single-stranded oligonucleotide on the resonator surface which hybridizes selectively with the complementary strand from solution. Well-known procedures are the modification of 5'-phosphate residues by thiol groups.^[56–60] In this way, DNA as well as RNA can be immobilized, and also peptide nucleic acids carrying a polyethylene-modified terminal cysteine.^[61] Suitable procedures solely for DNA and RNA are based on

electrostatic interactions of the negatively charged backbone and positively charged amine monolayers.^[62, 63] Frequently used methods, though quite complicated, are based on the binding of biotinylated oligonucleotides to surface-confined avidin or streptavidin. The coupling of proteins is accomplished by simple physisorption,^[64] by electrostatic interaction,^[63] or covalently linked through an amide bond between chemisorbed 3,3'-bispropionic acid and the amine residue of the protein.^[56, 65, 66] Unfortunately, the oligonucleotide coverage is not well determined in most cases; surface coverages are mostly calculated after adsorption of the nucleic acid in air or liquid using the proportional constant of the quartz as determined before. The average values obtained are well below 100 %, with typical coverages between 10 and 30 %. A correct correlation between the actual concentration of the nucleic acid at the surface and the frequency decrease, as experimentally determined by Su et al.,^[67] demonstrated that the surface coverage obtained from the frequency shift is 3 to 12 times larger than that determined by radioisotope labeling. The question is whether the common oligonucleotide immobilization techniques provide satisfactory surface coverages. The quality of the immobilization method is rather important for dealing with aspects such as sensitivity and specificity since the surface coverage reflects the sensitivity of the mass-sensing device. Moreover, nonspecific adsorption can be minimized by complete surface coverage. A desirable, user-demanded detection limit is approximately 10^{-18} M, but depends on the number of base pairs.^[68] In order to minimize the detection limit of a functionalized resonator surface quartz crystals with higher resonance frequencies were used, multilayers composed of nucleic acids and polymers/proteins were developed,^[63] DNA dendrimers were synthesized,^[69] and nucleic acids were amplified on the surface by using the polymer chain reaction (PCR).^[70] In a recent study Bardea et al.^[71] used anti-double-stranded DNA and anti-mouse F_c antibodies as a second antibody to amplify the signal. Despite these efforts, the detection limit is currently in the range of 10^{-8} – 10^{-7} M.^[56, 60, 69]

Besides difficulties of nucleic acid immobilization and sometimes unknown surface coverage the reason for a low detection limit can also be sought in the low hybridization yields on the surface, which do not exceed 10 %.^[57, 59, 67] Steric hindrance of the hybridization reaction explains this low value; however, the equivalence of mass change and frequency upon the detection of nucleic acids in solution is questionable. Although the translation of the frequency shift into mass using the Sauerbrey Equation is pursued in many publications, one should refer to the articles of Thompson and co-workers^[67, 72–74] and Fawcett et al.,^[68] who showed that oligonucleotides immobilized on the resonator surface in fluids do not behave like an ideal rigid mass. Thompson and co-workers revealed, for example, that the serial resonance f_s upon binding of DNA exhibits a frequency change which is 18 times larger than predicted by the Sauerbrey Equation. They attributed this discrepancy to an altered viscosity on the surface that was generated by hybridization. Network analysis revealed that the motional resistance R , which is indicative of energy loss, changes in the same fashion as the serial resonance frequency. For immobilized DNA, R is not only

dependent on the liquid's viscosity but is also influenced by the electrolyte effecting the electroacoustic coupling of the oscillation. Thus, R is additionally determined by the solution's conductivity and the electrochemical double layer, which makes a particularly large contribution in the case of highly charged DNA.^[67] These altered viscoelastic effects can be used to obtain kinetic data of the DNA hybridization on the surface.^[72] However, a systematic study remains to be performed.

Besides sensitivity, the specificity of oligonucleotide biosensors is also of paramount importance. Bardea et al.^[60, 71] showed, that a seven base pair mutation within a 31-mer that occurs in the genome of the Tay–Sachs disease is sufficient to inhibit hybridization of the mutated DNA with surface-immobilized complementary strands. In contrast, 10-mers containing two terminal mutations hybridize almost completely (92 %), while DNA with one mutation positioned in the center of the strand hybridizes with a yield of only 30 %. However, nonspecific adsorption of oligonucleotides could not be excluded.^[57] An increased sensitivity was gained by using peptide nucleic acids instead of DNA on the surface. Functionalization of gold surfaces with peptide nucleic acids has the effect that a single point mutation of the complementary DNA strand is sufficient to inhibit DNA binding to the surface (Figure 18).^[61]

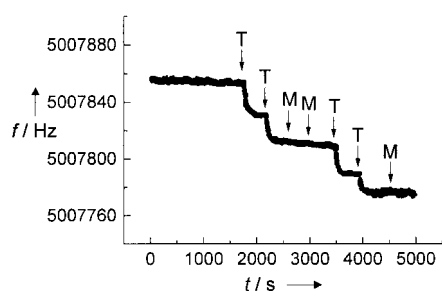


Figure 18. Frequency-time response of a QCM biosensor functionalized with a peptide nucleic acid of the sequence Cys-egl-GGC-AGT-GCC-TCA-CAA for multiple additions of $10 \mu\text{g mL}^{-1}$ of the 15-mer 5'-TTG-TGA-GGC-ACT-GCC-3' (T) and of $50 \mu\text{g mL}^{-1}$ of 5'-TTG-TGA-GAC-ACT-GCC-3' (M). The mismatch base is indicated in bold.^[61]

A recent study of Wang et al.^[75] illustrates the use of the quartz-crystal microbalance to monitor real-time enzymatic activity of RNAses and DNAses using surface-confined poly(U) single strands and (dA)₂₀–(dT)₂₀ double strands. The specificity of both enzymes was preserved on the surface.

3.3. Adsorption of Proteins at Functionalized Surfaces

The primary area of application of the quartz-crystal microbalance today is the investigation of protein adsorption at functionalized surfaces. For instance, the basic principle of piezoelectrochemical sensors is the detection of the binding of antibodies to surface-confined antigens. For a long time it was postulated that a direct quantification of the adsorbed amount of protein would be feasible by using the Sauerbrey Equation. However, a number of publications established that protein adsorption performed in liquid leads to larger

frequency shifts than in air. In order that the quartz-crystal microbalance can be used as a universal tool it is desirable to find reasons for the different frequency changes. A study from 1993 found for the first time a direct correlation between mass load and frequency shift by adsorbing human serum albumin (HSA) on the resonator.^[76] Muratsugu et al. quantified the mass load by using radioisotope-labeled HSA in combination with the determination of the frequency shift by using the quartz-crystal microbalance. This result is a hallmark with respect to the sensitivity of shear-wave resonators. While $\Delta f / \Delta m$ is supposed to be $0.183 \text{ Hz cm}^2 \text{ ng}^{-1}$ for a 9 MHz quartz as predicted by Sauerbrey, the results revealed values of $(0.375 \pm 0.012) \text{ Hz cm}^2 \text{ ng}^{-1}$ for HSA and a value of $(0.716 \pm 0.066) \text{ Hz cm}^2 \text{ ng}^{-1}$ for anti-HSA. On the one hand, these numbers are considerably larger than that expected from the Sauerbrey Equation, and on the other hand, they depend on the investigated protein. In order to clarify this discrepancy, Kasemo and co-workers developed a measuring device that allowed frequency shifts to be monitored simultaneously with energy loss, which was represented as the dissipation factor with a time resolution of 1 s.^[52] Energy loss can occur within the adsorbed film, where included water also has to be taken into account, or at the interfaces as a result of friction. Their experimental results in aqueous solution confirmed that energy loss, that is, an increase in the dissipation factor, can be detected.^[77–79] Thus, binding of methemoglobin (met-Hb) and hemoglobin-CO (HbCO) on a hydrophobic methyl-terminated monolayer exhibits the expected frequency decrease as well as an alteration of the dissipation factor D (Figure 19 A, B). Plotting the frequency shift versus change in D gives rise to two distinct slopes, which indicates that a two step adsorption process occurs (Figure 19 C).

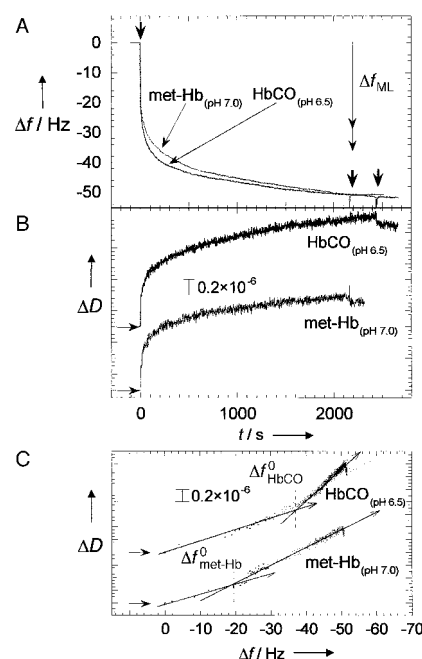


Figure 19. A) Changes in frequency and B) dissipation as a function of time during the adsorption of met-Hb and HbCO at pH 7.0 and pH 6.5, respectively, onto a methyl-terminated thiol monolayer (ML). C) Plot of the change in the dissipation factor versus the change in frequency. Two different slopes, which indicate a two-step adsorption process, are discernable for the adsorption of both met-Hb and HbCO.^[78]

These results have led to the conclusion that energy loss does not only occur at the interfaces but also in the protein layer itself; dissipation might be attributed to conformational changes within the adsorbed protein layer and tightly bound water; they are induced by periodic shear movement. The hypothesis that enclosed water contributes considerably to the frequency shift was confirmed by Rickert et al.^[80] Upon deposition of multilayers composed of alternating layers of biotinylated BSA and streptavidin they obtained a sensitivity that was four times larger than the integral mass sensitivity as predicted from the Sauerbrey Equation. By determining the thickness of the layer they could demonstrate that approximately 75 % of the overall mass is water. They excluded viscoelastic effects since a nonchanging frequency shift per protein layer up to 20 monolayers was observed. A decrease in the frequency response by a factor of approximately 0.7 would be expected according to Rickert et al. if the protein layer showed viscoelasticity.^[80]

Although the frequency shift cannot be translated into mass load, it is nevertheless conceivable that the quartz-crystal microbalance could be used for *in situ* measurements of binding events. Concentration-dependent measurements of the frequency shift together with the assumption of a linear relation between frequency shift and mass load allow the binding and rate constants of the protein and peptide adsorption to be determined.^[66, 81–85] The technique is well suited for the quality control of multilayers prepared by the Langmuir–Blodgett technique^[86] or self-organization processes.^[80, 87–94]

3.4. Lipid–Protein Interactions at Solid-Supported Lipid Membranes

Protein–receptor interactions at lipid membranes, for example ganglioside–toxin interactions play an essential role in biological processes. The first contact of a protein, virus, or bacterium with its receptor at a biological membrane initiates a variety of reactions at the cell membrane. Artificial membrane systems which are variable in their lipid composition are necessary for investigating these kind of interactions at a lipid membrane.^[95, 96] Nowadays, common model membrane systems are vesicles and black lipid membranes. Lipid membranes immobilized in a highly ordered fashion on solid supports are of great interest for the quartz-crystal microbalance. Different preparation techniques are available to prepare so-called solid-supported membranes. The Langmuir–Blodgett and Langmuir–Schäfer techniques, for example, allow the transfer of highly ordered lipid monolayers from the air–water interface onto a pretreated quartz surface. Instead of using these equipment-intensive methods, techniques based on the chemisorption of thiol or disulfide components are well suited for immobilizing lipid bilayers on gold-covered quartz plates. In a first step gold surfaces are functionalized using sulfur-containing components. These self-organized monolayers serve as starting points for the preparation of lipid bilayers. Common methods for the preparation of lipid bilayers are the vesicle-fusion technique,

detergent dilution method, or painted lipid membrane procedure.^[96]

Okahata and co-workers^[97–100] immobilized lipid monolayers at the air–water interface of a quartz surface by dipping it horizontally from the air-side at the interface. The hydrophilic head groups are oriented towards the water subphase so that proteins and peptides dissolved in the subphase may interact with them. These functionalized quartz plates allowed the binding and dissociation constants of the interaction of melittin and β -globulin with dipalmitoylphosphatidylethanolamine membranes to be determined; moreover the binding of the lectine concanavalin A to glycolipid monolayers was quantified.^[101] It is very easy to incorporate various numbers of receptor molecules in those lipid monolayers at the air–water interface. With the same preparation technique it was feasible to investigate the binding behavior of the influenza A virus and wheat germ agglutinin with G_{M3} -doped monolayers composed of sphingomyeline and/or glucosylceramide. Variation of the lipid matrix and the dopant concentration revealed that only these parameters are pivotal for virus binding and that the binding rate is influenced considerably. The system allows the inhibition of the virus binding by adding sialyllactose (Neu5Ac- α -2-3Gal- β -1-4Glc).^[97, 99, 100, 102]

The above-mentioned methods based on the self-organization of lipids on surfaces make it possible to readily prepare lipid membranes that are composed of two single leaflets. A lipid bilayer composed of a first chemisorbed alkanethiol monolayer and a second lipid monolayer can be obtained by fusing unilamellar vesicles on the hydrophobic monolayer. These vesicles fuse on the surface so that their composition also determines that at the surface. Impedance analysis of the mono- and bilayers allows an exact quality control of each layer, thereby ensuring highly reproducible membrane preparations (Figure 20).

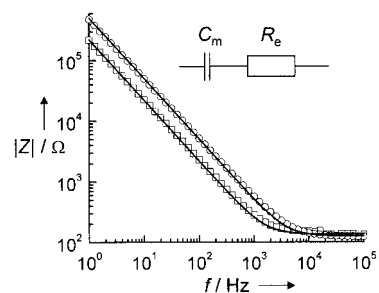


Figure 20. Impedance spectra of an octanethiol monolayer ($\square$) and a lipid bilayer composed of octanethiol and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC; $\circ$). The solid lines show the results of fitting the parameters of the simple equivalent circuit depicted.^[103]

A comparison of two different QCM investigations demonstrates that the choice of the artificial membrane system significantly influences the obtained results. Both studies dealt with the adsorption of melittin to phospholipid membranes. Whereas Okahata and co-workers used lipid monolayers at the air–water interface and obtained an ideal Langmuir adsorption isotherm for melittin concentrations of up to 100 μM ,^[100] Steinem et al.^[103] used lipid bilayers and

obtained adsorption isotherms which indicated multilayer adsorption started at a concentration of $8\ \mu\text{M}$. The immobilized membrane was solubilized by melittin at concentrations larger than $20\ \mu\text{M}$, these effects were not observed at the air–water interface.

In principle, the quartz-crystal microbalance in combination with lipid membranes composed of an alkanethiol monolayer and a second lipid monolayer obtained by vesicle fusion allows an easy determination of thermodynamic and kinetic parameters of protein–ligand couples without the use of labels. The example of the interaction of peanut agglutinin (PNA) with gangliosides shows the suitability of this approach. A lipid membrane is doped with different concentrations of the receptor lipid G_{M1} and the frequency shift is monitored upon addition of PNA. Figure 21 shows that a dopant concentration of 1.3 mol % of the receptor lipid G_{M1} is sufficient to achieve maximum coverage of the protein

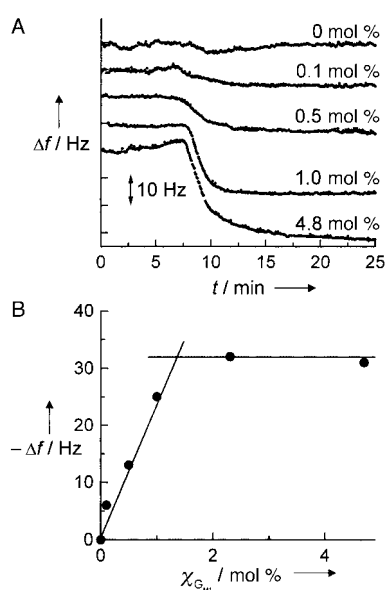


Figure 21. A) Frequency shifts of a 5 MHz quartz functionalized with solid-supported octanethiol/POPC lipid bilayers containing different amounts of G_{M1} upon addition of $2\ \mu\text{M}$ of PNA. B) Dependence of the equilibrium value of the resonance frequency Δf on the mole fraction $\chi_{\text{G}_{\text{M1}}}$ within the phospholipid monolayer. The solid lines intersect at $\chi_{\text{G}_{\text{M1}}} = 1.3$ mol %.^[107]

surface. A calculation of the theoretical value of the minimum number of necessary G_{M1} molecules within the lipid matrix, assuming a homogenous distribution of the receptor lipids and correct values for the geometry of the protein, leads to a value of 1.5 mol %. A comparison of the theoretical value with the one obtained experimentally implies that the monomeric protein coverage on the surface has to be close to one. Similar maximum protein coverage using a lipid matrix doped with 2 mol % of the receptor lipid was corroborated by Ebato et al.^[104] who investigated the streptavidin–biotin couple with the quartz-crystal microbalance. The frequency shift Δf has to be monitored at various concentrations of the protein c_0 in solution to determine the binding constant of a protein–receptor couple (Figure 22). By assuming that the binding sites on the surface are energetically equivalent and that there is a

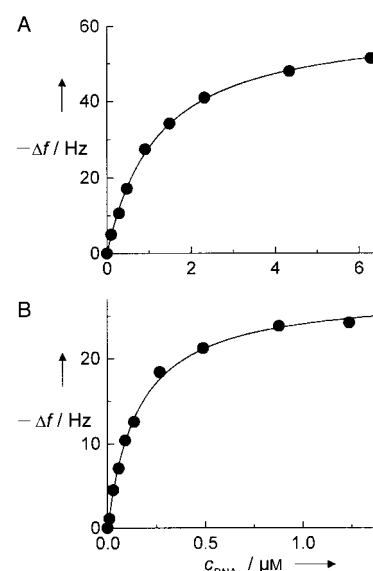


Figure 22. Isotherm of the binding of PNA to a G_{M1} -doped lipid bilayer (A) and to an *asialo*- G_{M1} -doped octanethiol/POPC bilayer (B) immobilized on a gold-covered AT-cut quartz. The dependence of the equilibrium resonance frequency shift Δf on the PNA concentration in solution is shown. The POPC monolayer was doped with 4.8 mol % of the receptor lipid. The solid line is the result of fitting the parameters of a Langmuir isotherm according to Equation (17) with $K_a = (0.8 \pm 0.1) \times 10^6$ and $(6.5 \pm 0.3) \times 10^6\ \text{M}^{-1}$, respectively, and $\Delta f_{\text{max}} = -(61.0 \pm 0.5)$ and (28.0 ± 0.5) Hz, respectively.^[109]

homogeneous distribution of the receptor lipids,^[105] the binding constant K_a can be obtained by fitting the parameters of a Langmuir adsorption isotherm [Eq. (17)] to the data.

$$\Delta f = \Delta f_{\text{max}} \frac{K_a c_0}{1 + K_a c_0} \quad (17)$$

The established binding constants present information about the chemical structure of the receptor essential for an appropriate binding, as demonstrated by the adsorption of PNA to G_{M1} and *asialo*- G_{M1} . While the binding constant of PNA to G_{M1} is $K_a = (0.83 \pm 0.04) \times 10^6\ \text{M}^{-1}$, it is determined to be almost a factor of 10 larger at $K_a = (6.5 \pm 0.3) \times 10^6\ \text{M}^{-1}$ for *asialo*- G_{M1} .^[105] This difference is attributed to the fact that *N*-acetylneuramic acid of G_{M1} is not necessary for or even disrupts the binding of PNA. However, it has been demonstrated that the affinity of PNA to the trisaccharide $\beta\text{-Galp-(1,3)-GalNac-(1,4)-}\beta\text{-Galp}$ is larger than to the dissacharide $\beta\text{-Galp-(1,3)-GalNac}$.^[106]

This is an example how the molecular structure of a receptor molecule can be illuminated by varying the receptor molecules embedded in the lipid membrane using the quartz-crystal microbalance. Besides quantifying the inhibition of binding in solution this method is capable of clarifying carbohydrate structures that play a pivotal role in receptor function. Monitoring the frequency shift upon binding of PNA to G_{M1} in the absence of an inhibitor (Figure 23) allows the binding constant K_I of the inhibitor in solution to be determined.^[107] A prerequisite for the determination of K_I is an appropriate ratio between K_I and K_a . If the binding constants have similar orders of magnitude an exact determination of the binding constant K_I is practicable since the

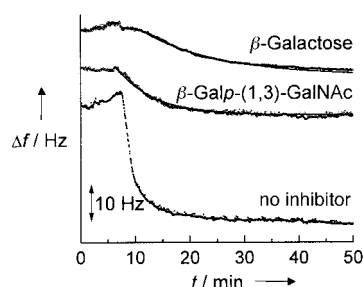


Figure 23. Time course of the resonance frequency shift upon addition of a 2 μM solution of PNA to a lipid bilayer composed of octanethiol and POPC doped with 4.8 mol % of G_{M1} without inhibitor, with 0.27 mM $\beta\text{-Galp-(1,3)-GalNAc}$, and 26.5 mM $\beta\text{-galactose}$.^[107]

frequency changes continuously with the inhibitor concentration in solution. If there are several orders of magnitudes between K_{I} and K_{a} , the protein binds either almost unaffectedly on the surface or not at all.

The PNA–ganglioside system displays the potential of the quartz-crystal microbalance combined with solid-supported membranes in regard to studying ligand–receptor couples and the parameters which can be calculated from the obtained data. In summary, it can be concluded that the QCM technique enables one to quantify binding constants and kinetics in a relatively simple fashion so that information can be gathered about the structure of natural receptor molecules. This feature was particularly demonstrated in a study dealing with the adsorption of bacterial toxins—cholera, tetanus, and pertussis toxin—on various gangliosides.^[104, 107–109]

Besides basic knowledge that can be garnered from lipid membranes immobilized on quartz crystals, this system might also be useful for biosensor applications. Solid-supported lipid membranes on gold surfaces are not only well-suited because they can be prepared reproducibly with an exactly adjustable composition, but also because they are of particular interest, since nonspecific protein adsorption arising from the lipid matrix is strongly suppressed. First experiments pointing in the direction of regenerating the sensor surfaces after binding of a protein were demonstrated by Janshoff et al.^[107] Upon addition of protease, the adsorbed protein can again be released from the surface (Figure 24).

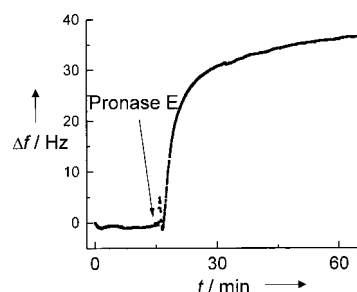


Figure 24. Time course of the resonance frequency shift upon addition of a pronase solution to a PNA-covered lipid bilayer composed of octanethiol and POPC doped with 4.8 mol % of G_{M1} . The PNA concentration was 2 μM , the concomitant frequency shift –36 Hz. The concentration of pronase E was 0.2 % (w/w). The frequency increase arising from the action of pronase was 35 Hz after incubation of the protein monolayer in solution for approximately 1 h.^[107]

3.5. Piezoelectroimmunosensors

The high specificity of antigen–antibody reactions and the ability to generate antibodies against a variety of biological and nonbiological substances opened up a way to develop immunosensors to address questions in areas ranging from clinical diagnosis, food control, to environmental analysis. During the last three decades radioisotope-labeled antibodies were mostly used for immunoassays.^[110] Disadvantages that emerge from work with radioactive components has led to the development of new marker systems, that is, fluorescence-labeled antibodies. Nowadays the enzyme-linked immunosorbent assay (ELISA) is the most widespread analysis tool to detect antibody–antigen reactions. The peculiarity of this detection method is the amplification of the antigen–antibody reaction using an enzyme that is bound to the antibody and catalyzes a detectable reaction, namely, the formation of a UV/Vis-active or fluorescence active dye.^[111] Despite this established technique, interest in discovering label-free and less time consuming online detection methods is still undiminished. Numerous articles were published dealing with the immobilization of antibodies or antigens on transducer surfaces whose signal is affected upon binding of the complement. Such immunosensors are valued with respect to their handling, their overall costs, and how the sensor surface can be recycled without loss of sensitivity.

Piezoelectric immunosensors, such as the quartz-crystal microbalance and SAW sensors, are suitable transducer surfaces which fulfill the above demands appropriately. Piezoelectric immunosensors in the gas phase have been investigated for quite some time^[112–116] whereas the online detection of antibody–antigen reactions in aqueous solutions originally started with the work of Roederer and Bastiaans on SAW sensors^[117] and Thompson et al.^[118] on AT-cut quartz plates. Contradictory to the classical understanding of piezoelectric sensors Thompson and co-workers found a frequency increase upon binding of immunoglobulin G (IgG) to the immobilized antigen in their first study. They concluded from this result that an alteration of the microviscosity at the interface was induced upon binding of the antibody.

Similar publications from this time also dealing with the piezoelectric detection of IgG established, however, that a frequency decrease was observed upon the binding of an antibody; hence the previously published frequency increase remains questionable.^[119, 120] All further studies supported the decrease in frequency upon the binding of an analyte to a surface.

Nowadays the applicability of piezoelectroimmunosensors to various fields has been proved. The spectrum ranges from medical applications for the detection of bacterial toxins^[121–123] and viruses^[114, 116, 124–128] through the determination of bacteria^[55, 115, 129–132] in the food industry, to environmental analysis for the detection of organic compounds by using an antigen–antibody reaction.^[122, 133–137]

For the development of a functional bioimmunosensor based on piezoelectric transducers, problems characteristic to all biosensors need to be solved: 1) functionalization of the electrode-covered surface, 2) sensitive, specific, and repro-

cible detection of the analyte in solution, and 3) regeneration of the sensor surface.

The immobilization technique of antigens or antibodies regularly leads not only to a functionalized sensor surface but also determines substantially the sensitivity and reproducibility of the sensor. A frequently used method is based on the simple physisorption of the desired compound on clean gold surfaces^[120, 124, 126–128, 131, 138, 139] and polymers.^[88, 115, 140] The stability of those immobilized molecules does not differ significantly from covalently coupled ones.^[129, 132, 138, 141, 142] However, problems arising from these techniques are the nondirectional orientation of the molecules on the surface, namely, that part of the antigens/antibodies is not accessible. An oriented immobilization of antibodies can be achieved by nonoriented physisorption of antibodies or covalent coupling of protein A followed by an oriented adsorption of the antibody through the F_{ab} domain. Thus, the F_{ab} domain points preferentially to the external medium.^[114, 119, 120] Another possibility is to utilize the linkage of the antibody to an individual thiol group.^[143] In this way Göpel and co-workers succeeded in the arranged linkage of an antigen—a synthetic peptide of the mouth and claw epidemic virus—on a ω -hydroxyundecanethiol monolayer.^[87, 144, 145] Another technique for the oriented immobilization of antibodies using Langmuir–Blodgett films with F_{ab} fragments bound to linker phospholipids was successfully demonstrated by Vikholm et al.^[143, 146]

However, a major problem remains: the nonspecific adsorption at the transducer surface that can only be minimized by improved immobilization techniques and cannot be influenced by the transducer itself. The functionalized surface is often blocked with a protein such as BSA or casein before the binding of the analyte so as to minimize nonspecific adsorption. Additionally, nonspecific adsorption, which is predominantly caused by hydrophobic interactions, can be reduced by the addition of detergents.^[124, 126, 128]

Apart from nonspecific interactions, which generate undesired signals independent of the choice of the transducer, viscous coupling of the liquid can cause severe problems if the solution's composition is varied during the experiment. The shear-wave resonator responds sensitively to variations in the solution's properties, namely, an altered viscosity. There are several possible ways of minimizing this bulk effect. If the viscosity of the solution under investigation is known, the viscosity of the medium can be adjusted by adding an appropriate amount of glycerol.^[128] Aberl and co-workers used diluted humane serum albumin as a medium to minimize nonspecific adsorption.^[124, 126] More general procedures to prevent this problem, which do not require the knowledge of the solution's composition, are the reference crystal method^[147] and the same-condition method.^[148] A new procedure was introduced by Zhang et al.,^[149] after they were able to distinguish the mass load of the quartz from viscoelastic effects by measuring the resonance frequency and the amplitude of the applied voltage simultaneously. The obtained frequency shift could be corrected by viscoelastic effects with this setup.

The minimization of nonspecific adsorption enhances the sensitivity of the piezoelectric immunosensor. Furthermore, the minimum concentration which can still be detected with

this method is determined by the binding constant of a given ligand–receptor couple. Ebato et al.^[135] showed that the immobilization of an antigen (fluoresceine-labeled lipid) decreases the binding constant on the surface by a factor of 300 relative to the reaction in solution.

Eventually, the question remains as to whether the surface can be regenerated after the binding of the analyte and allow further binding studies. Willner and co-workers used a reversible cleavage of antibodies which were bound to an antigen-covered gold surface of a shear-wave resonator. They functionalized gold surfaces of 9 MHz quartz plates with the photoisomerable substrate *N*-methyl-dinitrospiropyran, which exhibits an affinity to the antibody antidinitrophenyl-Ab. Light with a wavelength of $360 \text{ nm} < \lambda < 380 \text{ nm}$ induced an isomerization of *N*-methyl-dinitrospiropyran to *N*-methyl-dinitromerocyanin, which does not exhibit an affinity to the antibody. The resomerization occurs at $\lambda > 495 \text{ nm}$. The reversible binding of the antidinitrophenyl-antibody was impressive as demonstrated by QCM measurements.^[150–152] Another technique to achieve reversible antibody–antigen interactions at surfaces was presented by Sargent and Sadik.^[140] They immobilized anti-HSA antibodies on a conductive polypyrrole surface on a quartz plate and were able to induce a reversible binding by applying voltage steps.

In summary, piezoelectric immunosensors are an alternative to established ELISA methods. In contrast to ELISA techniques, which need a marker molecule and take approximately two hours, this method can be fully automated. Nowadays, the quartz-crystal microbalance provides a technique for analyzing phage libraries within a short time by using a flow injection system.^[153] The detection of the analyte can occur online and is label-free within 10 min; moreover, kinetic data can also be used to specifically detect an antibody. The specificity is determined predominately by the chosen immobilization techniques and is therefore equivalent for ELISA and quartz crystal microbalance measurements. However, as yet the obtained sensitivity of piezoelectroimmunosensors is lower than that of an ELISA assay.

3.6. Detection of Cellular Systems

The application of microgravimetric acoustic sensors for the detection and characterization of pro- and eukaryotic cells has led to a number of interesting experimental findings as a result of the abundant information provided by such an analysis. Complex and time-consuming methods of cell biology may one day be replaced by faster, more highly resolving, and simpler techniques using TSM resonators as piezoelectric sensors. These sensors are particularly important in the food industry for the routine determination of bacterial cell numbers in diets, but it is also desirable in clinical areas to be able to determine cell numbers in body fluids online. Table 5 gives an overview of bacteria whose cell numbers were determined using piezosensors.

Most piezosensors used for the detection of bacteria in solution are based on antigen–antibody reactions in which the bacterium binds to the corresponding surface-confined antibody and thus can be monitored. In this way, the highest

Table 5. Summary of cell types that were investigated using quartz resonators. The lowest detection limit as well as the surface reaction that was used for detecting the cells are given.^[186]

Cell species	Cell number ^[187]	Surface reaction
<i>Staphylococcus epidermidis</i> ^[187]	$1 \times 10^2 - 4 \times 10^7$ cells mL ⁻¹	liquidation of gelatin
<i>Listeria monocytogenes</i> ^[131]	$2.5 \times 10^5 - 2.5 \times 10^7$ cells per quartz	antigen – antibody
<i>Chlamydia trachomatis</i> ^[129]	$0.26 - 7.8$ μ g mL ⁻¹	antigen – antibody
<i>Vibrio cholerae</i> ^[122]	$> 10^5$ cells mL ⁻¹	antigen – antibody
<i>Candida albicans</i> ^[188]	$10^6 - 5 \times 10^8$ cells mL ⁻¹	antigen – antibody
<i>Enterobacteriaceae</i> ^[189]	$10^6 - 10^9$ cells mL ⁻¹	antigen-antibody
<i>Salmonella typhimurium</i> ^[190]	$10^5 - 10^9$ cells mL ⁻¹	antigen – antibody
<i>Salmonella typhimurium</i> ^[132]	$9.9 \times 10^5 - 1.8 \times 10^8$ CFU mL ⁻¹ [a]	antigen – antibody
<i>Salmonella typhimurium</i> ^[191]	$3.6 \times 10^6 - 102 \times 10^6$ cells mL ⁻¹	cell growing on SS-agar ^[b]
<i>Pseudomonas cepacia</i> ^[192]	$> 3 \times 10^5$ cells cm ⁻²	cell growing on gold
<i>Staphylococcus epidermidis</i> ^[193]	3.9×10^{-2} % (v/v)	antigen – antibody, agglutination in solution
<i>Pseudomonas aeruginosa</i> ^[130]	$10^5 - 10^8$ cells mL ⁻¹	antigen – antibody

[a] CFU = colony-forming unit (number of cells in a culture that can build a new colony in a layer). [b] SS = *Salmonella shigella*.

specificity is ensured. However, problems similar to those of immunosensors occur, such as nonspecific adsorption, which can only be abolished by blocking the free binding sites prior to the binding assay. In most cases a linear correlation between the bacterial cell number and frequency shift can be found, thus enabling one to calibrate the system for bacterial cell numbers. These studies are based on the assumption that the frequency shift upon cell binding can be attributed to a simple mass change as described by the Sauerbrey Equation. However, in 1993 Gryte et al.^[154] and Redepenning et al.^[155] were the first to point out that cells on quartz resonators in a medium cannot be treated like an ideal rigid mass. Cells are more likely to be presented as a viscous load similar to a fluid. A single cell layer on a 5 MHz quartz would give rise to a theoretical frequency shift of 5600 Hz according to the Sauerbrey Equation.^[155] The actual values are in general at least one order of magnitude lower than that. This observation is attributed to the concept that a cell body behaves like a viscoelastic body (for example, Voight or Kelvin body)^[156] under shear stress, which leads to the fact that besides an increase in the kinetic energy as a result of a mass change (increase in inductance L) an energy dissipation (increase in R) also occurs. It was shown that the shear wave within the system composed of the cell, extracellular matrix, and a water layer vanishes. This result was confirmed by experimental results in which cell multilayers and the adsorption of silica beads do not cause a detectable frequency shift.^[157]

In order to draw conclusions from the cell parameters assuming that they behave as viscoelastic bodies, an impedance analysis in the range of the resonance frequencies of the quartz is necessary. Complex shear moduli can be obtained by fitting electromechanical models to the obtained data. In Figure 25 the impedance behavior of a 5 MHz quartz in the presence and absence of adherent Madin darbine canine kidney (MDCK II) cells is shown. The quartz is highly damped because of the attached cells—an indication that they do not behave like a rigid mass. Modeling the cells attached to the resonator as a Newtonian liquid turns out to be too simplified to account for its impedance behavior on the resonator. In fact it is apparent from the spectra that an elastic contribution has to be considered.^[158] A detailed analysis of the individual components of an adherent cell monolayer on a quartz plate illuminates the complexity of the system. Besides

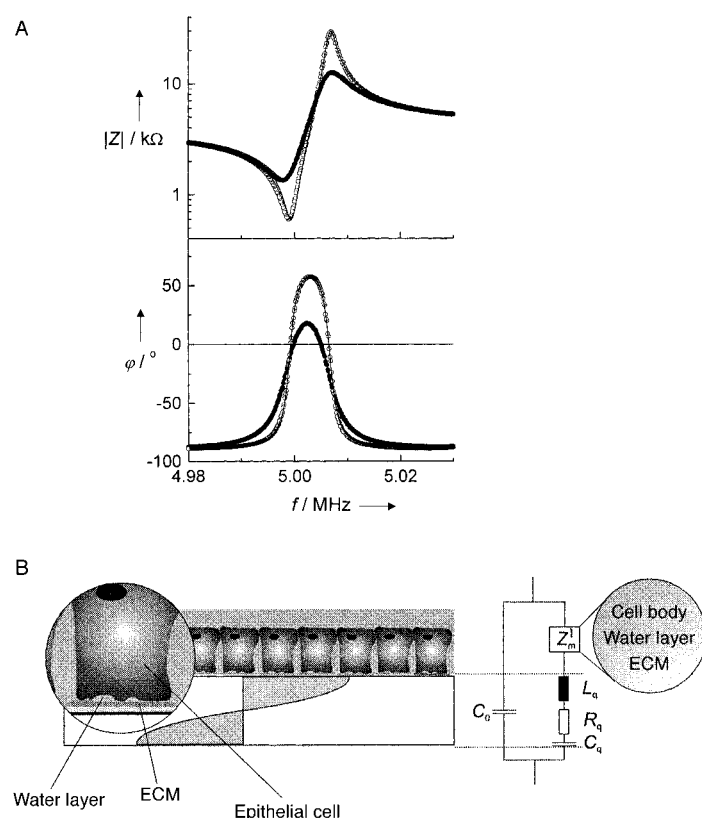


Figure 25. A) Influence of a confluent MDCK II cell monolayer on the impedance spectrum of a 5 MHz quartz resonator ($|Z|$ and ϕ); the spectra before (●) and after (○) scraping the cell layer from the surface are shown. The solid lines are the results of curve fitting using the BVD-equivalent circuit. B) Equivalent circuit of an adherent cell monolayer including extracellular matrix (ECM) and water layer. Z_m^l represents the combined viscoelastic properties of adherent cells as well as the ECM and an adjacent water layer between the cell and the surface.^[167]

the actual cell layer, the extracellular matrix (ECM), a water film between the ECM and cell, and the medium on top of the cells need to be considered. It was shown that the influence of the extracellular matrix plays a pivotal role in the shear oscillation, while the mechanical properties of a cell-covered quartz resonator remain unaffected by the supernatant medium. The contribution of the ECM is mainly inductive and can therefore be considered as a simple mass added to the

resonator. Energy dissipation, however, could not be observed.^[159]

Damping of the shear oscillation by the presence of a confluent cell monolayer is dependent on the particular cell species and their ability to adhere on the surface. Loosely attached bovine aortic cells have little effect on the motional resistance R , while strongly adhered MDCK cells damp the oscillation of the quartz considerably (Table 6). This difference can be explained by the presence of a variable, thin water layer between the cell and the surface. Diminishing the thickness of the water layer by applying hyperosmotic stress, in which the osmolyte does not penetrate the cell layer (saccharose), leads to a dramatically increased damping of the shear oscillation (Figure 26).

Table 6. Change of the motional resistance R and inductance L of different cell species. The parameters were obtained from the fitting of the BVD-equivalent circuit before and after mechanical removal of the confluent cell layer.^[195]

Cell species	ΔR [Ω]	ΔL [μH]
MDCK I	755 ± 36	6.7 ± 0.7
MDCK II	992 ± 36	10.5 ± 0.8
Plexus epithelia	804 ± 43	16 ± 1.5
BAEC	55 ± 12	3.1 ± 0.5
3T3-fibroblasts	277 ± 20	2.5 ± 0.5

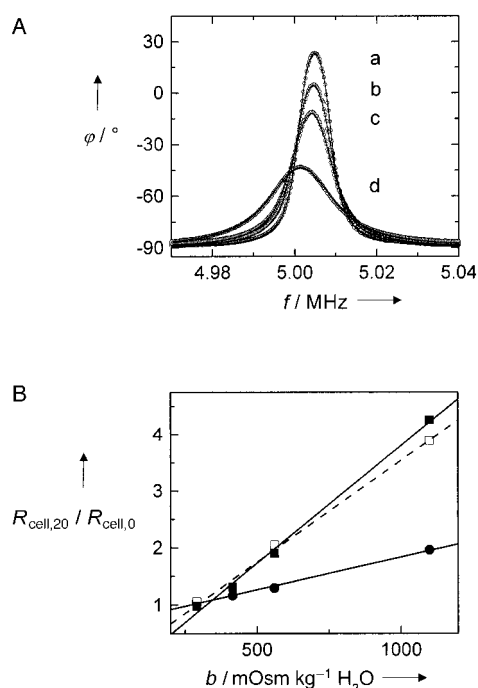


Figure 26. A) Phase spectra obtained from a quartz resonator covered with a confluent MDCK I cell layer upon sequential increase in the concentration of the osmotically active substances; a) 290, b) 415, c) 560, d) 1100 mOsm per Kg H₂O. The solid lines are the results of a fitting procedure using the BVD-equivalent circuit. B) Change of the relative motional resistance as a function of the molality of the osmotically active substances for MDCK I ($\square$), MDCK II ($\blacksquare$), and 3T3 cells ($\bullet$). The motional resistance $R_{\text{cell},20}$ was obtained 20 min after exchange of the isotonic medium for the indicated molality of the osmotically active substances, and is related to the initial resistance $R_{\text{cell},0}$. The solid lines are the results of linear regressions.^[159]

Wegener et al.^[159] demonstrated that the observed increase in damping may probably not be attributed solely to a reduction of the cell volume;^[160] instead, an osmotic-driven water flow out of the cell–substrate interspace presumably occurs, and this moves the cells more closely to the quartz surface and increases the damping.^[161]

On the basis of the previous considerations, a quantitative analysis of the impedance data should ascribe a cell-covered quartz surface as a three layer model consisting of a rigid mass (ECM), a thin liquid layer between the ECM and cell body, and a semi-infinite viscoelastic body, the cell. A detailed analysis of this multicomponent system is the subject of current investigations.

Besides from data of confluent cell monolayers obtained under static conditions, time-resolved processes, such as cell adhesion or the influence of pharmacological substances, can also be monitored using the quartz-crystal microbalance in the active or passive mode. The process of cell adhesion is rather complex and characterized by several distinct processes which influence the oscillation behavior of the quartz resonator. Starting with the first physical contact of the cell with the surface, processes such as cell spreading, that is, the enlargement of the contact area of the cell with the surface, modification of the adhesion properties, excretion of extracellular matrix components, and changes of the cell's cytoskeleton influence the oscillation in various ways. A detailed study of cell adhesion based on a time-resolved frequency analysis was performed by Wegener et al.^[162] using different cell lines (MDCK I and II cells as well as 3T3-fibroblasts). Wegener et al. confirmed the presumptions of Redepennig et al.^[155] and Matsuda et al.^[163] that the frequency decrease is correlated with the number of cell–substrate contacts. Cells that do not form contacts with the surface as a result of the presence of contact-inhibiting peptides (RGD sequences) or cell death that prevent adhesion, do not change the resonance frequency of the quartz (Figure 27).

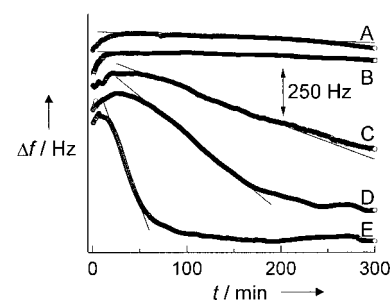


Figure 27. Time course of the resonance frequency during the adhesion of MDCK II cells starting with seeding densities of $(8 \pm 1) \times 10^5 \text{ cm}^{-2}$ in the presence of A) 1 mM GRGDS; B) 1 mM RGDS; C) 2 mM RGD; D) 1 mM RGD, and E) 1 mM SDGRG. The solid lines are linear regressions to enable the apparent adhesion rates to be determined.^[162]

If a comprehensive impedance analysis of the quartz oscillation is not possible, or a higher time-resolution than provided by impedance analysis is required, changes in the dissipation factor $D = Q^{-1}$ of the oscillation, which represents the energy dissipation upon the binding of cells to the resonator surface, can be monitored. In this way Kasemo and

co-workers were able to detect the adsorption of monkey kidney epithelial (MKE), Chinese hamster ovary (CHO) cells, and neutrophils by measuring the frequency shift of the quartz and the time-resolved energy dissipation simultaneously.^[164, 165] Otto et al.^[166] used the same technique to investigate the interaction of *E. coli* with surfaces varying in their hydrophobic behavior. These experiments showed that contact area, surface energy, ionic strength, and the cell surface influence the frequency and dissipation changes considerably.

If cell monolayers exhibit interesting barrier-forming properties as a consequence of the presence of tight junctions, the quartz-crystal microbalance can be combined with common electrochemical techniques. Such a setup allows the performance of a so-called quasi-simultaneous double-mode impedance analysis, as described by Janshoff et al.^[167] (Figure 28).

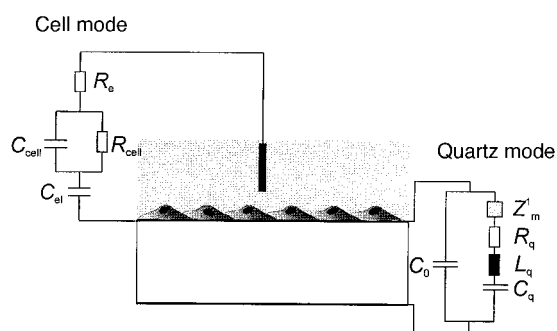


Figure 28. Schematic drawing of cells that are adhered to a quartz plate and the corresponding equivalent circuits used for the impedance spectra obtained in quartz mode and cell mode.^[167]

With this setup it is possible to detect electrical and viscoelastic properties of cell monolayers with one device. A cell monolayer can be described by a parallel RC element in series with a capacitance C_{el} , which represents the electrode–solution interface; the corresponding equivalent circuit is depicted in Figure 28. Figure 29A shows the time course of the transepithelial and motional resistance after the seeding of MDCK II cells on a quartz resonator. Damping of the shear oscillation occurs immediately after seeding, whereas the transepithelial resistance can only be detected after 15 h. The adhesion of cells, which predominately determines the damping behavior of the cells, occurs long before the formation of tight junctions. Morphological changes of the cells induced by changes of the cytoskeleton can be monitored time-resolved with this device, as demonstrated by the example of cytochalasin D (alkaloid), which inhibits the polymerization of actin filaments (Figure 29B, C). The induced rounding of the cells by the degradation of the actin filaments results in a decreased contact area of the cells with the resonator and an increase in the average distance of the cells to the substrate; as a consequence a decrease in the characteristic damping of the shear oscillation by a confluent cell monolayer occurs. Moreover, it was shown that the transepithelial resistance collapses as a result of the large morphological changes.

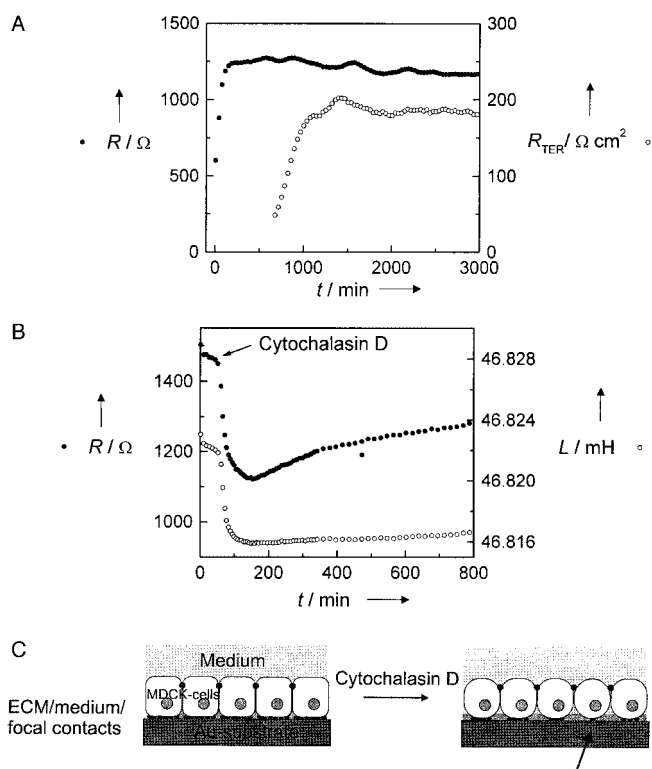


Figure 29. A) Time course of the motional resistance R (●) and transepithelial resistance R_{TER} (○) when a confluent MDCK II cell layer is formed on a 5 MHz quartz. The seeding density was $(1.5 \pm 0.2) \times 10^6$ cells per cm^2 . B) Time course of the motional resistance R (●) and the induction L (○) of a MDCK II-covered 5 MHz quartz upon addition of the alkaloid cytochalasin D with a concentration of $5 \mu\text{M}$. C) Conceivable scenario of the influence of cytochalasin D on a confluent cell layer. The arrow indicates the decreased contact area of the cells with the surface as a result of the rounding of the cell bodies.^[109]

3.7. Functionalized Vesicles as Model Systems for Cell Adhesion

As a consequence of the complexity of the cell and its contacts to the surface, it is quite difficult to address the question as to which properties of the cell mostly influence the resonance frequency shift. A simplified system to model the surface adhesion of cells in detail are receptor-doped lipid vesicles, which can interact with surface-confined ligands.^[168] Pignataro et al.^[169] scrutinized the interaction of biotinylated lipid vesicles with a streptavidin and avidin matrix. It turned out that the frequency decrease of the quartz was determined by the extent of biotinylation within the vesicles (Figure 30).

However, the frequency decrease could not solely be explained with the number of adsorbed vesicles on the surface. A complementary scanning force microscopy study demonstrated that an increase in the degree of biotinylation of the vesicles, that is, an increased number of biotin–streptavidin bonds, leads to a more pronounced flattening of the vesicles up to a biotin content where the vesicles disrupt and start to spread on the surface and form planar lipid bilayers. The authors drew the conclusion that the number of contacts influences the frequency response of the quartz. The connection between the contact area and frequency decrease was

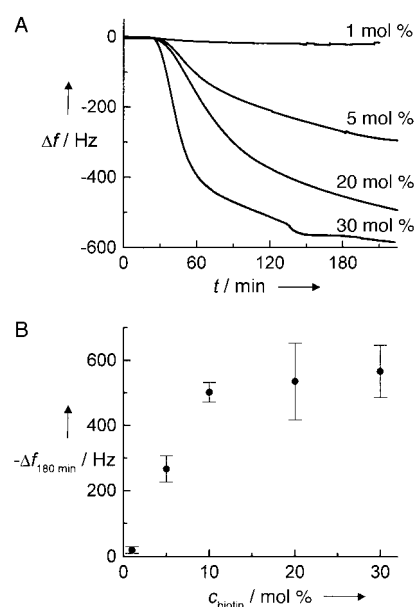


Figure 30. A) Frequency decrease of a 5 MHz quartz upon binding of vesicles doped with different amounts of biotin-X-DHPE to an avidin layer. B) Frequency shift 180 min after addition of vesicles.^[169]

also observed by Liebau et al.^[170, 171] They demonstrated that polymerized liposomes produce a significantly smaller frequency decrease than nonpolymerized ones. They explained this observation through a suppressed membrane fusion. As already demonstrated by Ohlson et al.^[172] and Janshoff et al.^[107] fusion of vesicles on the surface results in large frequency shifts of more than 230 Hz using a 5 MHz quartz. Presumably, a significant change in the surface energy might be responsible for the observed frequency shift.^[173] A more detailed explanation remains to be elucidated.

4. Development of Biosensors Based on Optical Transducers

In this section the acoustic resonators described above are compared with common state of the art optical transducers as used for bioanalytic purposes. In principle, information about the quantity of an analyte can be obtained from the characteristic properties of light, such as frequency, amplitude, phase, or polarization. In the area of biosensor development several label-free optical methods, such as surface plasmon resonance spectroscopy (SPR),^[174–176] grating coupler, reflectance interference spectroscopy (RIFS),^[177] and ellipsometry have been established.^[178] These techniques all detect the refractive index n and the film thickness d or the effective optical thickness (nd). The general principles of different optical techniques are depicted in Figure 31.

The specificity of those sensors is achieved by surface-immobilized receptive layers. The process of functionalization has to be adapted to the properties of the sensor surface. Thus, thiol chemistry is suitable for SPR and ellipsometry while silane chemistry is advantageous for glass surfaces (grating coupler, interferometry). In the following sections different techniques with significant commercial impact, such as SPR,

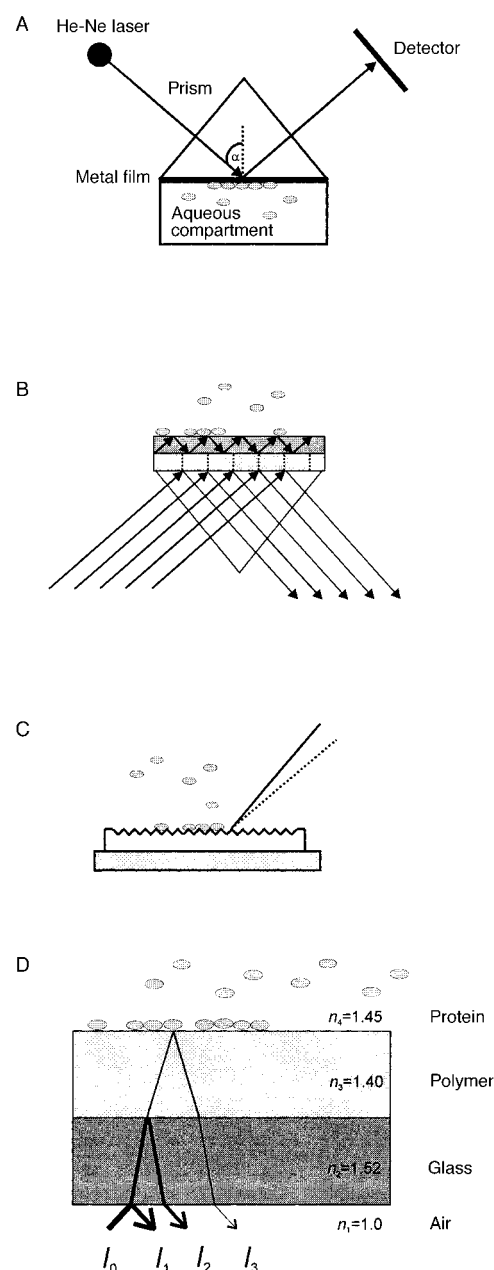


Figure 31. Schematic representation of the device principles of different optical label-free techniques. A) Surface plasmon resonance spectroscopy (SPR); B) resonance mirror (RM); C) grating coupler, and d) reflectometric interferometric spectroscopy (RIFS).

grating couplers, the resonance mirror technique, and interferometry (RIFS), are explained in more detail and different parameters, such as sensitivity, detection range, and their limitations, are discussed and compared to acoustic resonators.

4.1. Grating Coupler and Resonance Mirror Technique

Evanescent field techniques are based on multiple reflections at the interface between a substrate and a thin film ($d < 1 \mu\text{m}$) immobilized on a surface composed of biological receptive layers that show a lower refractive index than the

supporting substrate. The detection limit is considerably lowered when the system is equipped with an optical waveguide with multiple reflections since the relative propagation velocity of the guided wave depends not only on the refractive index of the medium but also on the number of reflections. Guidance of the wave is accomplished by an imprinted grating structure on the surface using a certain angle of incidence. Observing the intensity after the wave has traveled through the material at the end of the film is the easiest way of measurement.^[177]

However, in most cases this technique is used as a differential interferometer in which the different propagation velocities of the waves for different polarizations are considered. As *E*- and *B*-field vectors of the electromagnetic radiation are orthogonal, they will propagate with different velocity, dependent on the refractive index of the surface confined compound. The detectable phase difference, or strictly speaking its change, is a measure of the altered refractive index, for example, on the binding of an analyte to a receptive surface.

The resonance mirror (RM) technique^[177] is based on a prism coupler; the coupling conditions for two orthogonal waves of linear polarized light are met at different angles of incidence. The polarized waves experience a phase shift in the waveguide. Linear polarized light with an incident angle of 45° is used in a typical experiment. The observed ellipticity after travelling through the waveguide depends not only on the phase shift, and therefore considerably on the refractive index, but also on the adsorption layer at the interface. Thus, the ellipticity is again a direct measure of the amount of surface-bound analytes. The main applications of both methods in the field of biosensors are the detection of antigen–antibody reactions.

4.2. Surface Plasmon Resonance

Surface plasmon resonance spectroscopy (SPR) utilizes the evanescent field in the same fashion as the resonance mirror technique for the determination of the refractive index close to the sensor surface. Surface plasmons are longitudinal electron-density oscillations at the interface of a metal and a dielectric medium, for example. Surface plasmon resonance occurs by optical excitation only if the wave of the incoming light interacts with the free electrons of the metal and if the energy and momentum of the incident light beam correspond to those of the surface plasmons. The dispersion relation between frequency ω and wave number k of the plasmons at the interface [Eq. (18) ϵ_m and ϵ_d are the dielectric constants of the metal film and the dielectric medium, respectively] is decisive for excitation.

$$k = \frac{\omega}{c} \sqrt{\frac{\epsilon_d \epsilon_m}{\epsilon_d + \epsilon_m}} \quad (18)$$

Excitation is accomplished by attenuated total reflection, with the compound under investigation being immobilized on a glass prism covered with an evaporated metal film. In this setup, plasmons are excited by an evanescent electromagnetic field which induces oscillation of the free electrons in the

metal. Only p-polarized light is capable of exciting surface plasmons. However, these plasmons generate another electromagnetic field, which penetrates the dielectric medium closely attached to the metal surface. Only at a certain angle of incidence do the wave numbers of radiation and surface plasmons match, which leads to a resonance phenomenon in which the energy of photons is transferred to plasmons. Plasmon resonance can be observed as a sharp minimum in the reflectivity of the incident light beam. The angle of incidence of monochromatic light is varied during an experiment, and the reflectivity near the resonance monitored. As the wavelength is constant, the angle of the resonance minimum only depends on the dielectric constant of the medium covering the metal layer and is thus a measure of the concentration of an analyte adsorbed on the functionalized metal surface.

SPR can be used as a spectroscopic as well as a microscopic technique, which leads to an increasing number of applications of this optical method. Thus, SPR is used not only for studying classical ligand–receptor interactions in biochemistry but also for the detection of binding events at lipid membranes, similar to the quartz crystal microbalance technique. Special methods are required to modify these surfaces, which are essentially based on the fusion of liposomes onto hydrophobic surfaces. The interested reader is referred to the review articles of Salamon et al.^[175, 176] The high sensitivity at a distance up to 100 nm away from the gold surface allows the imaging of focal contacts of adherent cells by SPR microscopy.^[180]

4.3. Reflectometric Interference Spectroscopy

While both label-free methods discussed above detect changes in the refractive index upon analyte binding using an evanescent field, the reflectometric interference spectroscopy directly determines the effective thickness and thus the surface concentration of the analyte. This bound analyte layer exhibits a thickness of up to 10 nm in the case of biomolecules, but only 0.1 nm for low molecular weight compounds.^[177] The physical principle of this method is a wavelength modulation of the reflectivity of a thin transparent film (Figure 31 D). When a film is illuminated with white light through a substrate it is reflected at both interfaces of the film. If monochromatic light is used, either constructive or destructive interference is observed, dependent on the phase shift; the interference obtained using white light varies upon the wavelength, so that a periodic modulation of the reflecting light intensity—an interference spectrum—results. Thus, the positions of the maxima and minima only depend on the film thickness at a given refractive index and angle of incidence. The constructive interference obtained by illumination along an axis coincident with the surface normal is given by Equation (19), in which m is the order of the maxima.

$$2nd = m\lambda \quad (19)$$

An increase in the thickness of the transparent layer results in a shift of the spectrum to larger wavelengths and is strictly valid only for transparent films with a thickness in the range of

the wavelength of the incoming light. This is not true for high molecular weight biomolecules and low molecular weight analytes. Nevertheless, a simple experimental trick enables one to obtain defined interference spectra, for example, a glass substrate is covered with a thick interference layer of a polymer material such as gels or oxides (SiO_2). This layer, which exhibits an interference pattern, is then functionalized by receptor molecules. As a consequence, a change in the thickness because of the adsorption of the complementary binding partner can be measured. The thickness change is far below the wavelength of radiation. This method allows measurements of thickness changes down to 1 pm resolution in effective optical thickness. Gauglitz and co-workers used this technique to quantify antigen–antibody reactions as an example of high molecular weight analytes as well as the binding of pesticides (triazine) as an example for low molecular weight analytes. They immobilized anti-pesticide antibodies on an interference layer and determined the thickness change, which was partially below 1 pm.

In special cases porous surfaces obtained from *p*-type silicon are used as an alternative to polymer films.^[181] These thin meso- to macroporous films serve not only for the generation of interference maxima and minima but also provide a signal enhancement arising from the large increase in the surface area within the porous matrix. The inner cavities of the pores need to be properly functionalized, which is achieved either by using trialkoxysilanes or Lewis acids and Grignard reagents to generate Si–C bonds.^[182–184] Reflectometry as well as the other optical methods can be applied to all kinds of binding problems and is an attractive alternative to common ELISA techniques, although it is less sensitive. Furthermore, reflectometry is suited to deal with thermodynamic and kinetic problems. Optical transducers as used in reflectometry reveal a high potential for detecting pharmaceutically relevant analytes.

5. Comparison of the Application of the QCM with Optical Methods

A detailed comparison of the QCM and SPR method was published in 1995 by Köblinger et al.^[185] In this work a QCM and SPR spectrometer were realized in one setup. The authors

claimed that no significant difference between both techniques could be notified in terms of sensitivity and cross reactivity. Both measurement devices are equally suited for the online detection of chemical and biological analytes and work without chemical modifications of the analyte. The essentially different measurement principles (mass density for QCM and dielectric constant for SPR), however, lead inevitably to different limitations with respect to sensitivity, signal to noise ratio, accuracy, reproducibility, regeneration of the sensor surface, suitability for different complex analyte systems, handling, and cost.

For SPR transducers, the metal film is evaporated on glass substrates, which are transparent for lasers in the visible region, or silicon chips, which are transparent in the IR region.^[174] Optical components such as simple glass fibers, ATR-prism couplers, grating couplers, or integrated layer systems can be used as optical waveguides. The cost of such a setup is about two times higher than that of a QCM, which comprises a frequency counter, voltage supply, and an oscillator circuit. Table 7 summarizes typical properties of the different measuring devices.

The comparison of Köblinger et al.^[185] basically demonstrates that both techniques are equally suited for bioanalytics. Affinity measurements can be satisfactorily performed with both devices (Figure 32).

The great advantage of SPR over QCM is its small sensor area ($5 \times 10^{-3} \text{ mm}^2$ in comparison to 5 mm^2 for QCM) and the higher sensitivity, namely, a greater number of particles is detectable on the effective sensor surface (10^{-17} mol for SPR and 10^{-14} mol for QCM). The quartz-crystal microbalance is, however, more suited for the determination of material properties such as the viscoelasticity of polymer films.

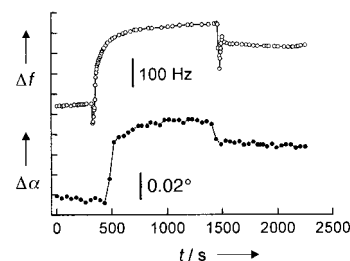


Figure 32. Change in the resonance frequency (○) and SPR angle (●) upon nonspecific adsorption of bovine serum albumin to a gold surface.^[185]

Table 7. Comparison of surface plasmon resonance spectroscopy (SPR) with the quartz-crystal microbalance (QCM; 20 MHz quartz) on the basis of their special properties.^[185]

	QCM	SPR
wave equation	Christoffel equation	Maxwell equation
wave type	elastic shear waves	surface plasmons
basic physical quantities	mechanical impedance Z	reflectivity
measuring quantities	frequency, quality factor, impedance	angle of reflectivity minimum
material parameter	film thickness, density, viscosity, surface tension, viscoelasticity, ionic strength	dielectric constant, film thickness
penetration depth in water	126 nm	150 nm ($\lambda = 1300 \text{ nm}$)
influence of ionic strength	can be neglected	large
sensitive area	5 mm^2	0.005 mm^2
detection limit	10^{-14} mol	10^{-17} mol
thickness sensitivity/protein film	184 Hz nm^{-1}	$0.0263^\circ \text{ nm}^{-1}$
minimal significant change of the measuring quantity	20–25 Hz	0.005°
minimal detectable mass	30 ng per 5 mm^2	$0.5\text{--}5 \text{ ng per } 5 \times 10^{-3} \text{ mm}^2$
sensitivity calibration	electrolysis	–

6. Summary and Outlook

In the last few years the quartz-crystal microbalance has developed from a pure mass sensor in the gas phase into a versatile tool in chemo- and bioanalytics which not only provides information about binding events at surfaces, but also reveals material-specific quantities, such as elasticity moduli, surface charge densities, and viscosity. Indirectly, one can also deduce conformational changes of proteins as well as the water content and net charge of biomolecules from the data. Potential applications are quantification of cell adhesion and the determination of viscoelastic properties of adherent cells. It is expected that TSM resonators will become to be a real alternative to conventional cell-specific techniques, such as cell counting and optical microscopy, to control cell culture. Many active substances directly influence cell adhesion. Hence, the quartz-crystal microbalance is a fast and sensitive device providing information about cell–substrate interactions, the alteration of the number of focal contacts, or the distance of the cell from the substrate. Moreover, the possibility of combining the quartz-crystal microbalance with other techniques, for example, optical (SPR) or electro-mechanical techniques (EQCM), will increase the knowledge about processes at interfaces in biochemistry as they occur at cell surfaces. In this way double mode impedance analysis allowed alterations of the barrier properties and cell substrate interactions of cell monolayers to be correlated in a time-resolved fashion and quasi-simultaneously. While the electrochemical QCM is already routinely used in electrochemistry for monitoring deposition and corrosion processes, a combination of voltammetry, chronoamperometry, and impedance analysis with QCM is rarely used in bioanalytics.

In summary, the detection limit of the quartz-crystal microbalance is poorer than that of optical methods such as SPR or RIfS, which is partially a result of the larger sensor surface area, and partially a result of the inherent sensitivity. In most cases, a lower sensitivity does not hinder the investigation of biological problems since equilibrium thermodynamic and kinetic data cannot be obtained beyond picomolar concentrations as a consequence of the limited volume. A continuous depletion in solution would result—this is also true for surface plasmon resonance spectroscopy, though SPR spectroscopy needs a considerably smaller detection area, since the sensor area covered with adsorbed molecules is in general larger than the detected one. As far as the QCM is concerned miniaturization is naturally limited, which plays an important role with respect to the desired automation because of the need for small reaction chambers. The power of piezoelectric sensors is definitely the vast quantity of available information that can be obtained and the reliable determination of thermodynamic and kinetic data. The method allows the use of almost any material from metals to dielectric surfaces. In order to achieve lower detection limits for minor component analysis it is necessary to use piezoelectric sensors with higher mass sensitivity, such as APM and FPW sensors, though these methods need to be further developed.

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- [13] In addition, in the case of small resonators with large thickness, the resonance frequency depends considerably on the lateral dimensions of the circular-shaped plate as apparent from the second term.
- [14] An AT-cut quartz crystal exhibits thickness excitation (TE) and lateral field excitation (LFE); the first one generates transversal the last one longitudinal waves.
- [15] Notably, Equation (2) describes basically the serial resonance ($Z \rightarrow 0$). The difference between the antiresonance ($Z \rightarrow \infty$) and serial resonance is manifested in the electroacoustic constant k_i : $\omega_a^2 - \omega_s^2 = 8k_i(\bar{\epsilon}_{66}/\rho_q d_q^2)$.
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New Developments in the Origins of the Homochirality of Biologically Relevant Molecules**

Helmut Buschmann, Richard Thede, and Detlef Heller*

In numerous review articles models have been compiled to explain why the essential molecular building blocks for life, proteinogenic amino acids and the ribose or deoxyribose units in nucleic acids, both have the same chiral sense.^[1] Competing autocatalysis, asymmetric adsorption, and the symmetry fractures during crystallization belong to the “abiotic” explanations, which describe the occurrence of homochirality in biomolecules as accidental. The basic concept of the alternative “determined mechanism” is an external physical interaction, which is able to produce an enantiomerically enhanced product from an optically inactive starting material, or which utilizes chirality already intrinsic to the molecule. Typical examples of this are photochemistry with circularly polarized light—either as asymmetric destruction, as photo deracemization, or as asymmetric synthesis—and the energy differences between molecules which can usually be regarded as enantiomers. The latter originates from the parity violation of the electroweak interaction. Herein we will present new results on the energy difference between enantiomers and on asymmetric autocatalysis as an amplification model.

Since the prediction and the experimental confirmation that the weak interaction (responsible for the β -decay of atomic nuclei) is, in contrast to the other fundamental physical forces (gravitation, electromagnetism, and the strong interaction), parity violating,^[2] the intrinsic “handedness” of certain elementary particles has been known.^[3] In the universe presently accessible there are only left-handed neutrinos and only right-handed anti-neutrinos. A unified discussion of the electromagnetic and the weak interaction leads to the parity violating electroweak interaction. The

resulting z force interacts between the electrons and the atomic nucleus and is able to differentiate between right and left because of its parity violating character. Quantum mechanical calculations which consider the z force show that an energy difference between enantiomers exists. This energy difference is called *parity violating energy difference* (PVED).^[4] However, so far it has not been possible to measure the PVED values of enantiomers although possible experiments have been suggested for several years.^[5]

Since the beginning of the 1980s *ab initio* calculations have been available, particularly from Mason and Tranter,^[6] that show the energy difference between enantiomers resulting from parity violation is in the order of 10^{-14} J mol⁻¹ and leads to an energetic stabilization of the L-amino acids and the D-sugar, these are the isomers that we find in nature. For peptides consisting of the achiral amino acid glycine an energetic advantage for the naturally occurring secondary structures, the α -helix as well as the β -pleated sheet, was calculated.^[6b,d] Results for ribose show how sensitive the PVED value is to conformational changes (C_2 -endo compared to C_3 -endo),^[6e] with the known consequences in the α -helix. Also noteworthy is that considerably larger stabilization-energy values are to be expected^[6i] for sulfur-substituted DNA analogues.

To the question of whether these types of energy differences, corresponding to a value of approximately 10^{-15} % *ee*, are the reason for the homochirality found in relevant biomolecules there are contradictory answers, even when assuming highly effective amplification mechanisms. While there are estimations that a period of 10 000 years should be sufficient for enantiomeric purity to be reached in a lake of 4 million m³ by an autocatalytic process (Kondepudi–Nelson scenario)^[7], many other authors, however, dismiss this process as being too inefficient.

Recently published *ab initio* results from Quack et al. showed for the first time, that with improved methods PVED values could be obtained which are one order of magnitude larger than previously thought.^[8] Results, from another research group, concerning biologically relevant molecules such as alanine, valine, serine, aspartate, and glyceraldehyde, confirm larger PVED values between enantiomers and again indicate an energetic preference for the L-amino acids and the D-sugar.^[9] However, it must be pointed out that for the example of alanine Quack et al. and also Schwerdtfeger et al.

[*] Priv.-Doz. Dr. D. Heller
Institut für Organische Katalyseforschung
an der Universität Rostock e.V.
Buchbinderstrasse 5/6, 18055 Rostock (Germany)
Fax: (+49) 381-4669324
E-mail: detlef.heller@ifok.uni-rostock.de

Dr. H. Buschmann
Grünenthal GmbH, Aachen, Forschungszentrum
Zieglerstrasse 6, 52078 Aachen (Germany)

Priv.-Doz. Dr. R. Thede
Institut für Chemie und Biochemie der E.-M.-Arndt-Universität
Greifswald
Soldtmannstrasse 23, 17487 Greifswald (Germany)

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have questioned the energetic preference for the L-enantiomer.^[8b,e] Besides the differences in calculation techniques applied, which lead to systematic deviations extensively discussed in ref [8d], the various possible conformations of biologically relevant molecules complicate the unequivocal interpretation of results. Interestingly, the results for glyceraldehyde in the hydrated form always lead to an energetic stabilization of the D-enantiomer, regardless of the torsion angles (PVED values between $0.5\text{--}2.6 \times 10^{-13} \text{ J mol}^{-1}$).^[9a,b]

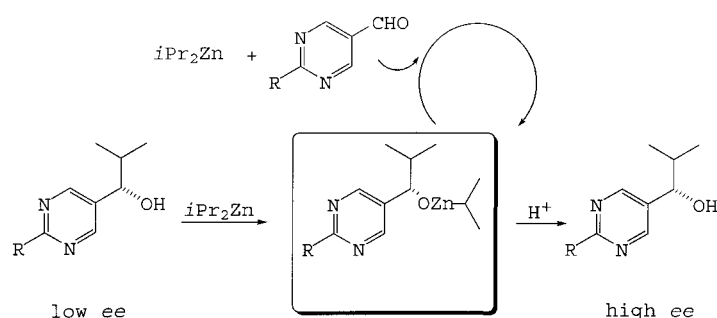
In spite of the unanswered questions, it remains undisputed that the PVED values calculated with the improved methods are clearly larger than those previously accepted. Today it seems increasingly more likely that the cause for the homochirality of biologically relevant molecules can be assigned to the intrinsic chirality present at the elementary particle level. The higher PVED values also lead to an increase of the characteristic critical temperature in the model suggested by Salam which describes the development of homochirality of amino acids on the basis of the Bose–Einstein condensation.^[10]

Recent reports concerning the possible demonstration of parity violation during the crystallization of racemates of tris(ethylenediamine)cobalt(III) and analogues of iridium complexes are described in ref [11].

An essential element in the chain of logic to relevant homochiral biological molecules is (after successful symmetry fractures) the amplification of the very small, according to most models, enantiomeric excess. Nonlinear effects in asymmetric synthesis, the result of which is diastereomeric interactions, offer an interesting potential to considerably increase the small enantiomeric excess of a catalyst. This topic was reviewed very recently by Kagan et al.^[12] Analogous amplification phenomena also result when optically enriched initial materials react (“meso-effect”).^[13]

A process is described as autocatalytic^[14] if the product and the catalyst are identical. In an asymmetric synthesis the chiral auxiliary is normally structurally different from the chiral target material. The advantages of an asymmetric autocatalytic reaction are clear; the chiral product and the chiral catalyst do not have to be separated, they are identical. In addition no chiral source, other than the product, is necessary. The kinetics of asymmetric autocatalysis were formally described in 1953 by Frank as a model for the generation of homochirality.^[15] Assuming the conditions of a “specific mutual antagonism” for the enantiomers formed autocatalytically, the system is in an unstable racemic state. A slight disturbance of the racemic composition—for example by a statistical fluctuation or because of the energy difference between the enantiomers^[7]—moves the system into one of the enantiomerically pure states.^[16]

An autocatalysis that amplifies the enantioselectivity was first reported by Soai et al (Scheme 1).^[17] The alkylzinc complex reacts first with the optically enriched chiral alcohol. The optically active isopropylzinc alkoxide formed in situ clearly catalyzes its own formation by an enantioselective addition of $i\text{Pr}_2\text{Zn}$ to the aldehyde. Subsequent hydrolysis leads finally to the initial chiral alcohol, but with an increased enantiomeric excess.



Scheme 1. Amplifying asymmetric autocatalysis according to Soai et al.^[17a,b] the asymmetric autocatalyst is shown in the middle.

Today these first results do not seem overwhelming, but they could be enhanced effectively by the consequent optimization of the system to ideality.^[18] Using, for example, 1-(3,3-dimethyl-1-butyl-5-pyrimidyl)-2-methyl-1-propanol (R in Scheme 1: $\text{Me}_3\text{C}-\text{C}=\text{C}$) leads under optimal conditions to yields of $>99\%$ and to enantioselectivities of $>99.5\%$ ee. When the alcohol formed is used as starting material in a subsequent cycle an amplification factor of 6×10^7 is achieved!^[18c]

Other “chiral initiators” can also be used, however, it is no longer an asymmetric autocatalysis in the strictest sense. Thus the conversion of the achiral compound 2-methylpyrimidine-5-carbaldehyde (R in Scheme 1: Me) with $i\text{Pr}_2\text{Zn}$ in the presence of L-leucine with an enantiomeric excess of only 2% (molar ratio: 1.0:2.4:0.02) produces the respective 1-propanol derivative in a yield of 82% and with an ee-value of 21%.^[19] The alcohol produced can be “replicated” by an amplifying autocatalysis as described above. Even enantiomeric excesses as small as 0.1% in a chiral initiator ((S)-2-butanol) yield positive results. Racemates, naturally, only produce the racemic pyrimidyl alcohol.

Recently, enantiomorphic inorganic crystals,^[20] (d)- and (l)-quartz (see Figure 1),^[21a] and (d)- and (l)- NaClO_3 ,^[21b]—were used very successfully as initiators for the first time by Soai et al. Yields $>90\%$ and enantioselectivities ranging from 93–98% ee could be attained in the known (3,3-dimethyl-1-butyl)pyrimidine-5-carbaldehyde/ $i\text{Pr}_2\text{Zn}$ system by adding portions of the components in the presence of enantiomorphic

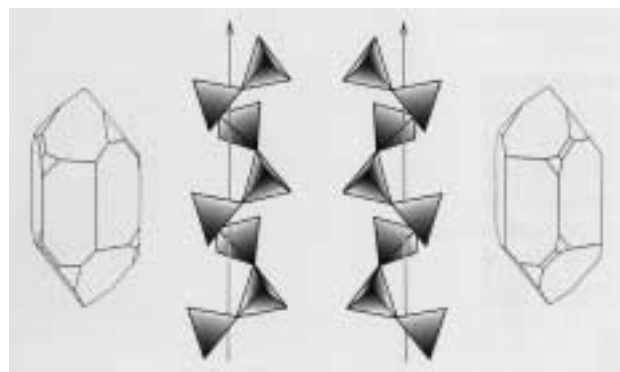


Figure 1. Macroscopic (outer) and microscopic (inner) views of the two enantiomers of quartz, (l)-quartz (left) and (d)-quartz (right). Reprinted from *Rechts oder links In der Natur und anderswo*, Wiley-VCH, Weinheim, 1999, p. 170.

quartz or NaClO₃ crystals. The chirality of the enantiomorphic crystals determines reproducibly the chirality of the alcohol generated. Mixtures from (*d*)- and (*l*)-quartz (80% *ee*) are employed with equal success. These results are significant in two respects. First, these findings clearly demonstrate that an amplified asymmetric synthesis has occurred and that it is comparable with the example quoted in ref [12]. Second, this result is also significant for the question concerning the origin of homochirality; a possible connection between the energy difference of enantiomers from parity violation and the naturally occurring excess of (*l*)-quartz is discussed in ref [22], but ref [23] should also be studied. Whatever the contradictory opinions might be about a global excess of one quartz enantiomer,^[24] it is acknowledged that there are local excesses of one of the quartz enantiomers. A discussion of the preliminary results^[25] anticipates that further highly interesting relationships between crystallization and parity violation can be expected in the near future.

The subject of chirality amplification, as described above, has developed rapidly during the last few years.^[26] That the newly calculated energy differences between the enantiomers of relevant biomolecules is about one order of magnitude larger than previously assumed increases the probability that the homochirality of relevant biomolecules, and consequently the origin of our existence, might be determined by an intrinsic chirality at the elementary particle level.^[27]

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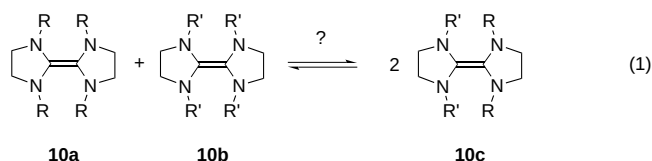
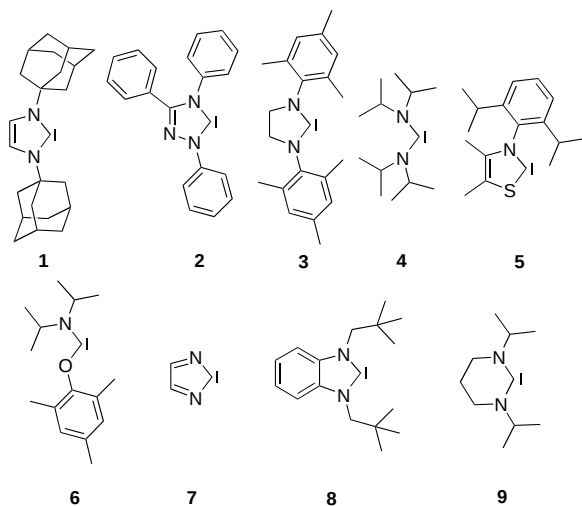
The “Wanzlick Equilibrium”**

Volker P. W. Böhm and Wolfgang A. Herrmann*

Dedicated to Professor Manfred Regitz on the occasion of his 65th birthday

Carbenes have attracted considerable attention in both organic and inorganic preparative literature due to their unique properties as reagents and reaction intermediates.^[1] The isolation of a stable carbene was pursued for a long time until 1,3-bis(1'-adamantyl)imidazolin-2-ylidene (**1**) was pre-

pared in 1991.^[2] In the following years, related carbenes, such as **2–9**, were characterized.^[3–9] Among these, imidazolidin-2-ylidenes like **3**,^[2f, 10] had been postulated by H.-W. Wanzlick in 1960 as intermediates in the formation of electron-rich tetraaminoethylenes.^[11] At that time, Wanzlick already suspected these carbenes to be rather stable, although they could only be identified by characteristic trapping reactions.^[12] Wanzlick's assumption of an equilibrium between the carbene and the olefin [Eq. (1)] was based on simple molecular weight measurements.^[11]



Before their isolation, carbenes were seen stable only as ligands in metal complexes. The first two complexes of N-heterocyclic carbenes of type **1** were isolated independently by Wanzlick in Berlin and by Öfele in Munich in 1968.^[13, 14] In the meantime, the coordination chemistry of these ligands has attracted attention: A broad scope of synthetic routes to their metal complexes was established.^[15] N-heterocyclic carbenes (NHCs) were also used as ligands in the catalytic hydrosilylation.^[16] It was shown that asymmetric induction is also possible with NHC ligands in catalysis.^[17] However, major interest in catalysts that bear NHC ligands started when applications in ruthenium-catalyzed olefin metathesis^[18–20]

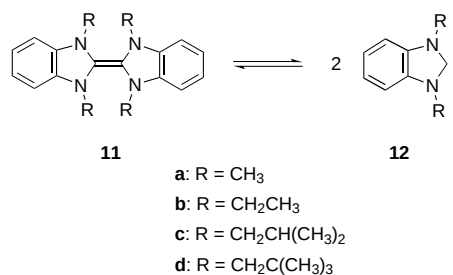
[*] Prof. Dr. W. A. Herrmann, Dipl. Chem. V. P. W. Böhm
Anorganisch-chemisches Institut
Technische Universität München
Lichtenbergstrasse 4, 85747 Garching (Germany)
Fax: (+49) 89-289-13473
E-mail: sekretariat.ac@ch.tum.de

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and in palladium-catalyzed cross-coupling reactions were discovered.^[21–23] Meanwhile, materials science also took advantage of the strong metal–ligand bond.^[24] The unique properties of the carbenes **1–9** and their metal complexes is documented by a variety of review articles about this class of compounds.^[15, 25]

Electron-rich tetraaminoethylenes are prepared by dimerization of the corresponding carbenes.^[26] Although these olefins can be used to prepare carbene complexes with electrophilic metal precursors,^[15] the equilibrium of the carbene and the tetraaminoethylene has only been speculative.^[11, 12] Early work excluded this equilibrium for NHCs of type **3**.^[27] The crossed product **10c** of two different tetraaminoethylenes **10a** and **10b** was only observable in the presence of catalytic amounts of electrophiles [Eq. (1)].^[27b, 28] Even in the absence of electrophiles, the reaction mechanism does not necessarily involve carbene intermediates, as the results could also be explained by a [2+2] cycloaddition/[2+2] cycloreversion mechanism.^[29] In contrast for NHCs of type **1**, the corresponding tetraaminoethylene products become unstable^[30] and can be observed only in bridged systems.^[31]

The equilibrium of a tetraaminoethylene and its corresponding carbene would only be proved unequivocally if both compounds were detected as a mixture while starting from the analytically pure olefin in the absence of any catalytic electrophiles. This task was solved recently by Hahn et al. and Lemal et al. Both groups submitted their results in June^[32] and July^[33] of 1999, respectively. The essential change in regard to previous experiments is the use of dibenzotetraazafulvalenes **11** (Scheme 1). Sterically less demanding substituents like methyl (**a**) and ethyl (**b**) moieties favor the formation of the tetraaminoethylene **11**, whereas sterically demanding substituents like isobutyl (**c**) and neopentyl (**d**) groups shift the equilibrium towards the free carbene **12**.



Scheme 1. Compounds used to demonstrate the “Wanzlick equilibrium”.

Recrystallized, electrophile-free samples of **11a–11c** were examined by ¹H and ¹³C NMR spectroscopy. In the case of **11c**, an equilibrium mixture of **11c:12c** = 1:9 is obtained after 24 h even at room temperature (Figure 1).^[32] In the case of **11a** and **11b**, characteristic signals of the carbenes **12a** and **12b** could be observed at 140 °C but not at room temperature.^[33] The van't Hoff plot for the dissociation equilibria of **11b** and **12b** at different temperatures reveals the thermodynamic parameters for the dissociation: $\Delta H^\circ = 13.7 \pm 0.6 \text{ kcal mol}^{-1}$ and $\Delta S^\circ = 30.4 \pm 1.7 \text{ cal mol}^{-1} \text{ K}^{-1}$.^[33]

Now, 40 years after the first experimental and theoretical treatment of “stable” *N*-heterocyclic carbenes by H.-W. Wanzlick, the last remaining important question is answered:

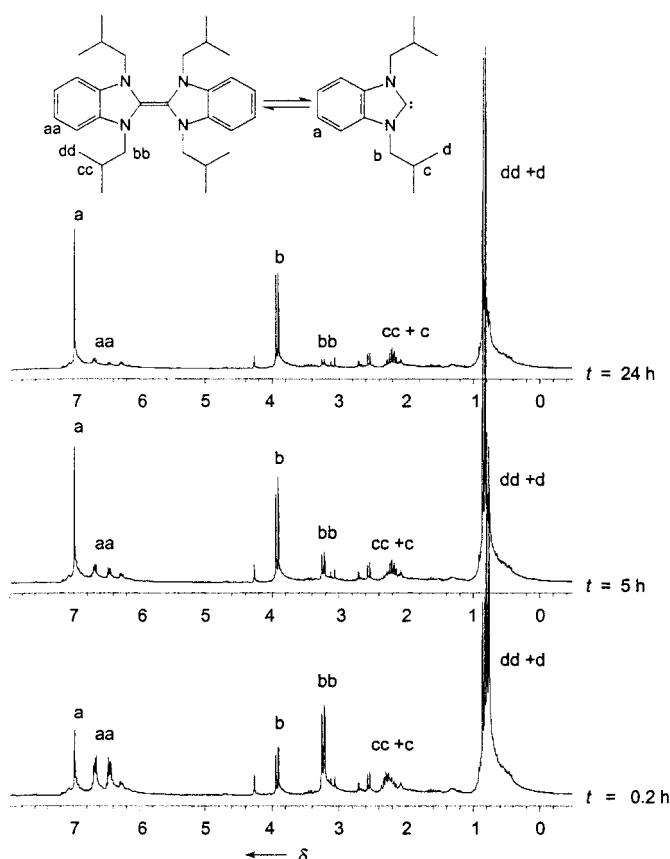


Figure 1. Equilibration of **11c** and **12c**, as observed by ¹H NMR spectroscopy (200 MHz, [D₈]-toluene, 298 K).

The simultaneous spectroscopic identification of **11** and **12** in a solution originally containing pure **11** has proved that the “Wanzlick equilibrium” between a tetraaminoethylene and its corresponding carbene exists. Along with the isolation of various stable carbenes **1–9** in previous work, Wanzlick’s ideas and reaction models have finally been proved to be reality. The “Wanzlick equilibrium” is likely to yield novel chemistry, especially in the light of recent successful results in homogeneous catalysis.^[18–23]

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Comments on the Terminology for Applications of Temporarily Attached Solubility-Modifying Moieties in Combinatorial Chemistry

Andreas Link*

In their excellent article on glycopeptide and oligosaccharide libraries published recently in this journal, St. Hilaire and Meldal carefully explain the distinction they feel is necessary between combinatorial libraries and sets of compounds prepared in parallel by methods not leading to exponentially growing numbers of chemical entities in each synthetic cycle.^[1] However, their understanding of the term “combinatorial” in this respect is much stricter than the IUPAC recommendations and therefore might not be generally acceptable.^[2]

Nevertheless, the constant struggle for precision in scientific terminology is highly desirable and the article by St. Hilaire and Meldal can be regarded as an invitation to start a discussion on terms used in the dynamic field of library synthesis that should be reconsidered.

High-throughput chemical transformation strategies used in combinatorial approaches require that products can be easily isolated from reaction mixtures without needing to resort to time-consuming work-up operations. Strategies that make use of solid supports and other temporarily attached solubility-modifying moieties have gained considerable impact in the generation of molecular libraries.^[3]

The range of solid supports currently available is already very broad and it is still growing fast. Virtually all kinds of material originating from man-made or naturally occurring resources, such as organic or inorganic polymers, as well as nonpolymeric material, have been suggested as supports in solid-phase organic synthesis (SPOS).^[4]

The most important feature that is shared by these different supports from a synthetic point of view is their ability to modify the solubility of the attached growing product in order to facilitate work-up procedures. A simple phase-separation step, such as the collection of solid material by filtration, is usually envisioned to separate support-bound molecules from excess reagents or impurities in solution. During this compulsory phase-separation step the temporarily attached solubility-modifying groups typically lead to the formation of the desired solid phase. The physical state of the support during reactions is of minor importance in SPOS and need not

necessarily be a solid phase.^[5] On the contrary, much effort has been undertaken to design supports that display functional groups in a “solutionlike” environment to circumvent the inherent difficulties that are connected with syntheses in heterogeneous phases or on-bead screening protocols.^[5]

Unlike virtually nonswelling, macroporous, highly cross-linked polystyrene (PS) or inorganic systems, for example, controlled-pore glass supports, swelling is crucial for materials such as cellulose derivatives and the frequently used standard resins, such as polystyrene with 1 % cross-linking, for their utility as supports in organic synthesis. Hence, it is generally accepted that reactions occur more or less in a gel-type phase, and not literally on a solid phase. This is true as well for polystyrene beads with polyethylene glycol (PEG) tentacles or second-generation grafted PS-PEG supports with various molecular architecture or the cross-linked PEG copolymers introduced by Renil and Meldal as well as Rademann et al., respectively.^[6]

For the reasons given above it is not advisable to concentrate on the proper physical characterization of phases during synthesis. Clearly a focus on the ability of the supports to “phase switch” as suggested by Curran is much more helpful, and the “phase-switching/phase-trafficking” terminology developed by Flynn is comprehensible and practical for this purpose, but may only be appreciated by specialists working in the field.^[7]

However, despite its shortcomings the term solid-phase organic synthesis is widely accepted and generally understood by the scientific community. This is not the case for the term liquid-phase organic synthesis (LPOS), which is meant to indicate that a soluble polymer is used as a support that can aid product isolation from liquid reaction mixtures by using membrane technologies, ultrafiltration, or, more commonly applied, by conventional filtration following precipitation. Thus, the name LPOS clearly suffers from a vague description of the underlying concept. In fact, this term neither indicates that solubility-modifying supports such as linear polystyrene, polyethylene glycol, or block copolymers are used nor that a phase separation by filtration is envisioned.

LPOS reactions take place, as most of the reactions in organic synthesis do, in a liquid solution formed by dissolving a solid, namely a polymer, in a solvent. These solutions are, of course, “liquid phases”, but this is not a special feature that justifies the preservation of this term.^[8] For example, appro-

[*] Dr. A. Link

Institut für Pharmazie der Universität Hamburg
Bundesstrasse 45, 20146 Hamburg (Germany)
Fax: (+49) 40-42838-6573
E-mail: link@uni-hamburg.de

priate cheap waxlike solid polyethylene glycol mono methyl ethers can be dissolved in a broad range of solvents, for example, toluene, and reactions on molecules attached to the hydroxyl function can be performed and monitored in solution. After a given synthetic operation, the polymer-bound molecules are precipitated by the addition of diethyl ether to the reaction mixture, and can easily be separated from the residual solution by filtration using standard laboratory equipment. Thus, the use of the soluble polymer supported synthesis rediscovered and pioneered by Janda et al. combines the advantages of synthesis in solution, such as straightforward reaction monitoring, and operative advantages brought about by the ease of phase separation of the solid formed upon precipitation.^[8d]

Recently a technique that applies small organic molecules as temporarily attached solubility-modifying groups was reported and entitled "Liquid-phase synthesis with solid-phase workup".^[9] Again, reactions take place on a temporarily dissolved solid in solution; a phase switch for work-up is achieved by precipitation upon protonation of a covalently attached heterocycle, a small molecule instead of a soluble polymer, called a "quinoline precipitation device".^[9] Consequently, the term synthesis in the "liquid phase" can no longer be regarded as the equivalent term for the commonly accepted and descriptive term "soluble polymer-supported organic synthesis (in solution)", as initially suggested and lately used side by side with the term LPOS by Janda et al.^[10] Thus, it seems practical to indicate the use of soluble polymer-supports in organic synthesis as such, that is, by the abbreviation SPOS instead of LPOS when necessary.

Given that it is not generally accepted that the terms solid-phase synthesis or immobilization should be applied in such a loose way to strategies using small molecule precipitation devices (which can not be called supports), the use of soluble organic polymers and fluorinated hydrocarbons as covalently attached solubility modifiers are covered, it might be desirable to establish a general term that may serve as a keyword for the entire heterogeneous arsenal of built-in product-separation strategies. Discussion on this topic seems highly desirable. We propose the keywords "solubility control auxiliary" for all kinds of solubility-modifying devices attached to products or reagents in combinatorial or general organic chemistry as alternatives to the term "phase labeling" suggested by Curran.^[7a]

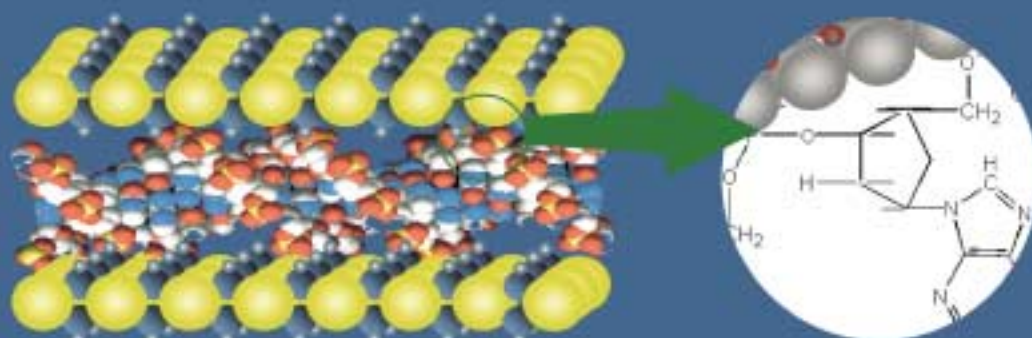
Since it is advisable to eliminate ambiguous terms before they lead to permanent misconceptions that are hard to eradicate the terms liquid-phase organic synthesis or "synthesis in the liquid phase" and the abbreviation LPOS should not be selected anymore to describe synthesis using soluble polymer supports as a solubility control auxiliary.

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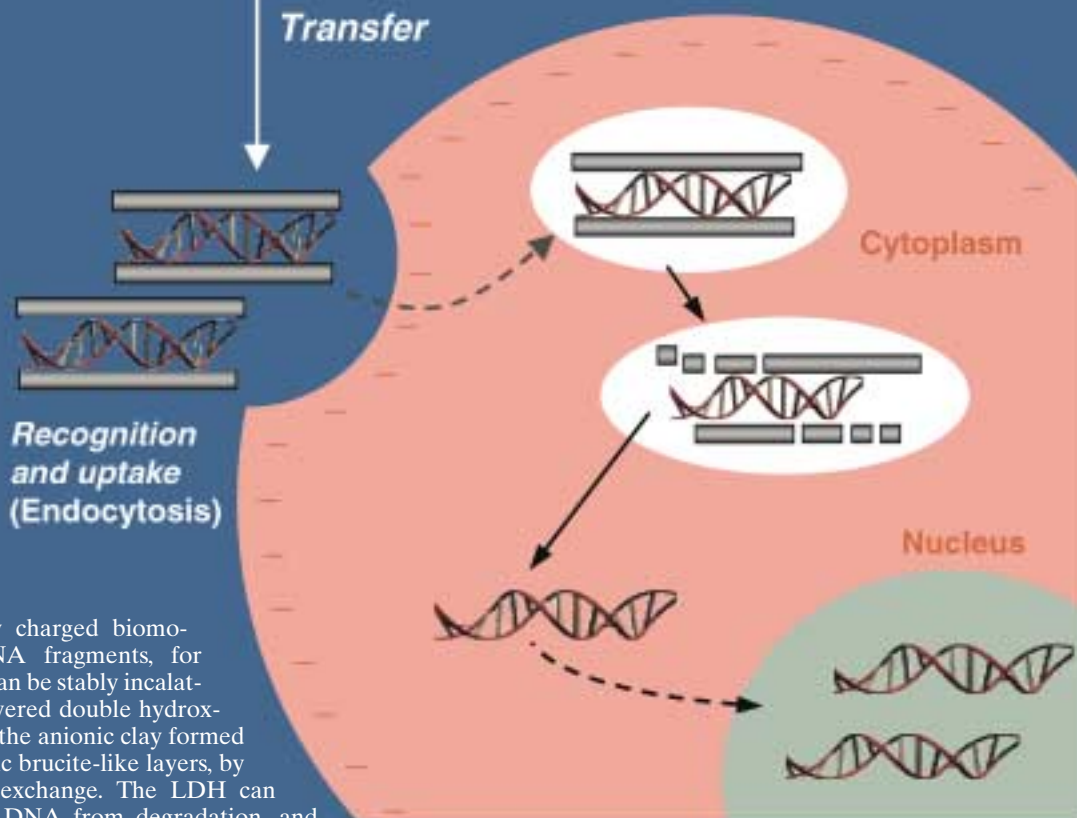
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Soluble Inorganic Vector



DNA-LDH nanohybrid

Transfer



Negatively charged biomolecules—DNA fragments, for example—can be stably intercalated into a layered double hydroxide (LDH), the anionic clay formed from cationic brucite-like layers, by simple ion exchange. The LDH can protect the DNA from degradation, and the charge neutralization enhances the transfer of the DNA-LDH hybrid into mammalian cells through endocytotic means. Once within the cells however, the slightly acidic lysosome dissolves the LDH and thereby exposes the intercalated molecule: The biomolecular-inorganic hybrid can deliver drugs or genes from the noncytotoxic carrier. Find out more on the following pages.

Inorganic Layered Double Hydroxides as Nonviral Vectors**

Jin-Ho Choy,* Seo-Young Kwak, Yong-Joo Jeong, and Jong-Sang Park

Layered double hydroxides (LDHs), the so-called anionic clays, consist of cationic brucite-like layers and exchangeable interlayer anions (Figure 1 A).^[1] The unique anion exchange capability of LDHs meets the requirement of inorganic matrices for encapsulating functional biomolecules with negative charge in aqueous media (Figure 1 B). Such biomolecules can be incorporated between hydroxide layers by a simple ion-exchange reaction to form bio-LDH nanohybrids.^[2] The negatively charged biomolecules intercalated in the gallery spaces would gain extra stabilization energy due to the electrostatic interaction between cationic brucite layers and anionic biomolecules, as depicted in Figure 1 C. Here, the hydroxide layers can play the role of a reservoir to protect intercalated DNA from DNase degradation. However, if desired, the hydroxide layers can be intentionally removed by

dissolving in an acidic media, which offers a way of recovering the encapsulated biomolecules. We have also found that the hybridization between cationic layers and anionic biomolecules would greatly enhance the transfer efficiency of biomolecules into mammalian cells or organs, as illustrated in Figure 1 D. The charge neutralization through hybridization of hybrids into cells through endocytosis,^[3] since it greatly reduces the electrostatic repulsive interaction between negatively charged cell membranes and anionic biomolecules during this process. Once bio-LDH hybrids are introduced into cells, the hydroxide layer in the bio-LDH hybrids would then be removed slowly in the lysosome, where the pH is slightly acidic (pH 4–5), because the hydroxide layers of Mg and Al dissolve in an acidic environment. At the same time, interlayer biomolecules would be partially replaced by other anions in the cell electrolyte in such a way that the encapsulated biomolecules could be released in the inside of a cell from an LDH hybrid. In this report, our primary attention was focused on verifying the possible applications of bio-LDH hybrids as gene or drug delivery carriers by a systematic experimental approach.

The first step is to prepare a two-dimensional LDH compound with an appropriate ion-exchange capacity and bio-compatibility. LDHs can be simply prepared by coprecipitation of mixed metal ions ($\text{Mg}^{2+}/\text{Al}^{3+} = 2/1$) with base (0.1N NaOH, pH ≈ 10) from aqueous solutions under nitrogen. The resulting white precipitate was washed with decarbonated water for several times, freeze-dried for chemical analysis,^[4] and then treated with each biomolecule. The pristine $\text{Mg}_{0.68}\text{Al}_{0.32}(\text{OH})_2 \cdot (\text{NO}_3^-)_{0.32} \cdot 1.2\text{H}_2\text{O}$ ($\text{Mg}_2\text{Al-NO}_3^-$ -LDH) has a well developed layer structure with the basal spacing of 8.7 Å (Figure 2 a), which corresponds to the sum of the gallery height of 3.9 Å (the thickness of ionic NO_3^-) and the thickness of brucite-like layer itself (4.8 Å). The second step is to replace interlayer nitrate ions in LDH with biomolecules or others. Upon replacement with herring testis DNA, adenosine triphosphate (ATP), fluoresceine 5-isothiocyanate (FITC), or *c-myc* antisense oligonucleotide (*As-myc*) by simple ion-exchange reaction,^[5] the basal spacings are expanded further to 23.9, 19.4, 18.8, and 17.1 Å,

respectively (Figure 2 b–e), to indicate that the biomolecules have been successfully intercalated into the hydroxide layers to form bio-LDH hybrids. The observed gallery height of 19.1 Å ($= 23.9 - 4.8$ Å) in the DNA-LDH hybrid is consistent with the thickness of a DNA molecule (≈ 20 Å)^[6] in a double helical conformation lying parallel to the basal plane of hydroxide layers. The slight discrepancy between observed and expected gallery height is surely due to the spatial confinement of DNA molecules through strong electrostatic interactions between the DNA phosphate groups and the positively charged hydroxide layers (Figure 1 C, inset).^[2] The

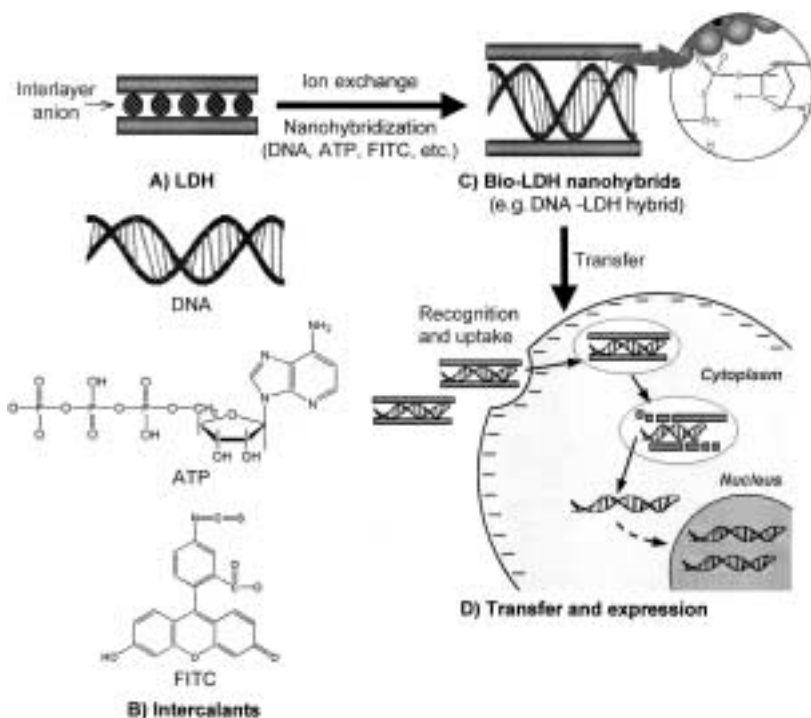


Figure 1. Schematic illustration of the hybridization and expected transfer mechanism of the bio-LDH hybrid into a cell.

[*] Prof. Dr. J.-H. Choy, Dipl.-Chem. S.-Y. Kwak, Dr. Y.-J. Jeong, Prof. J.-S. Park
National Nanohybrid Materials Laboratory (NNML)
School of Chemistry and Molecular Engineering
Seoul National University, Seoul 151-747 (Korea)
Fax: (+82)2-872-9864
E-mail: jhchoy@plaza.snu.ac.kr

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interlayer separations for the ATP-LDH and FITC-LDH hybrids (14.6 and 14.0 Å) are also in good agreement with the respective molecular dimensions of the guest species,^[7] assuming that they are oriented toward the hydroxide plane to maximize the electrostatic attractions.

We also performed cellular uptake experiments using FITC as a probe to verify the delivery potential. As shown in

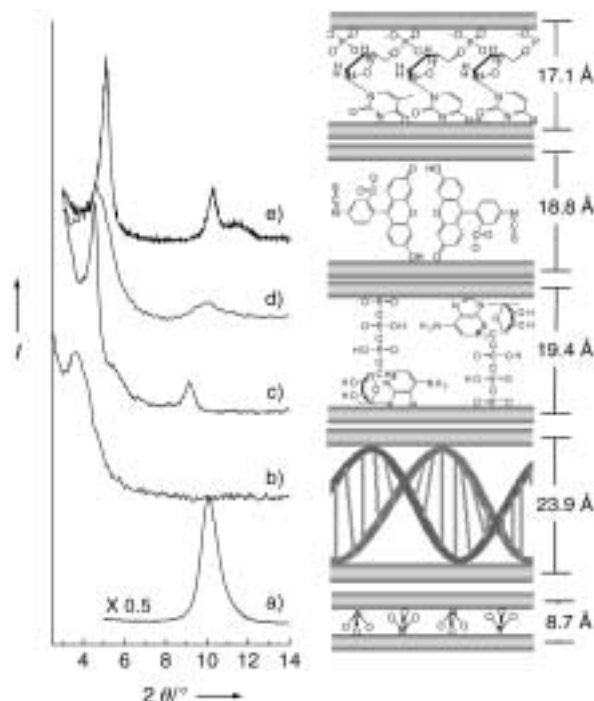


Figure 2. Powder X-ray diffraction patterns for a) pristine $\text{Mg}_2\text{Al}(\text{NO}_3)_2$ -LDH, b) DNA-LDH, c) ATP-LDH, d) FITC-LDH, and e) *As-myc*-LDH. The XRD patterns were obtained with a powder diffractometer (PW3710, Philips) equipped with Ni-filtered $\text{Cu}_{K\alpha}$ radiation ($\lambda = 1.5418 \text{ Å}$). The separation of the layers upon intercalation is shown schematically on the right.

Figure 3A, the uptake of cellular hybrid becomes more significant with increasing concentration. At the same time, the longer that the cellular incubation time is, the higher the cellular uptake rate becomes (Figure 3B). This implies that the hybrids can enter into cells effectively in both time- and dose-dependent manners. Further evidence on cellular uptake of the FITC-LDH hybrid was obtained directly from laser scanning confocal microscopy experiments.^[8] Solutions of FITC-LDH hybrids (1 and 3 μM) were added to NIH3T3 cells and then incubated for 1, 4, or 8 h. The cells were then washed with phosphate buffer solution (PBS) and fixed with formaldehyde (3.7%) prior to measurement. Figure 4 shows the cellular localization of the fluorophore obtained after a fixed incubation time. The fluorophores are detected in cells within an hour of incubation, and the fluorescence intensities continuously increase over 8 h. The fluorophores in the cells are distributed primarily in the peripheral and cytosol regions, with some in the nucleus. Moreover, as expected from Figure 3, the cells treated with 3 μM of FITC-LDH show more intense fluorescence than those treated with 1 μM (Figure 4). In contrast, the cells treated with 5 μM FITC remain dark regardless of incubation time because cells could not take up FITC itself even at high concentration. It is

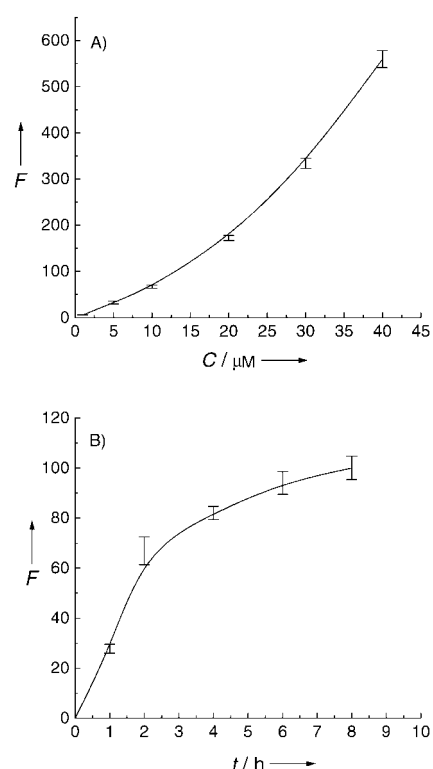


Figure 3. Cellular uptake kinetic profiles of FITC-LDH into cells at A) various concentrations of the FITC-LDH hybrid and at B) different incubation times.

obvious that LDH plays an important role in mediating the cellular uptake of FITC. All the cells can engulf the neutralized nanoparticles through phagocytosis or endocytosis. Further, FITC in the hybrid can be partially released by ion-exchange reactions, at physiological salt conditions, over 8 h and that LDHs, as encapsulating materials, dissolve gradually in a weak acidic solution. Therefore, we conclude that the intracellular fluorescence has been created by deintercalated FITC and some by FITC-LDH hybrids in the cell.

We are also able to demonstrate that antisense oligonucleotides enter into cells and participate in the process of cell division. The *As-myc* were intercalated into LDH and transferred into cells. LDHs themselves have no discernible cytotoxic effect on HL-60 cells, the human promyelocytic leukemia cell line, when administered at levels below 1000 $\mu\text{g mL}^{-1}$ for up to 4 days. The effect of *As-myc*-LDH hybrid on the growth of HL-60 cells is shown in Figure 5. The

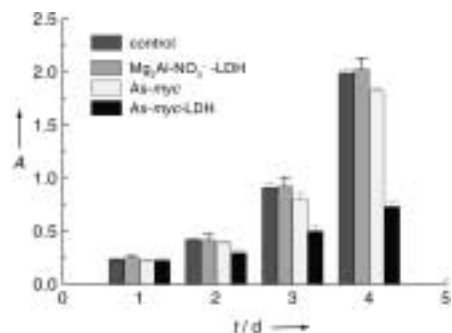


Figure 5. Effect of *As-myc* and the *As-myc*-LDH hybrids on the growth of HL-60. Each material was present at 20 μM .

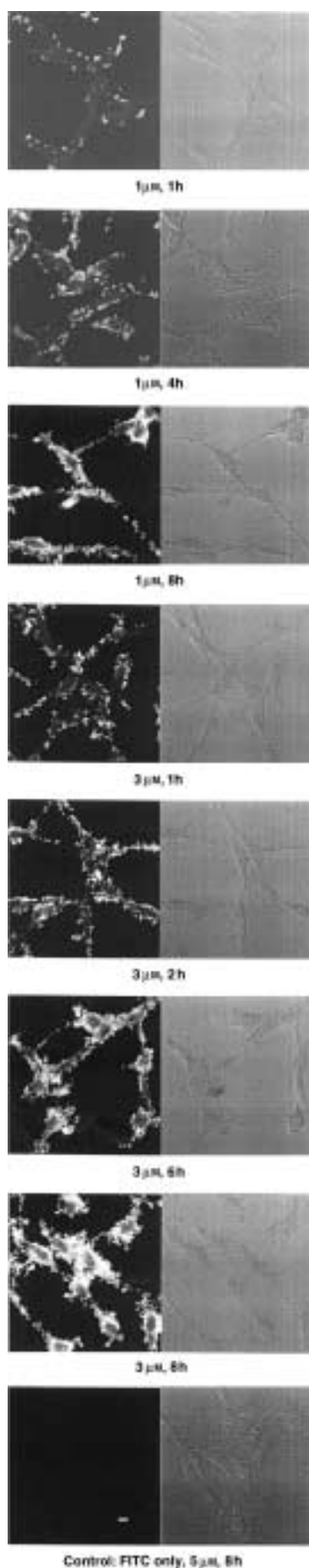


Figure 4. Laser confocal fluorescence microscopic images (left) and phase contrast micrographic images (right) of NIH3T3 cells. The scale bar is 10 μm .

cells have been exposed to As-myc-LDH hybrids at a concentration of 20 μM and incubated for 4 days (see Experimental Section and ref. [9]). During incubation, cell viability was measured daily by a MTT assay (a colorimetric assay that measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT reagent) by mitochondrial succinate dehydrogenase) and compared to a control free of As-myc. According to Figure 5, HL-60 cells in a culture treated with the As-myc-LDH hybrid exhibit time-dependent inhibition of proliferation, which indicates 65 % growth inhibition compared to the untreated cells. These results imply that As-mycs are internalized in the cells and eventually inhibit the growth of cancer cells. However, no significant inhibition effect is observed for the cells treated only with As-myc, as expected.

In conclusion, we demonstrated that inorganic supramolecules, such as the nanoscale LDHs, can act as biomolecule reservoirs and gene and drugs carriers. Cellular uptake experiments reveal that the FITC-LDH hybrid is effectively transferred into NIH3T3 cells and, when the HL-60 cells are incubated with the As-myc-LDH hybrid, a strong suppression of cancer cell growth (65 %) is observed. LDH itself is noncytotoxic towards HL-60, a type of leukemia cell. Therefore, LDHs can act as new inorganic carriers, completely different from existing nonviral vectors in terms of its chemical nature.

Experimental Section

Samples: Prior to the DNA exchange, the herring testis DNA (D-6898, Sigma) was dissolved in water (10 mg mL^{-1}). The solution was extracted by phenol and then by a phenol/chloroform (1/1) mixture. The recovered aqueous phase was passed rapidly through a 17-gauge hypodermic needle 15 times to shear DNA molecules to 500–1000 base pairs (bp). The sheared DNA was precipitated by adding ice-cold ethanol, followed by centrifugation to collect the purified DNA molecules, which were subsequently redissolved in deionized water (10 mg mL^{-1}). $\text{Mg}_2\text{Al-NO}_3$ -LDH was prepared by the aqueous coprecipitation of Mg^{2+} and Al^{3+} (0.024 and 0.012 M) with base (0.1 N NaOH) under nitrogen. During titration, the solution pH and temperature were adjusted to 10 ± 0.2 and 60°C . The resulting white precipitate was aged for 24 h, collected by centrifugation, then washed thoroughly with decarbonated water. Biomolecules (herring testis DNA, ATP, FITC) were intercalated into $\text{Mg}_2\text{Al-NO}_3$ -LDH at pH 7 for 48 h at 60°C .

Cellular uptake kinetic profiles: Various amounts of FITC-LDH were added to 8×10^4 NIH3T3 cells and incubated for 2 h. The FITC-LDH hybrid (10 mM) was added to the cells and incubated for 1, 2, 4, 6, or 8 h. After incubation, the samples were washed with PBS buffer, lysed with 1 % Triton X-100 in PBS, then incubated further at room temperature for 15 minutes. The samples were collected by scraping all areas of the plate surface. The detached cells were centrifuged to remove cell debris and to collect supernatant. The relative fluorescence intensity of the collected supernatant was measured ($\lambda_{\text{ex}} = 494$, $\lambda_{\text{em}} = 520$ nm) against a FITC-only control experiment.

Microscopy: NIH3T3 cells were grown on round coverslips in a 12-well culture plate and cultured for a day. FITC-LDH hybrids (1 and 3 mM) were added to the cells (6×10^4 per coverslip) and incubated for 1, 2, 4, 6, or 8 h. For comparison, control experiments were performed with only FITC (5 mM) itself under the same conditions. All of the samples were washed three times with the PBS buffer and fixed with formaldehyde in PBS (3.7 %). After washing again with PBS, the samples were observed with a laser scanning confocal microscope (LSM 410, Zeiss). The samples were excited by an argon laser ($\lambda = 488$ nm) and the images subject to a longpass filter (515 nm).^[8]

Cytotoxicity: HL-60 (human promyelocytic leukemia) cells were cultured in a 24-well culture plate (5×10^4 mL⁻¹). The cells were exposed to various amounts of LDH ($1-1000$ μ g mL⁻¹). A control experiment was performed without LDH under the same conditions. Cell viability was determined daily over four days by an MTT assay.^[9]

HL-60 cell growth: HL-60 cells were cultured in a 24-well culture plate (5×10^4 mL⁻¹). The cells were exposed to As-myc-LDH (20 μ M). The sequence of As-myc is 5'-d-(AACGTTGAGGGGCAT)-3' and complementary to the initiation codon and the next four codons of c-myc mRNA. Control experiments were performed with only As-myc (20 μ M) and without As-myc or As-myc-LDH complex under the same conditions. Cell viability was determined daily over four days by MTT assay.^[9]

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Epoxidation Catalysis by a Manganese Corrole and Isolation of an Oxomanganese(v) Corrole**

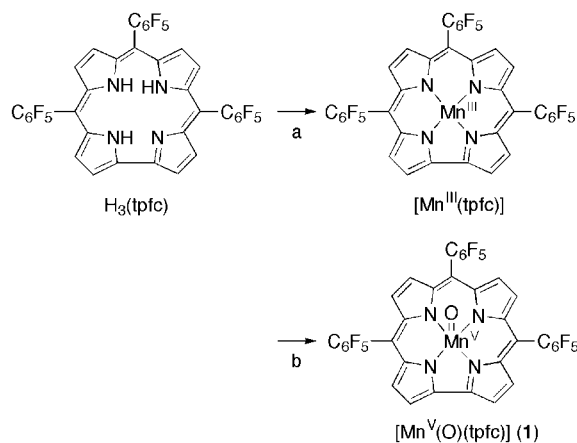
Zeev Gross,* Galina Golubkov, and Liliya Simkhovich

The milestones in metalloporphyrin-catalyzed oxygenation of hydrocarbons are the 1979 and 1980 publications of Groves, Hill, and their coworkers about the utilization of iron(III) and manganese(III) porphyrins as catalysts for the epoxidation of olefins by iodosylbenzene.^[1] Similar mechanisms were proposed in both cases, oxygen atom transfer from iodosylbenzene to the catalyst to form a *formally* oxometal(v) porphyrin

intermediate, which subsequently transfers its oxygen atom to the substrate.^[2] But, while trapping and extensive spectroscopic characterization of the intermediate in the iron porphyrin catalysis started as soon as 1981,^[3] oxomanganese(v) porphyrins remained elusive for a very long time.^[4] Only in 1999 did Jin and Groves succeed in obtaining the ¹H NMR spectrum of such a complex, the diamagnetism of which provides the strongest proof of the structure.^[5]

Recently we got involved in the chemistry of corroles; long known tetrapyrroles, whose skeleton may be viewed as either the one carbon atom short analogues of porphyrins or as the aromatic version of corrin, the ligand bound to cobalt in the coenzyme of Vitamin B₁₂.^[6, 7] We first introduced an extremely facile synthesis of 5,10,15-tris(pentafluorophenyl)corrole ($\text{H}_3(\text{tpfc})$),^[8, 9] from which a novel class of chiral macrocycles can easily be obtained.^[10] In addition, we have demonstrated that the metal complexes of $\text{H}_3(\text{tpfc})$ are potent catalysts.^[11] Specifically, the iron(IV) complex $[\text{Fe}(\text{tpfc})\text{Cl}]$ -catalyzed the oxygen-atom transfer from iodosylbenzene to both styrene and ethylbenzene. Assuming an analogy to metalloporphyrin catalysis, in which manganese complexes are equally or even more active than iron porphyrins,^[2a] we decided to investigate manganese corroles.^[12] Motivation for the present research was that the ability of corroles to stabilize high oxidation states is superior to that of porphyrins,^[13] the thermal stability of iron(IV) corroles being the most relevant example.^[14] Herein we report that the manganese(III) complex $[\text{Mn}(\text{tpfc})]$ is an interesting epoxidation catalyst and the successful isolation of an oxomanganese(v) corrole.

The metallation of $\text{H}_3(\text{tpfc})$ was achieved in high yields by reaction with excess $\text{Mn}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$ in DMF (Scheme 1).^[15]



Scheme 1. Synthesis of $[\text{Mn}^{\text{III}}(\text{tpfc})]$ and $[\text{Mn}^{\text{V}}(\text{O})(\text{tpfc})]$ (1) a) $\text{Mn}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$, DMF, reflux; b) CH_2Cl_2 , O_3 , -78°C .

[*] Prof. Dr. Z. Gross, G. Golubkov, L. Simkhovich
Department of Chemistry and
Institute of Catalysis Science and Technology
Technion – Israel Institute of Technology
Haifa 32000 (Israel)
Fax: (+972)-4-8233735
E-mail: chr10zg@tx.technion.ac.il

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Identification of the reaction product as $[\text{Mn}^{\text{III}}(\text{tpfc})]$ (corroles are trianionic ligands) is based on a combination of mass spectroscopy, NMR, and UV/Vis spectroscopy, and comparison to reported manganese corroles. Interestingly, both the green color and the UV/Vis spectrum of $[\text{Mn}(\text{tpfc})]$ (Figure 1a) are remarkably similar to that of manganese(III) porphyrins.^[16] Most relevant are the split Soret band and the

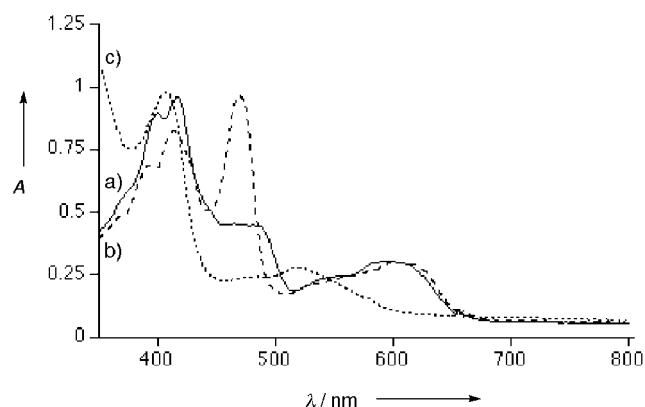
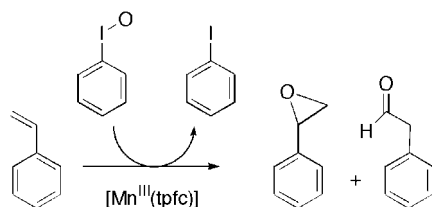


Figure 1. The electronic spectrum of a) $[\text{Mn}^{\text{III}}(\text{tpfc})]$ in CH_2Cl_2 (full line) and the changes upon addition of b) triphenylphosphane oxide (broken line), and c) iodosylbenzene (dotted line). A = absorbance.

changes in the relative intensities of the bands at 398 and 414 nm, and the band at 478 nm upon coordination of triphenylphosphane oxide (Figure 1a and b). But, the $[\text{Mn}^{\text{III}}(\text{tpfc})]$ -catalyzed epoxidation of styrene by iodosylbenzene (Scheme 2) proceeded very differently to the same



Scheme 2. The $[\text{Mn}^{\text{III}}(\text{tpfc})]$ -catalyzed epoxidation of styrene by iodosylbenzene. Reaction conditions: styrene (1.2 mmol), iodosylbenzene (0.12 mmol), catalyst (1.2 μmol), solvent (1 mL benzene or CH_2Cl_2), room temperature, 4–10 h, under Ar or aerobic. Results, in benzene, under Ar, 10 h: 77 % epoxide, 12 % aldehyde. In benzene, aerobic, 4 h, 74 % epoxide, 16 % aldehyde. In CH_2Cl_2 , under Ar, 6 h: 76 % epoxide, 22 % aldehyde. In CH_2Cl_2 , aerobic, 6 h, 80 % epoxide, 20 % aldehyde.

reaction with manganese(III), iron(III) porphyrins, or even $[\text{Fe}^{\text{IV}}(\text{tpfc})\text{Cl}]$ as catalysts. First, although the expected products—styrene oxide and phenyl acetaldehyde—were formed in high yields (Scheme 2), it took 4–10 h for completion of the reaction. More importantly, we noted an immediate color change from bright green to light red upon addition of iodosylbenzene, which persists until all the oxidant is consumed. Furthermore, the red color and the single Soret band at 412 nm (Figure 1c) are very similar to those of oxomanganese(IV) and oxomanganese(V) porphyrins.^[17]

Since we suspected that the observed species is an oxomanganese(V) corrole, we examined the low-temperature oxidation of $[\text{Mn}(\text{tpfc})]$ by spectroscopy. Instead of iodosylbenzene, which is very insoluble and also produces iodobenzene, ozone was chosen as the oxidant. The only byproduct from the reaction with ozone is the easily removable O_2 , and has been successfully utilized for the clean oxidation of iron(III) porphyrins to oxoiron(IV) porphyrin cation radicals.^[18] The large spectroscopic changes that occur upon the ozonolysis of $[\text{Mn}^{\text{III}}(\text{tpfc})]$ to $[\text{Mn}^{\text{V}}(\text{O})(\text{tpfc})]$ (**1**; Scheme 1) are shown in Figures 2 and 3.^[19] The ^{19}F NMR spectrum of

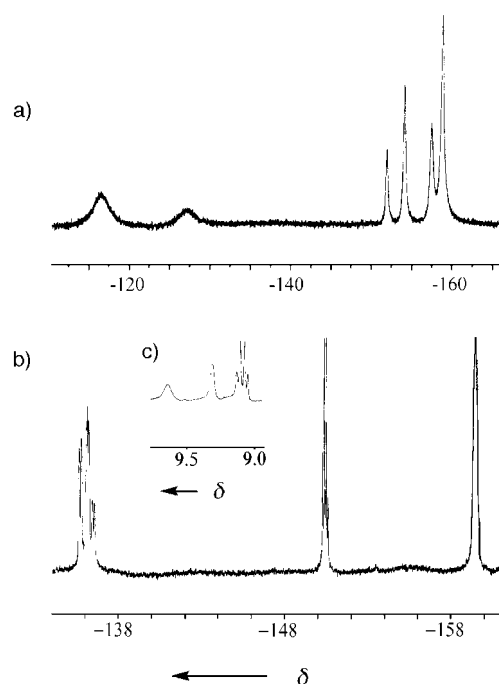


Figure 2. The $-80\text{ }^\circ\text{C}$ ^{19}F NMR spectra of a) $[\text{Mn}^{\text{III}}(\text{tpfc})]$ and b) **1**, c) the ^1H NMR spectrum of the same solution of **1**.

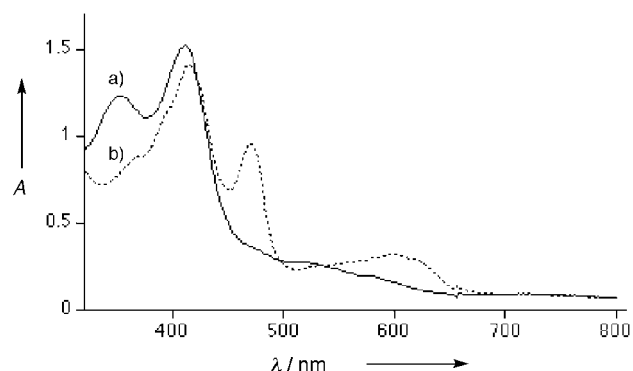


Figure 3. a) the room temperature UV/Vis spectrum of $[\text{Mn}^{\text{V}}(\text{O})(\text{tpfc})]$ (**1**) (full line) and b) after the addition of triphenylphosphane (dotted line). A = absorbance.

$[\text{Mn}^{\text{III}}(\text{tpfc})]$ displays paramagnetically broadened resonances (Figure 2a), which in the spectrum of **1** are fully converted into sharp signals containing the expected F/F coupling pattern (Figure 2b, and also Figure 2c which shows the ^1H NMR spectrum of the same solution). In addition, the color of the mixture changes from green to red, this is accompanied by large changes in the electronic spectrum (Figure 3a). Finally, the addition of excess triphenylphosphane to the ozonolysis product **1** resulted in its conversion into the triphenylphosphane oxide coordinated manganese(III) corrole $[\text{Mn}(\text{OPPh}_3)(\text{tpfc})]$, as may be appreciated by comparing Figure 3b and Figure 1b. All these results—the mass spectroscopy, the diamagnetism demonstrated in the NMR spectra, the red color, the single Soret band, and the oxidation of triphenylphosphane—lead to the safe conclusion that **1** is indeed an authentic oxomanganese(V) corrole, $[\text{Mn}^{\text{V}}(\text{O})(\text{tpfc})]$.^[20]

Importantly, the color and UV/Vis spectrum of **1** produced by ozonolysis (Figure 3a) are identical to those observed during the room-temperature catalytic reaction (Figure 1c). This observation suggests that the catalytic epoxidation of styrene proceeds by fast oxygen-atom transfer from iodosylbenzene to [Mn(tpfc)], followed by slow oxidation of the olefin by **1**. To check this postulate, we took aliquots of a concentrated solution of **1** in CH₂Cl₂ at low temperature and transferred them into a spectrophotometric cell with solvent (either benzene or CH₂Cl₂) at room temperature. Much to our surprise, the complex is stable under these conditions for many hours. Furthermore, even the addition of a large excess of styrene did not affect the spectrum (whereas **1** reacted with triphenylphosphane, see above). Accordingly, **1** can not be the reactive intermediate in the epoxidation of styrene by iodosylbenzene. A plausible explanation to account for all the observations—styrene being oxidized in the catalytic reaction, clear evidence for the formation of **1** during catalysis, and the inertness of isolated **1** toward styrene—is that a higher valent complex, formed by disproportionation of **1**, is the reactive intermediate. Supporting evidence for this proposal comes from the observation that the life time of **1** at room temperature depends very much on its concentration. In the dilute solutions used for the UV/Vis experiments (in the range of 10^{−5} M) it is stable for many hours, but it bleaches within less than one minute at higher concentrations (in the range of 10^{−3} M).

In conclusion, we have demonstrated that [Mn^{III}(tpfc)] is a mild epoxidation catalyst and an excellent precursor for preparation of an authentic oxomanganese(v) corrole, that is much more stable than corresponding porphyrin complexes. Further investigation of this and other high-valent metal corrole complexes will certainly lead to additional unique complexes, which will serve to address the puzzle of the peculiar stabilization of high oxidation states by corroles.

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- [20] The alternative formulation of **1** as an oxomanganese(IV) corrole radical, in which the d³ metal is low spin and antiferromagnetically coupled to the radical, is much more restrictive. Also, such a complex is expected to absorb in the > 700 nm region and to be green.

Structural, Electrochemical, and Photophysical Properties of Gallium(III) 5,10,15-Tris(pentafluorophenyl)corrole**

Jesper Bendix,* Ivan J. Dmochowski, Harry B. Gray,* Atif Mahammed, Liliya Simkhovich, and Zeev Gross*

We have recently reported the first facile synthetic route to *meso*-aryl substituted corroles,^[1,2] which is particularly efficient for the preparation of 5,10,15-tris(pentafluorophenyl)corrole ($H_3(tpfc)$).^[3,4] This was followed by a demonstration that the manganese, iron, and rhodium complexes of $H_3(tpfc)$ are potent catalysts for various transformations.^[5,6] In addition, we have shown that a water-soluble derivative of $H_3(tpfc)$ is superior to related porphyrins in cancer-related therapies.^[7] These intriguing developments call for an investigation of non-transition metal corroles for the following reasons: firstly, any mechanistic investigation of the catalytic action of transition metal corroles requires a working knowledge of the characteristics of corrole radicals, since during catalysis either the metal or the corrole might be oxidized. Secondly, for many potential applications it is important to know the photophysical properties of corroles, an almost completely unexplored field to date. We are only aware of investigations of corrole–corrole and porphyrin–corrole dyads, which are unstable to light.^[8] Zinc(II) complexes with porphyrins have been extensively utilized for both the preparation of cation radicals and for the examination of photophysical properties.^[9,10] Similar investigations of trianionic corroles require a trivalent non-transition metal, but until now only an unstable indium(III) corrole has been reported.^[11] According to density functional theory (DFT) calculations of corrole isomers, gallium(III) should be a perfect choice for regular corroles,^[12] but an experimental demonstration is still lacking. We have, therefore, prepared the gallium complex of $H_3(tpfc)$ and report herein its X-ray structure, electrochemistry, one-electron oxidation product, photophysical properties, and calculated electronic structure.^[13]

Using $GaCl_3$ as the metal source and pyridine (py) as solvent the synthesis of the desired complex was straightforward.

ward.^[14] Considerable effort was expended in attempts to grow X-ray quality crystals; success finally came when small amounts of pyridine were included in the recrystallization solvent mixture.^[15] The X-ray structure of $[Ga(tpfc)(py)]$ (**1**) shows that upon metal binding the corrole adopts a much more conventional geometry (Figure 1) relative to that of

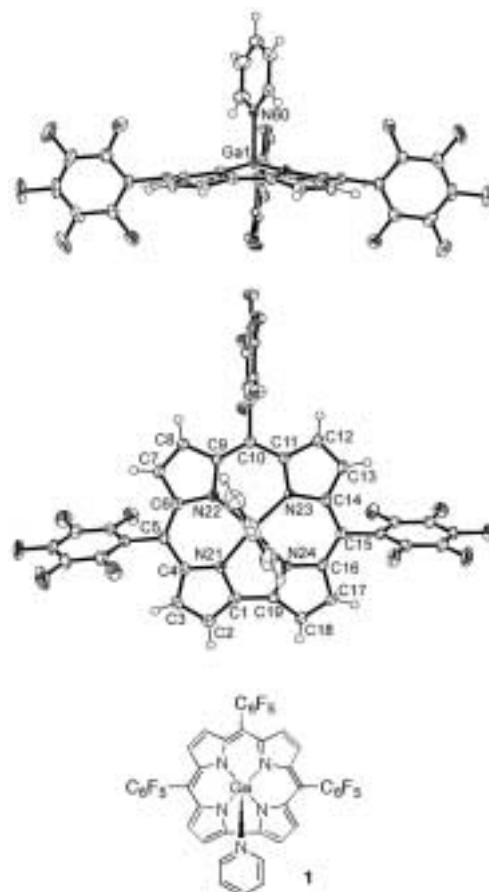


Figure 1. ORTEP plots and structural formula of **1**. Selected bond lengths [Å] and angles [°]: Ga–N21 1.9380(18), Ga–N22 1.9366(17), Ga–N23 1.9441(17), Ga–N24 1.9430(17), Ga–N60 2.0370(17), N21–Ga–N22 88.34(7), N22–Ga–N23 93.51(7), N23–Ga–N24 88.21(7), N21–Ga–N24 79.65(7), N21–Ga–N60 109.83(7), N22–Ga–N60 99.08(7), N23–Ga–N60 94.59(7), N24–Ga–N60 106.51(7). Only data for one of the two independent molecules have been given; they differ significantly only in the rotation of the pyridine ligand around the Ga–N_{py} bond.

$H_3(tpfc)$.^[3] The bonding geometry of the metal is pyramidal; the Ga center is displaced 0.4107(9) Å out of the N4 plane which is slightly domed (all the individual pyrrole rings are planar and point toward the metal). The Ga–N_{corrole} bond lengths (average 1.94 Å; Figure 1) are significantly longer than the computed values for unsubstituted gallium corrole (1.909–1.926 Å),^[13] but in the range (1.867–1.963 Å) calculated for planar $[Ga(tpfc)]$.^[16] The Ga–N_{py} bond is much longer (2.033 Å) than the Ga–N_{corrole} bonds, but, nevertheless, quite short for a gallium–pyridine bond (compare e.g. 2.121–2.151 Å in $[Ga(N_3)_3(py)_3]$).^[17] The binding of pyridine to the metal is strong enough for the high-field shifted resonances to be observed by ¹H NMR spectroscopy, but also weak enough for the ligand to be displaced by $[D_5]pyridine$.

[*] Dr. J. Bendix, Prof. Dr. Z. Gross, Dr. A. Mahammed, L. Simkhovich
Department of Chemistry and
Institute of Catalysis Science and Technology
Technion–Israel Institute of Technology
Haifa 32000 (Israel)
Fax: (+972) 4-8233735
E-mail: chr10zg@tx.technion.ac.il
Prof. Dr. H. B. Gray, Dr. I. J. Dmochowski
Beckman Institute
California Institute of Technology
Pasadena, CA 91125 (USA)
Fax: (+1) 626-449-4159
E-mail: hgcm@its.caltech.edu

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The cyclic voltammogram of **1** shows a reversible oxidation wave with an $E_{1/2}$ of 0.75 V versus SCE, much lower than the corresponding values of 1.13 and 1.20 V of the related five-coordinate complexes, [Ga(tpfc)(OH)] and [Sn(tpfc)(Cl)], respectively.^[18] This is reminiscent of similar observations in metalloporphyrins, in which the redox potential of the macrocycle correlates with the electronegativity of the metal ($\text{Ge}^{\text{IV}} \geq \text{Sn}^{\text{IV}} > \text{Ga}^{\text{III}} > \text{Zn}^{\text{II}} > \text{Mg}^{\text{II}}$).^[19] The oxidation potential of **1** suggests that it can be oxidized to the corresponding corrole π -cation radical complex, [Ga(tpfc[•])(py)]⁺ (**2**) by the commercially available compound tris(4-bromophenyl)-aminium hexachloroantimonate ($E_{1/2} = 1.11$ V). This is indeed the case; Figure 2 shows the UV/Vis spectra of **1** and **2**

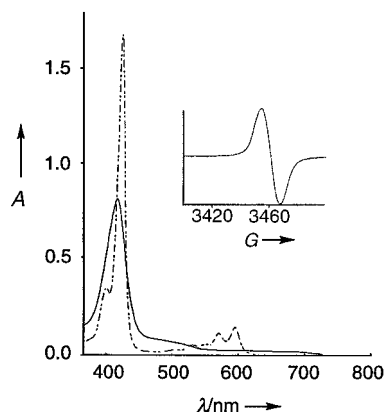


Figure 2. UV/Vis spectra of **1** (broken line) and **2** (full line), and the EPR spectrum of **2** (inset), all in CH_2Cl_2 solution at room temperature. A = Absorbance.

as well as the EPR spectrum of the latter. With respect to the color change from purple to green and the large decrease in the intensity of the Soret band, the corrole radical resembles porphyrin radicals. But, the appearance of new bands at $\lambda > 600$ nm is much less pronounced in **2** than in porphyrin radicals and there are no hyperfine splittings in the EPR spectrum from coupling to ^{14}N .

The EPR spectra of porphyrin radicals are traditionally analyzed in terms of the HOMO from which the electron is abstracted, with singlets obtained for $^2A_{1u}$ and characteristic nine-line hyperfine splitting for $^2A_{2u}$ states (under D_{4h} symmetry). To gain more insight into the electronic structure of the corrole radical, we have optimized the geometries of [Ga(tpfc)] and [Ga(tpfc[•])]⁺ (the full complexes were treated, including the pentafluorophenyl substituents) and calculated the two closely spaced HOMOs of both systems by use of gradient corrected DFT.^[16] The results for [Ga(tpfc)] are shown in a schematic presentation (Figure 3) and those for [Ga(tpfc[•])]⁺ are summarized in Table 1. Close inspection shows that the majority of spin density is located on the *meso*- and α -pyrrole-carbon atoms for the nearly degenerate $b_1(C_{2v})$ and $a_2(C_{2v})$ orbitals, respectively, thus *qualitatively* resembling the $a_{2u}(D_{4h})$ and $a_{1u}(D_{4h})$ orbitals of metalloporphyrins. But, the spin densities on the nitrogen atoms are much smaller in the corrole radical.^[20] Actually, the nitrogen splitting constants of the $^2B_1(C_{2v})$ state are calculated from the relation $a_N = 24\rho_N$ to be only 0.46–0.62 G, compared to 1.18 G for the $^2A_{2u}(D_{4h})$ state of porphyrins (they are much lower for

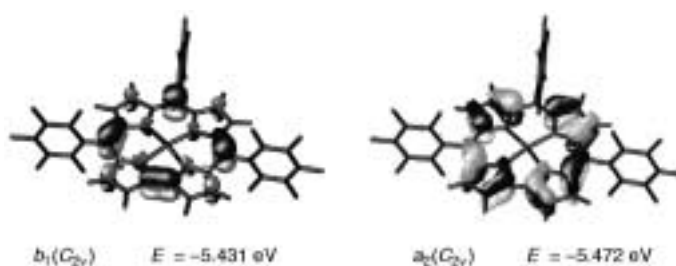


Figure 3. Perspective drawing of the calculated spin densities of the two highest occupied molecular orbitals of [Ga(tpfc)].

Table 1. Gross atomic spin populations of [Ga(tpfc[•])]⁺ (see Figure 1 for the numbering system).

Atom	$^2B_1(C_{2v})$ and	$^2A_2(C_{2v})$	Atom	$^2B_1(C_{2v})$	$^2A_2(C_{2v})$
C1 (C19)	0.098	0.063	C2 (C18)	−0.038	0.044
C5 (C15)	0.171	−0.015	C3 (C17)	0.083	−0.002
C10	0.225	−0.054	C7 (C13)	−0.007	0.050
C4 (C16)	−0.023	0.140	C8 (C12)	0.068	0.007
C6 (C14)	0.025	0.110	N21 (N24)	0.019	0.002
C9 (C11)	−0.045	0.151	N22 (N23)	0.026	−0.025
			Ga1	0.042	0.001

the $^2A_2(C_{2v})$ and $^2A_{1u}(D_{4h})$ states). Accordingly, we may conclude that the observed EPR singlets agree well with the computed electronic structure of [Ga(tpfc[•])]⁺. We also note that the $^2B_1(C_{2v})$ and $^2A_2(C_{2v})$ states of [Ga(tpfc[•])]⁺ are even closer in energy (0.041 eV) than in the (hypothetical) non-substituted gallium corrole (0.095 eV).^[13]

In the synthesis of $\text{H}_3(\text{tpfc})$, and even more so during its metallation by gallium, we noted a very strong fluorescence. Accordingly, we turned our attention to an examination of the photophysical properties of $\text{H}_3(\text{tpfc})$ and **1**, especially in comparison to the extensively studied $\text{H}_2(\text{tpp})$ and its metal complexes, $\text{Mg}(\text{tpp})$ and $\text{Zn}(\text{tpp})$.^[4, 21] These results are shown in Figure 4 and summarized in Table 2, which also includes data for various new corroles that we have recently reported.^[22, 23] The major points of interest are that the quantum

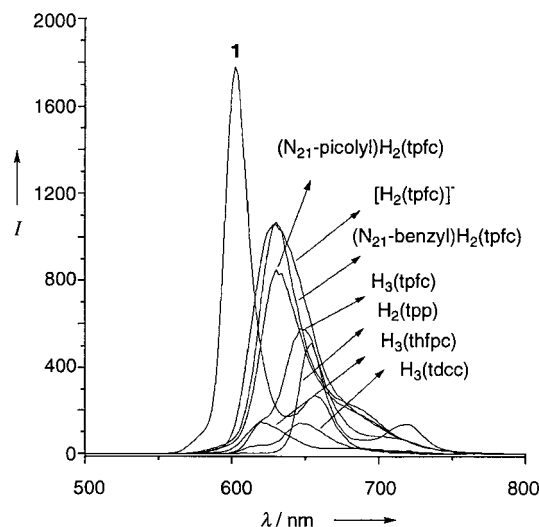


Figure 4. Emission spectra (I = emission intensity) of the porphyrin $\text{H}_2(\text{tpp})$, the corroles $\text{H}_3(\text{tpfc})$, $[\text{H}_2(\text{tpfc})]^-$, **1**, $\text{H}_3(\text{thfpc})$, $\text{H}_3(\text{tdcc})$, and the *N*-substituted corroles $(\text{N}_{21}\text{-picolyl})\text{H}_2(\text{tpfc})$, $(\text{N}_{21}\text{-benzyl})\text{H}_2(\text{tpfc})$, all in degassed toluene at room temperature (see Table 2 for the abbreviations).

Table 2. Emission data and quantum yields for several corroles and porphyrins.

Entry	Compound	Integrated intensity	Yield [%] ^[a]	λ_{max} [nm]
1	H ₂ (tpp)	18.0	11 ^[b]	654, 720
2	[Mg(tpp)]		16.5 ^[b]	
3	[Zn(tpp)]		3.3 ^[b]	
4	H ₃ (tpfc)	27.3	17	648 (622 sh, 704 sh)
5	[H ₂ (tpfc)] ^{−[c]}	56.9	35	628
6	[Ga(tpfc)(py)] (1)	51.2	31	602, 656
7	(N ₂₁ -picolyl)H ₂ (tpfc) ^[d]	41.8	26	630 (684 sh)
8	(N ₂₁ -benzyl)H ₂ (tpfc) ^[d]	49.3	30	630 (682 sh)
9	H ₃ (thfpc) ^[e]	6.33	3.9	620, 682
10	H ₃ (tdcc) ^[f]	7.09	4.3	646 (622, 704 sh)

[a] $\phi_f \times 10^2$. [b] Reference [21]. [c] A solution of H₃(tpfc) in the presence of Et₃N. [d] *N*-Substituted tris(pentafluorophenyl)corroles, see reference [22]. [e] thfpc = 5,10,15-tris(heptafluoropropyl)corrole, see reference [23]. [f] tdcc = 5,10,15-tris(2,6-dichlorophenyl)corrole, see reference [1].

yield of H₃(tpfc) (entry 4) is larger than that of H₂(tpp) (entry 1), that both deprotonation and metallation of H₃(tpfc) (entries 5 and 6, respectively) lead to an increase in the quantum yield (note the quenching effect of zinc in porphyrins, entries 3 and 1), and that *meso*-substitution by either heptafluoropropyl or dichlorophenyl (entries 9 and 10) produces a much lower quantum yield. All these features suggest that H₃(tpfc) and its metal complexes have advantages, yet to be explored, in the many applications where strong fluorescence and high quantum yields are desired. In this regard, noteworthy is that *N*-substitution of H₃(tpfc) (entries 7 and 8) does not quench the fluorescence,^[24] which indicates that H₃(tpfc) can be attached by one of its nitrogen atoms to a variety of solid supports without sacrificing useful photo-physical properties. Finally, we have also measured the lifetimes of both the excited singlet and triplet states of H₃(tpfc), which were found to be about 4 ns and 870(10) μ s, respectively, in degassed toluene.^[25] Importantly, the triplet lifetime was reduced to 4.8(3) μ s in aerobic solution, indicating an extremely high yield for the production of singlet oxygen.

In conclusion, an easily prepared gallium corrole has been fully characterized; it should serve as a prototype metallocorrole, very much as [Zn(tpp)] does in the metalloporphyrin family. Our study reports the unique photophysical properties of **1**, as well as the characteristic spectroscopic features of its π -cation radical complex and the calculated electronic structure.

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Evidence for the Formation of a Ru^{III}–Ru^{III} Bond in a Ruthenium Corrole Homodimer**

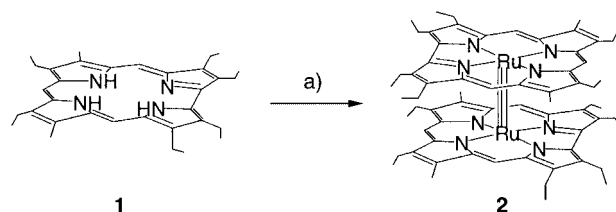
François Jérôme, Béatrice Billier, Jean-Michel Barbe, Enrique Espinosa, Slimane Dahaoui, Claude Lecomte,* and Roger Guilard*

The formation of a ruthenium(II) porphyrin homodimer was first mentioned by Whitten and co-workers in 1975.^[1] Nevertheless, the interaction between the two metal centers has been fully evidenced only in 1984 on the basis of an X-ray structure determination of $[(\text{Ru}(\text{oep}))_2]$.^[2, 3] It was shown later that this type of ruthenium porphyrin homodimer can be involved in several catalytic reactions as well as in the coordination of gaseous molecules such as ethylene and carbon monoxide.^[4, 5] Furthermore, these complexes are extremely air-sensitive and the one- or two-electron (chemical or electrochemical) oxidation of the dimer easily leads to the formation of the Ru^{II}/Ru^{III} and Ru^{III}/Ru^{III} species, respectively.^[6] However, until now, no bisruthenium(II) porphyrin homodimer has been obtained by a direct insertion of the Ru^{III} cation into the porphyrin cavity.

To study the reactivity of tetrapyrrole metal complexes in high oxidation states, we focused on the synthesis and

characterization of corrole metal complexes.^[7–9] Indeed, the corrole macroring possesses a small four-nitrogen cavity (because of the presence of only three meso carbon atoms) and three NH groups which enhance the stabilization of metal cations in a high oxidation state.^[10–12] Although as many as 18 different metal–corrole species are known, ruthenium corroles have never been synthesized. Moreover, many unsuccessful attempts have been made to metalate a corrole with ruthenium(III).^[13] Starting from RuCl₃ in dimethylformamide, Boschi et al. observed an insertion of a carbonyl group from the solvent into the pyrrole–pyrrole bond leading, after rearrangements, to a ruthenium(III) porphyrin. The same behavior was noted when using $[\text{Ru}_3(\text{CO})_{12}]$ in 2-methoxyethanol, again a carbonyl group insertion, this time from the metal carbonyl complex, occurred affording a ruthenium(III) porphyrin.^[13]

We have also attempted the metalation reaction of H₃(hedmc)^[3, 14] (**1**) with RuCl₃ in carbonyl group free solvents with different boiling points, such as pyridine, xylene, benzonitrile and trichlorobenzene. No metalation reaction was noted after 72 h at reflux. The complex $[(\text{cod})\text{RuCl}_2]_2$ ^[3] does not bear a carbonyl group and is more reactive than RuCl₃ in many metalation reactions.^[15] This Ru^{II} dimer is easily synthesized by the reaction of hydrated RuCl₃ with cod^[3] in boiling ethanol for 12 h. The reaction of **1** with an excess $[(\text{cod})\text{RuCl}_2]_2$ was carried out in refluxing 2-methoxyethanol with the presence of a trace amount of triethylamine (see Experimental Section). Triethylamine prevents the protonation of **1** by the HCl evolved during the metalation reaction. Furthermore, the reaction is easily carried out in an air atmosphere which enhances the oxidation of Ru^{II} to Ru^{III} necessary to form $[(\text{Ru}(\text{hedmc}))_2]$ (**2**, Scheme 1).^[16]



Scheme 1. The formation of $[(\text{Ru}(\text{hedmc}))_2]_2$; a) $[(\text{cod})\text{RuCl}_2]_2$, 2-methoxyethanol, triethylamine, reflux, 30 min.

By UV/Vis spectroscopy one can observe after 30 min of reaction the disappearance of the Q bands of the starting free base corrole and a large blue shift of the Soret band from 396 to 336 nm (Figure 1).

The formation of **2** is demonstrated by both the presence in the MALDI/TOF (matrix assisted laser desorption/ionization time of flight) mass spectrum of a peak pattern in the expected region (largest peak at 1186.53)^[16] and the results of a single-crystal X-ray diffraction study.^[17] No trace of a monomeric species is evidenced by mass spectrometry, thus confirming the presence of the pure, strongly bound dimer **2**. Figure 2a shows the molecular structure of **2** at $T = 112(2)$ K. In spite of the low-temperature X-ray diffraction measurements the atomic thermal parameters are quite large, resulting probably from a rotation of the dimer around the Ru–Ru axis. As

[*] Prof. Dr. R. Guilard, F. Jérôme, B. Billier, Dr. J.-M. Barbe, Dr. E. Espinosa
LIMSAG, UMR 5633, Université de Bourgogne
Faculté des Sciences "Gabriel"
6 Boulevard Gabriel, 21100 Dijon (France)
Fax: (+33)3-8039-6117
E-mail: roger.guilard@u-bourgogne.fr
Prof. Dr. C. Lecomte, Dr. S. Dahaoui
LCM3B, Université Henri Poincaré
Boulevard des Aiguillettes
BP 239, 54506 Vandœuvre-les-Nancy (France)
Fax: (+33)3-8340-6492
E-mail: lecomte@lcm3b.u-nancy.fr

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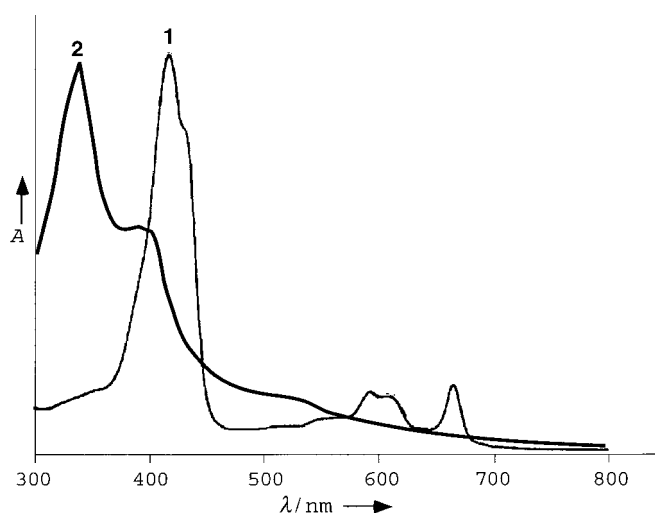


Figure 1. UV/Vis spectra of the corrole free-base **1** and the Ru dimer **2** recorded in dichloromethane.

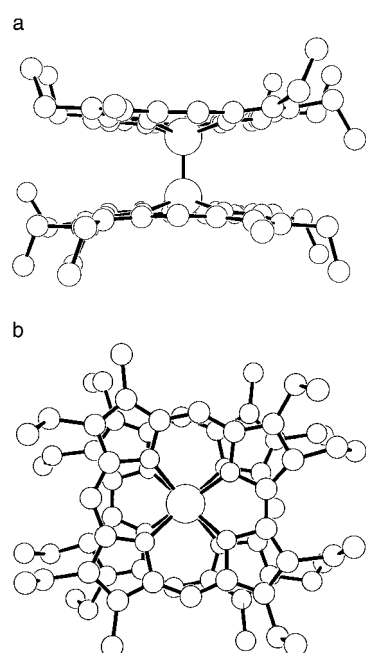


Figure 2. Structure of the ruthenium corrole homodimer **2**; a) viewed from the side, b) viewed from the top; hydrogen atoms are not shown; thermal ellipsoids are omitted for clarity (the corresponding ORTEP drawings are in the Supporting Information). Selected bond length [Å]: Ru–Ru 2.166(1).

suggested on the ORTEP view (see Supporting Information) a rotation about the Ru–Ru axis may be possible. But, as the actual thermal displacement parameter model is not totally satisfactory, a TLS+ Ω study (translation, liberation, and screw analysis of a particular group, if necessary)^[18, 19] at multiple temperatures from room temperature down to 15 K will be undertaken to clarify the dynamics of this system. On the other hand, inspection of the systematic absences leads unambiguously to the space group $P2_1/c$, indicating that only one half of the dimer constitutes the asymmetric unit. This result is in favor of a centrosymmetric C_s *anti* configuration of the ruthenium dimer. The crystal structure analysis^[17] leads to the following main features: a) a Ru–Ru separation of

2.166(1) Å, b) a planar N4 geometry (within 0.011 Å), c) a doming effect, the Ru center being displaced 0.514(4) Å out of the N4 plane, because of the strong Ru–Ru interaction and the steric repulsions (including π – π interactions) between the macrocycles.

The overall number of d electrons for the two ruthenium atoms is ten thus leading to a spin state of zero.^[6] One can reasonably assume that the local N4 geometry around the Ru center in **2** is close to that of [(p)Ru]₂²⁺.^[3] Therefore, from the qualitative MO diagram developed by Collman and Arnold the metal–metal bond order is expected to be three.^[6] These assumptions are corroborated by preliminary ESR measurements which do not show any signals in CH₂Cl₂ at 100 K. Moreover, the diamagnetic character of **2** is further confirmed by the ¹H NMR spectrum which shows sharp resonance signals, observed between δ = 1.64 to 8.78.^[16] Furthermore, the Ru–Ru bond length of 2.166(1) Å is in accordance with a triple bond and correlates with reported X-ray data.^[2, 20] Indeed, Ru–Ru bond lengths of 2.408(1) Å and 2.296(2) Å have been found for [(oep)Ru]₂ (bond order 2) and [(tpp)Ru]₂⁺^[3] (bond order 2.5), respectively.^[2, 20]

Complex **2** is the first ruthenium corrole derivative, and moreover, the first corrole complex exhibiting a metal–metal bond. Further investigations are underway to synthesize other Ru^{III}/Ru^{III} corrole dimers using the same reaction pathway. Such a procedure is applicable to other transition metals, for example, we have recently isolated a corrolatorrhodium(III) dimer.^[21] Using the “face-to-face” porphyrin–corrole bismacrocycles recently described by our group,^[9] the synthesis of heterobinuclear species should be possible.

Experimental Section

A solution of H₃(hedmc) (**1**; 100 mg, 0.2 mmol), [(cod)RuCl₂]₂ (226 mg, 0.4 mmol), and triethylamine (c.a. 100 μ L) in 2-methoxyethanol was heated under reflux for 30 min. The solvent was evaporated under vacuum, the resulting solid was dissolved in dichloromethane and the excess ruthenium complex was removed by filtration. The product was crystallized from a mixture CH₂Cl₂/CH₃OH (1/1) to give [(Ru(hedmc))₂] (**2**) in 72% yield (172.5 mg, 0.14 mmol).

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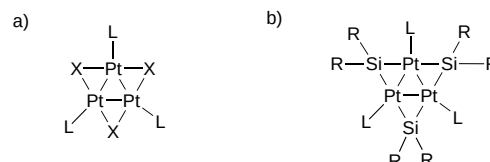
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- [17] X-ray data: C₆₆H₇₈N₈Ru₂, crystals were obtained by slow diffusion of methanol in dichloromethane; crystal dimensions 0.40 × 0.38 × 0.10 mm, monoclinic *P*2₁/*c*, *a* = 14.2080(8), *b* = 13.6160(8), *c* = 16.1907(7) Å, β = 113.322(3)°, *V* = 2876.3(3) Å³, *Z* = 2; ρ_{calcd} = 1.369 g cm⁻³, μ(Mo) = 5.73 cm⁻¹. The measurement of the reflection intensities was carried out on an Enraf-Nonius Kappa-CCD diffractometer (*T* = 112(2) K, MoK_α radiation λ = 0.71073 Å); φ and ω scans with κ offsets; 2θ_{max} = 55°; 15324 measured reflections (*R*_{int} = 0.053). The structure was solved with direct methods and refined with *F*² against all independently observed reflections (4507); heavy atoms with anisotropic, H atoms with isotropic temperature factors; *R*1 = 0.0957, *wR*2 = 0.2476 (for *I* > 2σ(*I*); total number of parameters 351, Δρ_{max} = 1.051 e Å⁻³, Δρ_{min} = -0.553 e Å⁻³. The programs used for solving and refinement of the structure were SHELXS-97 and SHELXL-97 (G. M. Sheldrick, University of Göttingen) respectively. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-141388. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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A Triangular Triplatinum Complex with Electron-Releasing SiPh₂ and PMe₃ Ligands: [{Pt(μ-SiPh₂)(PMe₃)₃}]³⁺*

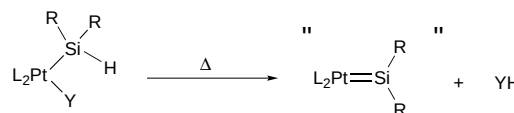
Kohtaro Osakada,* Makoto Tanabe, and Tomoaki Tanase

Triangular complexes composed of three zerovalent transition metal centers are among the most extensively studied cluster compounds. The trinuclear Pt⁰ complexes reported so far contain bridging CO or CNR ligands, which have significant π-acceptor character and stabilize complexes with low-valent metal centers (Scheme 1a).^[1] Organosilyl ligands



Scheme 1. Triangular trinuclear complexes of platinum: a) known complexes, where X = CO, CNR, or other neutral ligands; b) target complexes with organosilyl ligands.

have not been commonly used as ligands for these cluster compounds, partly because of the mismatched coordination of a ligand having a significant σ-donor character and negligible π-acceptor capability with electron-rich metal centers. The electronic state and structure of Pt⁰ complexes with organosilyl ligands would be of significant interest, but they have not yet been prepared.^[2–3] Since the target structure in Scheme 1b is a trimeric form of a platinum–silylene complex, a synthetic approach that uses such species as precursors might be successful. Owing to their intrinsic instability,^[4,5] mononuclear platinum–silylene complexes were obtained only by using well-designed precursors; however, they were postulated to arise by α-elimination from dialkyl- or diarylsilyl complexes of platinum (Scheme 2).^[6,7] We chose a diaryl-



Scheme 2. Postulated formation of mononuclear platinum–silylene complexes by α-elimination.

[*] Prof. Dr. K. Osakada, M. Tanabe
Research Laboratory of Resources Utilization
Tokyo Institute of Technology
4259 Nagatsuta, Midori-ku, Yokohama 226-8503 (Japan)
Fax: (+81)45-924-5224
E-mail: kosakada@res.titech.ac.jp

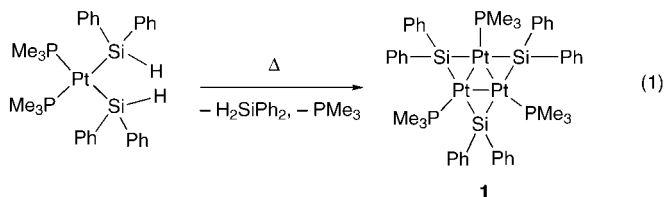
Prof. T. Tanase
Department of Chemistry, Nara Women's University
Kitaouya-higashi-machi, Nara 630-8285 (Japan)

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silylplatinum complex as the precursor for a trinuclear Pt⁰ cluster with bridging silylene ligands.

Heating [Pt(SiHPh₂)₂(PMe₃)₂]^[8] at 100 °C gave **1**, which was isolated as red crystals [Eq. (1)].^[9] The accompanying formation of H₂SiPh₂ and a smaller quantity of HSiPh₃ was



observed during the reaction. The reaction mixture also contained [(Me₃P)₂Pt(Ph₂SiOSiPh₂)] (**2**), which can be attributed to the reaction of adventitious oxygen with a disilene Pt complex generated in situ.^[10] Figure 1 shows the molecular

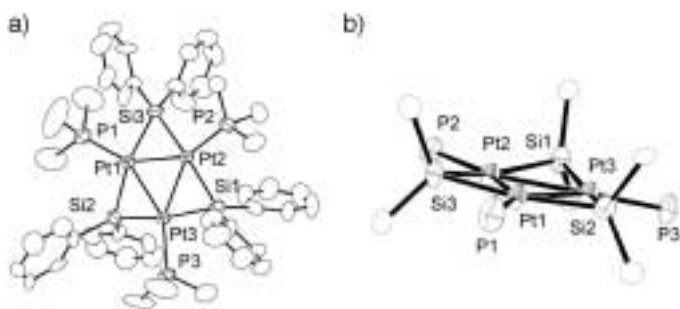


Figure 1. a) Top view and b) side view of the X-ray structure of **1**. Selected bond lengths [Å] and angles [°]: Pt1–Pt2 2.716(1), Pt1–Pt3 2.713(1), Pt2–Pt3 2.697(1), Pt1–P1 2.243(5), Pt2–P2 2.226(5), Pt3–P3 2.247(4), Pt1–Si2 2.364(5), Pt1–Si3 2.357(5), Pt2–Si1 2.355(5), Pt2–Si2 2.345(5), Pt3–Si3 2.343(5), Pt3–Si1 2.337(5); Pt1–Pt2–Pt3 60.17(3), Pt2–Pt3–Pt1 60.27(3), Pt3–Pt1–Pt2 59.57(3), Pt1–Pt2–Si2 55.1(1), Pt2–Pt1–Si2 54.5(1), Pt2–Pt3–Si1 55.2(1), Pt3–Pt2–Si1 54.6(1), Pt1–Pt3–Si3 55.0(1), Pt3–Pt1–Si3 54.5(1), Si2–Pt1–Si3 167.0(2), Si1–Pt2–Si2 163.4(2), Si3–Pt3–Si1 164.1(2), Si2–Pt1–P1 94.0(2), Si3–Pt1–P1 98.0(2), Si1–Pt2–P2 95.0(2), Si2–Pt2–P2 94.9(2), Si1–Pt3–P3 94.6(2), Si3–Pt3–P3 96.6(2), Pt1–Si2–Pt2 70.4(1), Pt2–Si3–Pt3 70.5(1), Pt2–Si1–Pt3 70.2(1).

structure of **1**, as determined by X-ray crystallography.^[11] The three Pt centers form a triangle with Pt–Pt distances of 2.697–2.716 Å and Pt–Pt–Pt angles of 59.6–60.3°. The metal–metal distances are similar to those in analogous trinuclear Pt⁰ complexes with bridging CNR and CO ligands and suggest the presence of Pt–Pt bonds. Interaction between transition metals with d¹⁰ configurations was proposed to account for the structure and electronic state of multinuclear complexes of Pd⁰, Pt⁰, and Cu^I. The acute Pt–Si–Pt angles (70.0–70.4°) and the almost perpendicular orientation of the phenyl substituents with respect to the coordination plane are similar to other silylene-bridged transition metal complexes.

The satellite signals of the PMe₃ hydrogen atoms in the ¹H NMR spectrum exhibit both three- and four-bond Pt–H coupling (³J_{Pt,H} = 43, ⁴J_{Pt,H} = 25 Hz). The ³¹P{¹H} NMR spectrum (Figure 2) exhibits long-range ³¹P–¹⁹⁵Pt and ³¹P–³¹P coupling. The presence of significantly stable Pt–Pt bonds, confirmed from the crystallographic and spectroscopic data, seems to be more consistent with Pt⁰ rather than the alternative Pt^{II} formulation.^[12]

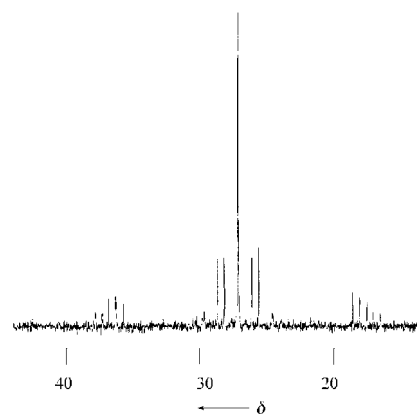


Figure 2. ³¹P{¹H} NMR spectrum of **1** (160 MHz at 25 °C in [D₆]benzene).

Complex **1** contains SiPh₂ and PMe₃ ligands, both of which are strongly electron donating, and is expected to have a unique electronic state. Modeling the electronic state of **1** by extended Hückel molecular orbital calculations on the C_{3v} model complex [Pt₃(PH₃)₃(μ-SiH₂)₃] (**I**) gave results that were consistent with the above structure.^[13] The electronic structure of **I** was consistent with the proposed 42-valence-electron cluster in which three Pt centers are bound with metal–metal bonds, in a similar manner to that observed in [Pt₃(PH₃)₃(μ-CNH)₃] (**II**).^[14, 15] The Pt–Pt bond overlap population in **I** (0.24) is comparable to that in **II** (0.20), whereas the energy of the HOMO is higher than that of the isocyanide complex **II** due to significant mixing of Si p orbitals.

Experimental Section

1: A solution of [Pt(SiHPh₂)₂(PMe₃)₂] (405 mg, 0.57 mmol) in toluene (20 mL) was heated for 24 h at 100 °C. The color of the solution changed from yellow to dark red. Analysis of the solution by ¹H NMR spectroscopy showed the formation of H₂SiPh₂ and a smaller amount of HSiPh₃. After evaporation of the solvent, hexane was added to the residue. This resulted in separation of an orange solid, which was repeatedly recrystallized from toluene/hexane to afford **1** as red crystals (96 mg, 28%). Cooling the hexane filtrate obtained after separation of **1** yielded **2** as pale yellow crystals (34%).

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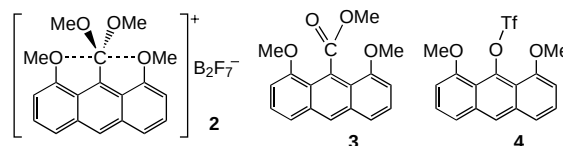
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- [10] Complex **2** gave satisfactory elemental analyses, NMR data, and X-ray crystallographic results. Analogous complexes [(dppe)Pt(R₂SiO-SiR₂)] (R = Me, *i*Pr) were prepared by reaction of O₂ with disilene Pt complexes [(dppe)Pt(R₂SiSiR₂)] (dppe = 1,2-bis(diphenylphosphanyl)ethane): E. K. Pham, R. West, *J. Am. Chem. Soc.* **1989**, 111, 7667; E. K. Pham, R. West, *Organometallics* **1990**, 9, 1517.
- [11] Crystallographic data for **1**: C₄₅H₅₇P₃Si₃Pt₃, *M_r* = 1360.69; triclinic, space group *P* $\bar{1}$ (no. 2), *a* = 12.914(3), *b* = 17.632(4), *c* = 12.830(4) Å, *α* = 111.01(2), *β* = 116.59(2), *γ* = 87.36(2)°, *V* = 2416 Å³, *Z* = 2, *μ* = 8.834 mm^{−1}, *F*(000) = 1296, *ρ*_{calcd} = 1.870 g cm^{−3}. The final *R* factor was 0.044 (*R_w* = 0.035) for 4183 reflections with *I* > 3σ(*I*). Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-141185. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [12] Pt^{II} and Pd^{II} complexes with a stable metal–metal bond are unknown. X-ray photoelectron spectroscopy (XPS) was applied to **1** and related Pt complexes to obtain more direct evidence for the valency of the metal centers. Complex **1** shows peaks at 72.3 and 75.6 eV (due to 4f_{7/2} and 4f_{5/2} states, respectively). The peak positions, however, are negligibly different to those of the triangular Pt⁰ complex [Pt₃(2,6-Me₂-C₆H₃CN)₆] (72.2 and 75.5 eV) and the mononuclear Pt^{II} complex [Pt(SiHPh₂)₂(PMe₃)₂] (72.3 and 75.7 eV). These data and the NMR data of the complexes did not provide additional useful information for determining the valence of Pt in **1**.
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Synthesis of a Versatile Tridentate Anthracene Ligand and its Application for the Synthesis of Hypervalent Pentacoordinate Boron Compounds (10-B-5)**

Makoto Yamashita, Yohsuke Yamamoto, Kin-ya Akiba,* and Shigeru Nagase

Hypervalent pentacoordinate boron compounds (10-B-5)^[1] have been postulated as transition states in S_N2-type reactions at a boron atom. For example, the reaction of the [BH₃-CO] complex with NMe₃^[2] as well as the intramolecular bond switch at the boron atom in compounds bearing a van Koten type ligand^[3] have been reported. There has been only one report of isolable hypervalent boron compounds (10-B-5 and 12-B-6),^[4] but the compounds bearing tridentate pyridine diol ligand(s) were characterized by ¹H, ¹³C, ¹⁹F, and ¹¹B NMR spectra in solution. The X-ray analysis of the compounds has not been reported. Here we report the synthesis and X-ray structures of 1,8-dimethoxy-9-borylanthracene (**1a–c**, see Scheme 2): the first fully characterized hypervalent 10-B-5 compounds.

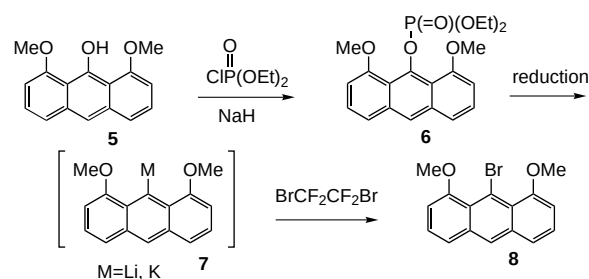
Recently, we reported the synthesis and the X-ray structure of the hypervalent five-coordinate carbon compound (10-C-5) **2** through the use of an 1,8-dimethoxy-9-anthracenyl ligand.^[5] Ester **3** was synthesized from the 9-OTf derivative **4** (OTf = trifluoromethanesulfonate) by carbon monoxide insertion in



methanol mediated by [Pd(PPh₃)₄]. However, several attempts to synthesize **1** from **4** were not successful. Thus, we designed a novel versatile precursor 1,8-dimethoxy-9-bromoanthracene (**8**). The synthetic pathway for **8** is illustrated in Scheme 1. After conversion of 1,8-dimethoxy-9-hydroxyanthracene (**5**)^[6] into the corresponding phosphate (**6**), reaction conditions for the reduction of **6** and the subsequent treatment of the resulting anion **7** with BrCF₂CF₂Br to yield **8** were examined (Table 1). Only the 9-H compound was obtained using Birch reduction conditions (entry 1) and only a trace amount of **8** was obtained by using lithium naphthalenide (entry 2). Fortunately, the reduction of **6** with lithium 4,4'-di-

[*] Prof. K.-y. Akiba, M. Yamashita, Prof. Y. Yamamoto
Department of Chemistry, Graduate School of Science
Hiroshima University, 1-3-1 Kagamiyama
Higashi-Hiroshima 739-8526 (Japan)
Fax: (+81)824-24-0723
E-mail: akiba@sci.hiroshima-u.ac.jp
Prof. S. Nagase
Department of Chemistry, Graduate School of Science
Tokyo Metropolitan University, 1-1 Minami-Osawa
Hachioji, Tokyo 192-0397 (Japan)

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Scheme 1. Synthesis of bromoanthracene **8**.

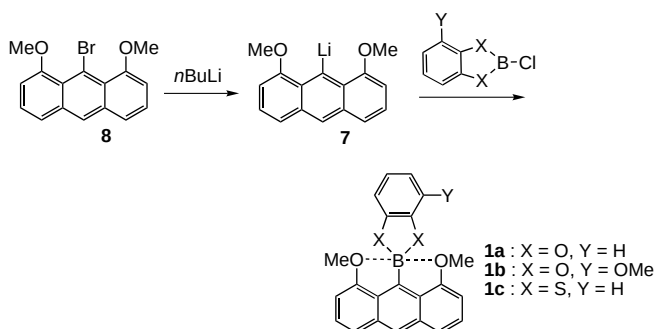
Table 1. Reaction conditions for the reduction of **6**.

Entry	Metal (equivalent)	Electron carrier	Solvent	T [$^{\circ}\text{C}$], t [h]	Yield of 8 [%]
1	K (2.2)	–	liq. NH_3	–78, 2	0
2	Li (2.5)	naphthalene	THF	0 → –25, 3.5	trace
3	Li (2.5)	DTBB	THF	0, 1	14
4	Li (2.5)	DTBB	THF	–20, 2	20
5	Li (2.5)	DTBB	THF	–30, 12	30
6	Li (4)	DTBB	THF	–20, 12	28
7	Li (10)	DTBB	THF	–20, 2	trace

DTBB = 4,4'-di-*tert*-butylbiphenyl

tert-butylbiphenylide (LDBB) gave **8**, albeit in low yields (up to 30%, entry 5).

Compound **8** was lithiated at -100°C in a dilute solution of THF and then the *B*-chlorocatecholborane derivatives added at the same temperature (Scheme 2). Catecholborane derivatives **1a–c** were obtained in acceptable yields (47% for **1a**, 25% for **1b**, and 32% for **1c**). These compounds were stable to atmospheric moisture and chromatographic treatment (SiO_2).



Scheme 2. Synthesis of hypervalent boron **1**.

Crystals of **1a–c** suitable for X-ray analysis were obtained by recrystallization from $\text{CH}_2\text{Cl}_2/n$ -hexane (Figure 1; Table 2).^[7] The sum of the bond angles around the central boron atom of **1a–c** are 360.0° , which indicates that the central boron atom is planar with sp^2 hybridization. Thus, one of the lone pairs on the oxygen atoms at the 1,8-positions in **1** interacts with the empty *p* orbital of the central boron atom at position 9 to form a three-center four-electron bond. Therefore, the structure around the central boron atom can be regarded as a slightly distorted trigonal bipyramid. The two B–O bond lengths (B1–O1, B1–O2) are 2.379(2) and 2.441(2) Å in **1a**, 2.398(4) and 2.412(4) Å in **1b**, and are

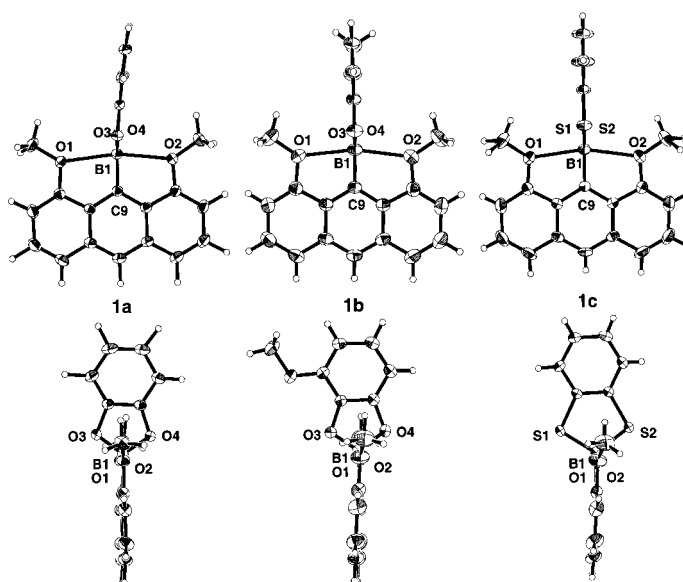


Figure 1. Crystal structures (30% thermal ellipsoids) of **1a–c**.

Table 2. Selected bond lengths [Å] and angles [$^{\circ}$] of **1a–c**.

	O1–B1	O2–B1	O1–B1–O2	O1–C1–C11	O2–C8–C14
1a	2.379(2)	2.441(2)	167.10(7)	113.2(1)	113.7(1)
1b	2.398(4)	2.412(4)	166.3(2)	113.2(2)	112.9(2)
1c	2.436(2)	2.436(2)	164.0(3)	113.5(2)	113.5(2)

identical (2.436(2) Å) in **1c**. The bonds are longer than those of the B1–O3 and B1–O4 bonds (1.397(2) and 1.403(2) Å in **1a**, 1.394(3) and 1.400(3) Å in **1b**) but shorter than the sum of the van der Waals radii (3.48 Å).^[8] The small difference in the B1–O1/2 bond lengths observed in **1a** may be a consequence of a packing effect or may be related to the electrophilicity of the central boron atom since **1b**, which bears a more electron-donating OMe substituent, showed a more symmetrical structure.

The ^{11}B NMR chemical shifts for **1a–c** are in the range of normal catecholborane derivatives (phenylcatecholborane: $\delta = +32.1$ (THF)),^[9] which indicates that the interaction between the central boron atom and the oxygen atoms at the 1,8-positions is weak. In contrast, upfield shifts were observed in the spectra of the boron compounds prepared by Lee and Martin (–20 to –41 ppm).^[4] The difference in the chemical shifts between the compounds of Lee and Martin and **1a–c** may be a consequence of the charge difference (the former being anionic systems, while **1a–c** are electronically neutral systems), and hence further investigation on the synthesis and chemical shifts of boron compounds bearing anionic oxygen atoms (O^-) at the 1,8-positions is in progress.

However, an attractive interaction between the central boron atom and the oxygen atoms at the 1,8-positions, even though weak, is confirmed by hybrid nonlocal density functional theory (DFT) at the B3LYP/6-31G* level using the Gaussian 98 program.^[10] The optimized geometry of **1a** is the symmetrical structure. The two B–O bond lengths are identical (2.447 Å) and are slightly longer than the experimental data (2.379(2) and 2.441(2) Å). The bond path is found between the central boron atom and the two oxygen

atoms, which indicates that these atoms are bonded.^[11] The B–O bond is weak and slightly ionic as shown by the small value of the electron density ($\rho(r)$: 0.021 e a₀^{−3}; a₀ = 0.529177 Å)^[11] and small positive Laplacian value ($\nabla^2\rho(r)$: 0.057 e a₀^{−5})^[11] at the bond critical points (BCP). These values, which include a large value of the ellipticity (ε = 0.220),^[11] are very similar to the values for the C–O bond in our hypervalent five-coordinate carbon compound **2** ($\rho(r)$: 0.022 e a₀^{−3}, $\nabla^2\rho(r)$: 0.078 e a₀^{−5}, ε : 0.220).^[5]

Experimental Section

6: Dry THF (10 mL) was added in an argon atmosphere at 0 °C to a mixture of **5** (509 mg, 2.00 mmol) and NaH (48.6 mg, 2.2 mmol; oil dispersion). After stirring the mixture for 15 min at 0 °C, diethyl chlorophosphate (0.32 mL, 2.2 mmol) was added dropwise and the mixture heated to reflux at 80 °C for 4 h. The solvent was then removed under reduced pressure, and the residue was poured into water, followed by extraction with CH₂Cl₂. The organic layer was collected and dried over K₂CO₃. Solvents were removed under reduced pressure to give a crude product, which was purified by cooled column chromatography (CH₂Cl₂/diethyl ether = 1/0–3/1, −10 °C) to give **6** (505 mg, 65 %) as a yellow solid. M.p. 115.8–117.0 °C; ¹H NMR (400 MHz, CDCl₃, 25 °C, CHCl₃): δ = 1.13 (dt, ⁴J(P,H) = 1 Hz, ³J(H,H) = 7 Hz, 6H; CH₃CH₂), 3.94–4.09 (m, 4H; CH₃CH₂), 4.02 (s, 6H; OCH₃), 6.75 (d, ³J(H,H) = 8 Hz, 2H; aromatic CH), 7.33 (t, ³J(H,H) = 8 Hz, 2H; aromatic CH), 7.47 (d, ³J(H,H) = 8 Hz, 2H; aromatic CH), 8.12 (s, 1H; aromatic CH); ¹³C NMR (100 MHz, CDCl₃, 25 °C, CDCl₃): δ = 15.54 (d, ³J(C,P) = 8.7 Hz, CH₃CH₂), 54.88 (s, OCH₃), 63.31 (d, ²J(C,P) = 6.6 Hz, CH₃CH₂), 103.21 (d, ¹J(C,P) = 1.6 Hz, aromatic CH), 117.28 (d, ¹J(C,P) = 4.2 Hz, quaternary C), 119.38 (d, ¹J(C,P) = 5.8 Hz, aromatic CH), 122.57 (d, ¹J(C,P) = 3.3 Hz, aromatic CH), 125.37 (d, ¹J(C,P) = 12.4 Hz, aromatic CH), 133.45 (d, ¹J(C,P) = 2.5 Hz, quaternary C), 141.40 (d, ¹J(C,P) = 9.1 Hz, quaternary C), 155.79 (d, ¹J(C,P) = 1.7 Hz, quaternary C); ³¹P NMR (162 MHz, CDCl₃, 25 °C, H₃PO₄): δ = −6.89 (s); elemental analysis calcd for C₂₀H₂₃O₆P: C 61.54, H 5.94; found: C 61.22, H 5.92.

8: THF (4 mL) was added to a mixture of 4,4'-di-*tert*-butylbiphenyl (668 mg, 2.5 mmol) and Li (178 mg, 2.5 mmol) under argon at 0 °C. The mixture was stirred for 4 h at 0 °C with a glass-coated stirring bar to give a solution of LDBB. A solution of **6** (390.5 mg, 1 mmol) in THF (5 mL) was added to the LDBB solution at −78 °C and the mixture stirred at that temperature for 1 h. The mixture was then allowed to warm to −30 °C and was stirred at the temperature for 12 h. BrCF₂CF₂Br (0.36 mL, 3 mmol) was added dropwise into the reaction mixture at −30 °C. The mixture was then allowed to warm to room temperature and was stirred for 1 h. Solvents were removed under reduced pressure. The crude product was dissolved in CH₂Cl₂ and was washed with CH₂Cl₂/H₂O. The organic layer was collected and was dried over K₂CO₃. Solvents were removed under reduced pressure to give a crude product, which was purified by column chromatography (CH₂Cl₂/*n*-hexane 0/1–1/15) to give **8** (99.1 mg, 30 %) as a pale yellow solid. M.p. 141–149 °C (decomp); ¹H NMR (400 MHz, CDCl₃, 25 °C, CHCl₃): δ = 4.04 (s, 6H; OCH₃), 6.90 (d, ³J(H,H) = 8 Hz, 2H; aromatic CH), 7.37 (t, ³J(H,H) = 8 Hz, 2H; aromatic CH), 7.54 (d, ³J(H,H) = 8 Hz, 2H; aromatic CH), 8.29 (s, 1H; aromatic CH); ¹³C NMR (100 MHz, CDCl₃, 25 °C, CDCl₃): δ = 55.75 (OCH₃), 101.78 (aromatic CH), 116.19 (aromatic CH), 119.63 (aromatic CH), 121.33 (quaternary C), 124.75 (quaternary C), 127.37 (aromatic CH), 131.68 (quaternary C), 155.77 (quaternary C); MS (FAB +): *m/z*: 316/318 [*M*⁺]; elemental analysis calcd for C₁₆H₁₃O₂Br: C 60.59, H 4.13; found: C 60.50, H 3.88.

1a: A solution of *n*BuLi in *n*-hexane (0.35 mL, 0.55 mmol) was added dropwise to a mixture of **8** (159 mg, 0.5 mmol) and THF (20 mL) at −100 °C. The reaction mixture was then stirred for 1.5 h at −100 °C. The solution of *B*-chlorocatecholborane (87.3 mg, 0.57 mmol) and THF (10 mL) was transferred to the reaction mixture at −100 °C. The mixture was stirred for 7.5 h at −100 °C and 9 h at room temperature. The reaction was quenched with aqueous 1N HCl (50 mL) and the mixture extracted with CH₂Cl₂. The organic layer was collected and dried over MgSO₄. The crude product was purified by HPLC to give **1a** (84.7 mg, 47 %) as a pale blue-green fraction (retention time = 74 min; Lc 908-C60. Column (Japan Analytical Industry), ClCH₂CH₂Cl eluent, 15 mL min^{−1} flow rate):

m.p. 278–288 °C (decomp); ¹H NMR (400 MHz, CDCl₃, 25 °C, CHCl₃): δ = 3.67 (s, 6H; OCH₃), 6.71 (d, ³J(H,H) = 7 Hz, 2H; aromatic CH), 7.12 (dd, ³J(H,H) = 6 Hz, ⁴J(H,H) = 2 Hz, 2H; aromatic CH), 7.30 (dd, ³J(H,H) = 6 Hz, ⁴J(H,H) = 2 Hz, 2H; aromatic CH), 7.38 (dd, ³J(H,H) = 7 Hz, ³J(H,H) = 8 Hz, 2H; aromatic CH), 7.64 (d, ³J(H,H) = 8 Hz, 2H; aromatic CH), 8.45 (s, 1H); ¹¹B NMR (128 MHz, CDCl₃, 25 °C, BF₃·OEt₂): δ = 28–39 (br); elemental analysis calcd for C₂₂H₁₇O₄B: C 74.19, H 4.81; found: C 73.78, H 4.67. A similar procedure was employed for **1b** and **1c**. **1b:** yield 25 %; pale blue fraction (retention time = 70 min): m.p. 262–273 °C (decomp); ¹H NMR (400 MHz, CDCl₃, 25 °C, CHCl₃): δ = 3.69 (s, 6H; OCH₃), 4.00 (s, 3H; OCH₃), 6.69 (d, ³J(H,H) = 8 Hz, 2H; aromatic CH), 6.76 (d, ³J(H,H) = 8 Hz, 1H; aromatic CH), 6.97 (d, ³J(H,H) = 8 Hz, 1H; aromatic CH), 7.06 (t, ³J(H,H) = 8 Hz, 1H; aromatic CH), 7.38 (t, ³J(H,H) = 8 Hz, 2H; aromatic CH), 7.62 (d, ³J(H,H) = 8 Hz, 2H; aromatic CH), 8.44 (s, 1H; aromatic CH); ¹³C NMR (100 MHz, CDCl₃, 25 °C, CDCl₃): δ = 56.57 (OCH₃), 56.66 (OCH₃), 102.66 (aromatic CH), 105.20 (aromatic CH), 106.74 (aromatic CH), 121.17 (aromatic CH), 121.64 (aromatic CH), 125.39 (aromatic CH), 127.77 (aromatic CH), 128.81 (quaternary C), 132.30 (quaternary C), 137.25 (quaternary C), 145.36 (quaternary C), 150.35 (quaternary C), 155.29 (quaternary C); ¹¹B NMR (128 MHz, CDCl₃, 25 °C, BF₃·OEt₂): δ = 30–37 (br); elemental analysis calcd for C₂₂H₁₉O₅B: C 71.53, H 4.96; found: C 71.18, H 4.68. **1c:** yield 32 %; pale blue fraction (retention time = 75 min): m.p. 240–250 °C (decomp); ¹H NMR (400 MHz, CDCl₃, 25 °C, CHCl₃): δ = 3.62 (s, 6H; OCH₃), 6.67 (d, ³J(H,H) = 7 Hz, 2H; aromatic CH), 7.25 (dd, ³J(H,H) = 6 Hz, ⁴J(H,H) = 3 Hz, 2H; aromatic CH), 7.39 (dd, ³J(H,H) = 7 Hz, ³J(H,H) = 8 Hz, 2H; aromatic CH), 7.61 (d, ³J(H,H) = 8 Hz, 2H; aromatic CH), 7.70 (dd, ³J(H,H) = 6 Hz, ⁴J(H,H) = 3 Hz, 2H; aromatic CH), 8.45 (s, 1H; aromatic CH); ¹³C NMR (100 MHz, CDCl₃, 25 °C, CDCl₃): δ = 54.50 (OCH₃), 103.24 (aromatic CH), 121.15 (aromatic CH), 124.34 (aromatic CH), 125.31 (aromatic CH), 125.53 (aromatic CH), 127.98 (quaternary C), 128.13 (quaternary C), 132.65 (quaternary C), 140.76 (quaternary C), 155.81 (quaternary C); ¹¹B NMR (128 MHz, CDCl₃, 25 °C, BF₃·OEt₂): δ = 55–68 (br); elemental analysis calcd for C₂₂H₁₇O₅S₂B: C 68.05, H 4.41; found: C 67.69, H 4.29.

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processed by using SCALEPACK. The structure was solved using the teXsan system and refined by full-matrix least-squares. (The programs DENZO and SCALEPACK are available from Mac Science Co., Z. Otwinowski, University of Texas, Southwestern Medical Center. The program teXsan is available from Rigaku Co.) Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-141764, -141765, and -141766. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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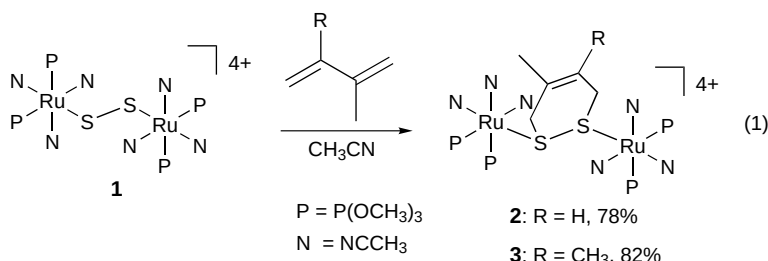
Diels–Alder Type Addition of 1,3-Dienes to a Disulfide Bridging Ligand in Diruthenium Complexes**

Hiroyasu Sugiyama, Yong-Shou Lin, and Kazuko Matsumoto*

Carbon–sulfur bond formation and C–S bond cleavage reactions have been the focus of research for several years because of their relevance to the biologically related metallo-sulfide proteins, the industrially important hydrodesulfurization (HDS) process, and the organic synthesis of sulfur-containing compounds.^[1] Reactions of transition metal sulfides with unsaturated organic substrates, such as alkynes, alkenes, and nitriles, have been reported, in which [2+3] cycloadditions occur between M(S)₂ and C–X double or triple bonds (X = C or N) to give metalacycles.^[2] The addition of olefins to a bridging sulfide ligand in the dimolybdenum complex has also been reported.^[3] Nevertheless, to the best of our knowledge, the

reaction of transition metal sulfides with dienes has not been reported. It is well known that in the HDS process thiophene is converted into butadiene and hydrogen sulfide.^[3d, 4] As a reverse reaction of the HDS process, the treatment of dienes with sulfur is especially noteworthy on transition metal centers. In our recent studies on the Ru₂S₂ core chemistry in the disulfide-bridged diruthenium complexes,^[5] we found that $[\{\text{Ru}[\text{P}(\text{OCH}_3)_3]_2(\text{CH}_3\text{CN})_3\}_2(\mu\text{-S}_2)]^{4+}$ (**1**, as CF₃SO₃ salt) reacts with unsaturated organic molecules, such as ketones and terminal olefins, to form C–S bonds on the disulfide ligand via the C–H bond activation reaction.^[6] These are very rare processes and only one analogous C–S bond formation reaction can be found in the literature: The reaction of acetone with a terminal sulfide ligand,^[7] which, however, seems to have been found by chance and no systematic study was carried out. Through our recent study of new reactions, we have found that the S₂ ligand in the electron-deficient Ru^{III} complex **1** and its analogues is activated to achieve C–H bond splitting both through electronic and steric effects. In our previous reports, we suggested that the addition of a C–H bond to the S=S double bond is the key step in the C–H bond activation process of acetone and monoolefins.^[6b,c] In the present report, we demonstrate directly the double bond character of the S=S bond between the two Ru centers by isolating the [2+4] cycloaddition products from the reaction of **1** with dienes.

Treatment of **1** with a conjugated diene, such as isoprene or 2,3-dimethylbutadiene in CH₃CN at room temperature, resulted in the formation of a pale yellow solution, from which $[\{\text{Ru}[\text{P}(\text{OCH}_3)_3]_2(\text{CH}_3\text{CN})_3\}_2(\mu\text{-SCH}_2\text{C}(\text{R})=\text{C}(\text{CH}_3)\text{-CH}_2\text{S})]^{4+}$ (**2**, as CF₃SO₃ salt, R = H, 78%; **3**, as CF₃SO₃ salt, R = CH₃, 82%) was obtained after standard work-up [Eq. (1)]. When the complex **1** was treated with 1,3-penta-



diene, a similar color change was observed, however, the product was a mixture according to the ¹H NMR spectrum. Separation of these products was not successful. Furthermore, the reaction of **1** with 2,4-hexadiene was also examined but no sign of the C–S bond formation was observed: Only $[\{\text{Ru}[\text{P}(\text{OCH}_3)_3]_2(\text{CH}_3\text{CN})_3\}_2(\mu\text{-S}_2)](\text{CF}_3\text{SO}_3)_3$ ^[5a] was recovered. Therefore, it seems that the C–S bond formation reaction of **1** needs at least one of the two double bonds at the terminal position. The structure of **3** was determined by X-ray diffraction, as shown in Figure 1.^[8, 10]

Analogously, the reaction of the dicationic complex $[\{\text{Ru}[\text{P}(\text{OCH}_3)_3]_2(\text{CH}_3\text{CN})_2\}_2(\mu\text{-S}_2)(\mu\text{-Cl})_2]^{2+}$ (**4**, as CF₃SO₃ salt) with 2,3-dimethylbutadiene gave $[\{\text{Ru}[\text{P}(\text{OCH}_3)_3]_2(\text{CH}_3\text{CN})_2\}_2(\mu\text{-SCH}_2\text{C}(\text{CH}_3)=\text{C}(\text{CH}_3)\text{CH}_2\text{S})(\mu\text{-Cl})_2]^{2+}$ (**5**, as CF₃SO₃ salt)

[*] Prof. Dr. K. Matsumoto, H. Sugiyama, Dr. Y.-S. Lin
Department of Chemistry
Advanced Research Center for Science and Engineering
Waseda University
3-4-1, Ohkubo, Shinjuku, Tokyo 169-8555 (Japan)
Fax: (+81) 3-5273-3489
E-mail: kmatsu@mn.waseda.ac.jp

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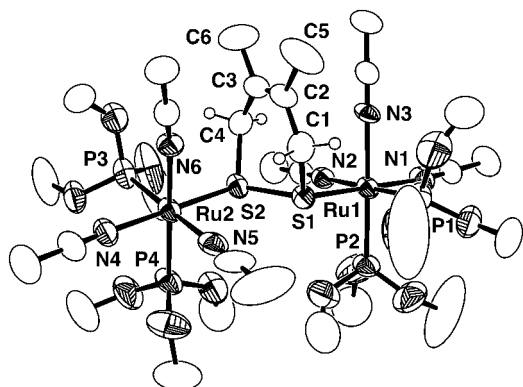


Figure 1. Structure of the cation **3** drawn at a 50% probability level. Methyl H atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Ru1-S1 2.3629(18), Ru2-S2 2.3707(18), Ru1-P1 2.257(2), Ru1-P2 2.256(2), Ru2-P3 2.275(3), Ru2-P4 2.277(2), S1-S2 2.099(3), S1-C1 1.827(8), S2-C4 1.808(8), C1-C2 1.517(14), C2-C3 1.295(14), C3-C4 1.518(13), C2-C5 1.511(15), C3-C6 1.520(15); Ru1-S1-S2 112.77(9), Ru2-S2-S1 111.18(9), Ru1-S1-C1 111.8(3), Ru2-S2-C4 113.1(3), S1-C1-C2 121.0(6), S2-C4-C3 121.4(6), C1-C2-C3 126.3(8), C1-C2-C5 108.9(10), C5-C2-C3 124.7(11), C2-C3-C4 126.1(8), C4-C3-C6 107.9(10), C2-C3-C6 125.9(10).

as yellow crystals in 49% yield [Eq. (2)]. The structure of **5** was also determined by X-ray diffraction, as shown in Figure 2.^[9, 10]

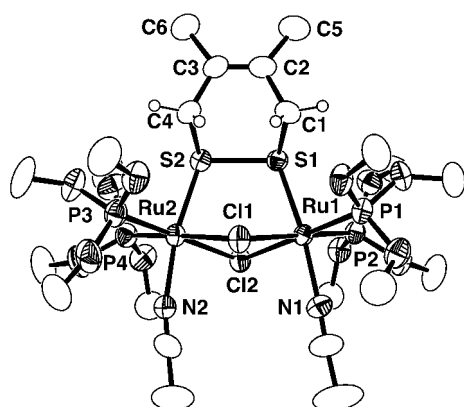
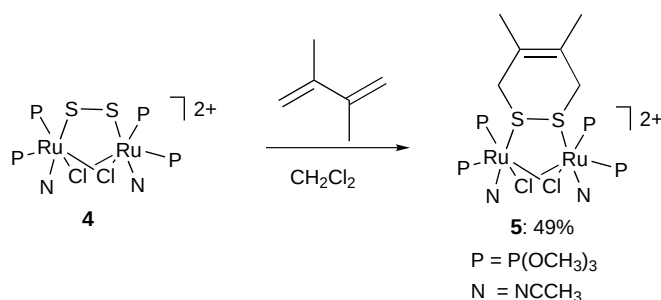


Figure 2. Structure of the cation **5** drawn at a 50% probability level. Methyl H atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Ru1-S1 2.317(2), Ru2-S2 2.316(2), Ru1-P1 2.230(2), Ru1-P2 2.233(2), Ru2-P3 2.236(2), Ru2-P4 2.231(2), Ru1-Cl1 2.468(2), Ru1-Cl2 2.472(2), Ru2-Cl1 2.484(2), Ru2-Cl2 2.480(2), S1-S2 2.176(3), S1-C1 1.821(8), S2-C4 1.825(8), C1-C2 1.519(11), C2-C3 1.304(12), C3-C4 1.527(12), C2-C5 1.507(12), C3-C6 1.508(11); Ru1-S1-S2 108.12(10), Ru2-S2-S1 107.27(10), Ru1-S1-C1 114.4(3), Ru2-S2-C4 114.6(3), S1-C1-C2 110.3(6), S2-C4-C3 110.4(6), C1-C2-C3 117.6(8), C1-C2-C5 116.0(8), C5-C2-C3 126.4(9), C2-C3-C4 118.4(8), C4-C3-C6 116.3(8), C2-C3-C6 125.3(9).

According to the structures of **3** and **5**, the C2–C3 bond distances (1.295(14) (**3**) and 1.304(12) Å (**5**)) fall in the range of C=C double bond distances, whereas the C1–C2 and C3–C4 distances (1.517(14)–1.527(12) Å) are comparable to a C–C single bond. Through the C–S bond formation process, the bond multiplicities in the diene moiety are changed. The S1–S2 bond distances (2.099(3) (**3**) and 2.176(3) Å (**5**)) are considerably longer than the corresponding ones in their respective starting complexes (1.933(11) (**1**) and 1.973(7) Å (**4**)). The C–S bond distances (1.808(8)–1.827(8) Å) are comparable to those known.^[6a, 11] A significant difference is observed between **3** and **5** in the conformations of the C₄S₂ six-membered ring (chair for **3**, boat for **5**) and the bridging mode of the S₂ ligand between the two Ru centers (*trans* for **3** and *cis* for **5**). The C1–S1–S2–C4 torsion angles (48.3(4)° (**3**) and 0.4(4)° (**5**)) also reflect the conformation differences. It is noteworthy that **3** crystallizes in the noncentrosymmetric space group *Pn*. This is due to the existence of the two chiral sulfur atoms, whose *R/R* and *S/S* configurations alone are in the single crystal lattice. As the diastereomers of *R/S* (or *S/R*) were not found in the NMR spectrum of **3**, the ring formation reaction is diastereospecific.

We reported previously that 1-pentene reacts with **1** and forms a C₃S₂ five-membered ring through the C–H bond activation reaction.^[6c] We also extended the reaction of terminal olefins to **4**, however, no reaction took place. Therefore, terminal olefins react only with the RuSSRu core of the open structure, such as in **1**, to form the five-membered C₃S₂ ring via a C–H activation reaction and do not react with **4**, which has a closed RuSSRu core. In contrast, dienes can readily react with both **1** and **4** to form two C–S bonds of the C₄S₂ ring. The mechanism of these reactions involves no C–H bond activation reaction but rather another reaction type, as explained later.

The chemistry of a multiple bond between heteroatoms has been extensively studied.^[12] In one of the typical reactions, the unstable heteroatomic multiple bond is trapped by an appropriate conjugated diene.^[13] The reaction is regarded as of the Diels–Alder type [2+4] cycloaddition, in which the X=Y bond (X, Y = heteroatoms) acts as a dienophile. Neutral S₂ is a known but, as yet, uncharacterized species.^[12] Several precursors types and preparative methods for disulfur have been reported, as well as its reactions with dienes. On the other hand, the S₂ units in **1** or **4** are stabilized by the two Ru moieties but they still exhibit reactivity towards dienes to give the [2+4] cycloadduct and the reaction suggests the double bond character of the S₂ ligand. With regard to the electronic structure of the RuSSRu⁴⁺ core, three types of formulation have been suggested in the literature, namely a) two Ru^{III} centers bridged by a disulfide S₂^{2−}, b) two Ru^{II} centers bridged by a singlet disulfur S₂⁰, or c) an Ru^{II} center and an Ru^{III} center bridged by a S₂[−] (supersulfide). Our cycloaddition reaction suggests that the type (b) contribution is significantly involved. In contrast, the structural analyses and an electrochemical study show that the major contribution of [(H₃N)₅RuSSRu(NH₃)₅]⁴⁺^[14, 15] is type (c). The electronic structure would be dependent on the electron densities on

the Ru centers, which are determined by the ligands. Similar reactions of 2,3-dimethylbutadiene with $[\text{Cp}(\text{Ph}_3\text{P})\text{RuSSRu}(\text{PPh}_3)\text{Cp}]^{2+}$,^[16] and the PMe_3 analogues of **1** and **4**^[17] did not take place under the similar condition. Only the disulfur in **1** and **4** are sufficiently activated to react with dienes. The stabilization and activation of S_2 seem to depend on the donating abilities of the ligands on the Ru centers. The steric effect of the bond angles within the RuSSRu core with respect to the S_2 may be small, since both **1** (*trans* type) and **4** (*cis* type) can react with dienes. In the reactions, it may seem possible that the $\text{C}=\text{C}$ bond coordinates to the Ru center by replacing CH_3CN , as suggested previously in the monoolefin reactions.^[6c] However, even if such diene substitution with CH_3CN occurs in **4**, the diene molecule is located away from the S_2 and the following reaction would be impossible. Therefore, the mechanism involving coordination of the $\text{C}=\text{C}$ double bond to the Ru atom can be ruled out and a Diels–Alder-type mechanism is supported.

We can successfully show that the stable disulfide ligands in **1** and **4** can react with dienes via a Diels–Alder reaction. The reaction shows that the S_2 bond has a double bond character, which is noteworthy, since only in situ prepared unstable S_2 was used in all the previous Diels–Alder reactions of S_2 with dienes.

Experimental Section

All experiments were carried out under a dry atmosphere of nitrogen, using standard Schlenk techniques or in a dry N_2 box. Dry solvents were purchased from Kanto chemical. CD_3CN was dried over CaH_2 and then trap-to-trap distilled prior to use. The NMR spectra were recorded on a Lambda 270 spectrometer (JEOL) operating at 270 MHz for ^1H , 109 MHz for ^{31}P , and 68 MHz for ^{13}C . The chemical shifts are reported in δ units (ppm) downfield from Me_4Si for ^1H and ^{13}C , and H_3PO_4 (85%, external reference) for ^{31}P . Elemental analyses were carried out on a PE 2400II Elemental Analyzer (Perkin–Elmer).

2 and **3**: **2** and **3** were prepared similarly, thus only the synthesis of **2** is described here. Isoprene (1 mL) was added to a CH_3CN (2 mL) solution of $[\{\text{Ru}[\text{P}(\text{OCH}_3)_3]_2(\text{CH}_3\text{CN})_3(\mu\text{-S}_2)\}^{4+}]$ (**1**, as CF_3SO_3 salt; 33.9 mg, 0.021 mmol). The immediate color change from dark blue to pale yellow was observed and the solution was stirred for 1 h at room temperature. Addition of Et_2O (8 mL) resulted in the formation of a pale yellow precipitate and the supernatant solution was removed via syringe. The residue was again dissolved in CH_3CN (0.5 mL) and slow addition of DME (ca. 3 mL) gave the pale yellow crystals of $[\{\text{Ru}[\text{P}(\text{OCH}_3)_3]_2(\text{CH}_3\text{CN})_3(\mu\text{-SCH}_2\text{C}(\text{CH}_3)=\text{CHCH}_2\text{S})\}^{4+}]$ (**2**, as CF_3SO_3 salt; 27.5 mg, 0.016 mmol, 78%). Elemental analysis for $\text{Ru}_2\text{S}_6\text{P}_4\text{C}_{33}\text{H}_{62}\text{N}_6\text{O}_{24}\text{F}_{12}$: calcd: C 23.69, H 3.73, N 5.02; found: C 23.69, H 3.61, N 4.94. ^1H NMR (270 MHz, CD_3CN): δ = 1.9 (s, 3H; $\text{SCH}_2\text{C}(\text{CH}_3)$), 2.48 (s, 6H; CH_3CN *trans* to S), 3.15 (d, $^2J(\text{H,H})$ = 17.6 Hz, 1H; eq $\text{SCH}_2\text{C}(\text{CH}_3)$), 3.40 (m, 1H; eq SCH_2CH), 3.85 (m, 36H; $\text{P}(\text{OCH}_3)_3$), 3.99 (d, 1H; ax $\text{SCH}_2\text{C}(\text{CH}_3)$), 4.11 (m, 1H; ax SCH_2CH), 5.99 (m, 1H; SCH_2CH). $^{13}\text{C}\{^1\text{H}\}$ NMR (68 MHz, CD_3CN): δ = 4.5 (CH_3CN *trans* to S), 25.8 ($\text{SCH}_2\text{C}(\text{CH}_3)$), 34.9 (SCH_2CH), 36.2 ($\text{SCH}_2\text{C}(\text{CH}_3)$), 55.8 (m; $\text{P}(\text{OCH}_3)_3$), 117.5 (SCH_2CH), 130.3 ($\text{SCH}_2\text{C}(\text{CH}_3)$). $^{31}\text{P}\{^1\text{H}\}$ NMR (109 MHz, CD_3CN): δ = 124.8 (d, $^2J(\text{P,P})$ = 78 Hz, 1P), 125.6 (d, 1P), 124.9 (br. s, 2P).

$[\{\text{Ru}[\text{P}(\text{OCH}_3)_3]_2(\text{CH}_3\text{CN})_3(\mu\text{-SCH}_2\text{C}(\text{CH}_3)=\text{C}(\text{CH}_3)\text{CH}_2\text{S})\}^{4+}]$ (**3**, as CF_3SO_3 salt) was prepared from treatment of **1** (124 mg, 0.077 mmol) with 2,3-dimethylbutadiene (0.1 mL). Yield: 107 mg, 0.063 mmol, 82%. Elemental analysis for $\text{C}_{34}\text{H}_{64}\text{F}_{12}\text{N}_6\text{O}_{24}\text{P}_4\text{Ru}_2\text{S}_6$: calcd: C 24.20, H 3.82, N 4.98; found: C 24.23, H 3.80, N 4.95. ^1H NMR (270 MHz, CD_3CN , 40 °C): δ = 1.92 (s, 6H; $\text{SCH}_2\text{C}(\text{CH}_3)$), 2.50 (s, 6H; CH_3CN *trans* to S), 3.20 (d, $^2J(\text{H,H})$ = 16.5 Hz, 2H; eq SCH_2), 3.87 (d, $^3J(\text{P,H})$ = 10.8 Hz, 36H; $\text{P}(\text{OCH}_3)_3$), 4.12 (d, 2H; ax SCH_2). $^{13}\text{C}\{^1\text{H}\}$ NMR (68 MHz, CD_3CN , RT): δ = 4.5 (CH_3CN *trans* to S), 20.9 ($\text{SCH}_2\text{C}(\text{CH}_3)$), 37.8 (SCH_2), 55.8 (m; $\text{P}(\text{OCH}_3)_3$), 128.6

($\text{SCH}_2\text{C}(\text{CH}_3)$). $^{31}\text{P}\{^1\text{H}\}$ NMR (109 MHz, CD_3CN): δ = 124.7, 125.6 (d, $^2J(\text{P,P})$ = 75 Hz)

5: To a CH_3CN (3 mL) solution of $[\{\text{Ru}[\text{P}(\text{OCH}_3)_3]_2\text{Cl}_2(\mu\text{-S}_2)(\mu\text{-Cl})_2\}]$ (90 mg, 0.1 mmol), AgCF_3SO_3 (54 mg, 0.2 mmol) was added and the solution was stirred for 3 h at room temperature. The resulted green solution was centrifuged to remove the AgCl and evaporated to dryness. The residue was washed with Et_2O (8 mL) and dried under reduced pressure to give the crude product of $[\{\text{Ru}[\text{P}(\text{OCH}_3)_3]_2(\text{CH}_3\text{CN})_3(\mu\text{-S}_2)(\mu\text{-Cl})_2\}^{2+}]$ (**4**, as CF_3SO_3 salt; 111 mg, 0.091 mmol, 91%). This material was again dissolved in CH_2Cl_2 (1 mL) and 2,3-dimethylbutadiene (0.5 mL) was added. An immediate color change from green to yellow was observed. To the reaction mixture, Et_2O (16 mL) was added to give a gummy solid after 3 h with stirring. The supernatant was removed and dried. The residue was recrystallized from $\text{CH}_2\text{Cl}_2/\text{DME}$ to give yellow needles of $[\{\text{Ru}[\text{P}(\text{OCH}_3)_3]_2(\text{CH}_3\text{CN})_3(\mu\text{-SCH}_2\text{C}(\text{CH}_3)=\text{C}(\text{CH}_3)\text{CH}_2\text{S})(\mu\text{-Cl})_2\}^{2+}]$ (**5**, as CF_3SO_3 salt; 53 mg, 0.045 mmol, 49% yield based on **4**). Elemental analysis for $\text{C}_{24}\text{H}_{52}\text{N}_2\text{Cl}_2\text{F}_6\text{O}_{18}\text{P}_4\text{Ru}_2\text{S}_4$: calcd: C 22.24, H 4.04, N 2.16; found: C 22.41, H 4.09, N 2.12. ^1H NMR (270 MHz, CD_3CN , –30 °C): δ = 1.88 (s, 6H; $\text{SCH}_2\text{C}(\text{CH}_3)$), 2.46 (s, 6H; CH_3CN *trans* to S), 3.44 (d, $^2J(\text{H,H})$ = 14.0 Hz, 2H; SCH_2), 3.74 (dd, $J(\text{P,H})$ = 8.9 Hz, 10.2 Hz, 36H; $\text{P}(\text{OCH}_3)_3$), 4.01 (d, 2H; SCH_2). $^{31}\text{P}\{^1\text{H}\}$ NMR (109 MHz, CD_3CN): δ = 130.1, 131.6 (d, $^2J(\text{P,P})$ = 75 Hz).

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- [9] Crystal data for **5**: $\text{C}_{24}\text{H}_{52}\text{Cl}_2\text{F}_6\text{N}_2\text{O}_{18}\text{P}_4\text{Ru}_2\text{S}_4$, F_w = 1295.84, yellow needles, monoclinic, space group $P2_1/c$, a = 9.1280(4), b = 22.4015(10), c = 24.6537(11) Å, β = 96.2770(10)°, V = 5011.0(4) Å³, Z = 4, ρ_{calc} = 1.718 g cm⁻³, R_1 = 0.0584; wR_2 = 0.1484 for 5456 reflections ($F_o^2 > 2\sigma(F_o^2)$), R_1 = 0.1436; wR_2 = 0.1991 for all 11514 reflections.
- [10] Diffraction data of complexes **3** and **5** were collected on a SMART 1000 CCD diffractometer (Bruker) using MoK_α radiation (λ = 0.71069 Å). All the intensity data were processed with the SAINT plus program package. Absorption corrections were applied

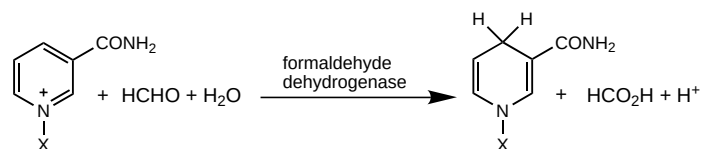
to the integrated intensity with a SADABS program. The structure solution was performed on a SHELXTL software package. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-142966 and -142967. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Regiospecific Hydride Transfer from *cis*-[Ru(bpy)₂(CO)(CHO)]⁺ to NAD⁺ Model Compounds: A Model for Enzymatic Reactions by Aldehyde Dehydrogenases**

Hideo Konno, Kazuhiko Sakamoto, and Osamu Ishitani*

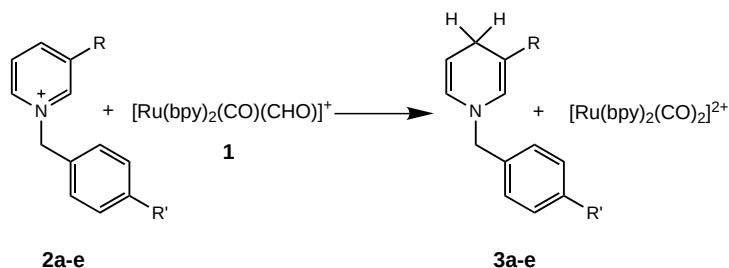
The nicotinamide adenine dinucleotide coenzyme NAD⁺ is required for the clearance of potentially toxic aldehydes by the enzymes of the aldehyde dehydrogenase (ALDH) family.^[1,2] For example, the oxidation of formaldehyde by NAD⁺ occurs in the presence of the formaldehyde dehydrogenase (EC 1.2.1.46.) to give formic acid and 1,4-NADH (Scheme 1).^[3] A crucial point of this reaction is the direct and



Scheme 1. The oxidation of formaldehyde by NAD⁺ in the presence of formaldehyde dehydrogenase. For clarity, only the pyridine ring of NAD⁺ and the 1,4-dihydropyridine ring of NADH are shown; the remainder of the molecule in each case is represented by X.

regiospecific hydrogen transfer from the carbonyl carbon of the aldehyde to the 4-position of the pyridinium ring of NAD⁺.^[4] In nonbiological systems, however, only a few nonenzymatic models for this important biological reaction have been reported so far.^[5] Herein we report the regiospecific reduction of NAD⁺ model compounds **2a–e** by a ruthenium formyl complex *cis*-[Ru(bpy)₂(CO)(CHO)]PF₆ (**1**; bpy = 2,2'-bipyridine) which acts as a mimic of the formaldehyde dehydrogenase reaction.

In a typical run, the reaction of **1** (7.6 mmol) with **2a** (11.4 mmol) was carried out in CD₃CN (0.75 mL) at 0 °C under an argon atmosphere in dim light, and the progress of the reaction was monitored by ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectroscopy. Within a few minutes, 1-benzyl-1,4-dihydronicotinamide (**3a**) and an equimolecular amount of *cis*-[Ru(bpy)₂(CO)₂]²⁺ were produced in a quantitative yield based on the quantity of **1** used in the reaction (Scheme 2). The other isomers of dihydronicotinamide, that is, the 1,2-dihydro and 1,6-dihydro forms, were not detected at all.



Scheme 2. The reduction of NAD⁺ models **2a–e** by the ruthenium complex **1**. For respective R and R' groups, see Table 1. For reaction conditions, see text.

Other NAD⁺ models with various electron-withdrawing groups at the 3-position were also regioselectively reduced by **1** to give the corresponding 1,4-dihydro forms (Scheme 2). Table 1 contains a summary of the results of the reactions, together with the reduction potentials of **2a–e**. The reductions of **2a–d** were completed within 15 min along with the quantitative formation of the corresponding 1,4-dihydro forms. In the case of **2e**, the reaction was negligible at 0 °C but occurred significantly at 25 °C to result in selective formation of **3e**, albeit in lower yield than was obtained from the other NAD⁺ models.

In order to identify the hydrogen origin for the selective reduction of **2a–e**, isotope experiments were carried out by two methods. The reduction of **2a** with *cis*-

[*] Prof. O. Ishitani, H. Konno, Prof. K. Sakamoto
Graduate School of Science and Engineering
Saitama University
255 Shimo-Okubo, Urawa, Saitama 338-8570 (Japan)
Fax: (+81) 48-858-3818
E-mail: ishitani@apc.saitama-u.ac.jp

[**] bpy = 2,2'-Bipyridine. We acknowledge helpful discussions with Dr. Chyongjin Pac (Kawamura Institute of Chemical Research). We also thank the Kojima Chemical Company for a generous gift of pure RuCl₃·3H₂O.

Table 1. Results of the reduction of NAD⁺ model compounds **2a–e** by *cis*-[Ru(bpy)₂(CO)(CHO)]PF₆ (**1**; see Scheme 2) and the reduction potentials *E*_{cp} of the models.^[a]

NAD ⁺ models	R	R'	<i>t</i> [min]	Conversion of 1 [%]	Yield of 3 [%] ^[b]	<i>E</i> _{cp} [V] ^[c]
2a	CONH ₂	H	15	100	100	−1.36
2b	CF ₃	H	15	100	100	−1.23
2c	COCH ₃	H	15	100	100	−1.26
2d	CONH ₂	CH ₃	15	100	100	−1.40
2e	CONEt ₂	H	30	15	20	−1.43
2e	CONEt ₂	H	300	100	20	−1.43

[a] The reactions of the NAD⁺ model compounds **2a–2d** with **1** were carried out at 0 °C in a CD₃CN solution. With model compound **2e** the reaction was performed at 25 °C. [b] Based on the amount of **1** consumed during the reaction. [c] Reduction potentials were measured against Ag/Ag⁺ and were irreversible. The scan rate was 200 mV s^{−1}.

[Ru(bpy)₂(CO)(CDO)]⁺ (> 95 % D) resulted in almost quantitative deuterium incorporation (> 95 %) at the 4-position of **3a**. By contrast, no deuterium incorporation into the dihydropyridine ring of **3a** occurred in the reaction of **2a** with **1** in a CD₃CN solution containing a drop of D₂O, while the exchangeable protons of the carbamoyl group were quantitatively deuterated. This clearly demonstrates that regioselective hydrogen transfer occurs from the formyl ligand to the 4-position of the pyridinium ring of **2a**. This is probably true for the reduction of the other NAD⁺ models. Since the formyl ligand of **1** can be regarded as an activated form of formaldehyde coordinated to the Ru^{II} center, the present reactions of the NAD⁺ model compounds with **1** might provide a useful mimic for the formaldehyde dehydrogenase reaction.^[6] In this regard, it should be noted that “free” formaldehyde is essentially unreactive with NAD⁺ models.^[7]

Recently, Kanomata and co-workers reported that a magnesium 1,1-diolate compound can reduce some particular NAD⁺ models with relatively high regioselectivities.^[5] This might be considered as the first example of an aldehyde (D-glyceraldehyde-3-phosphate) dehydrogenase model. In this model, however, the regioselective reduction occurs only for particular NAD⁺ models possessing a bulky tertiary amide group, while the reactions of conventional model compounds are accompanied by significant formation of other isomeric products, for example, the 1,6-dihydro form in 5–27 % yield. This is in contrast to the present model reactions that have accomplished the efficient regioselective reduction of the typical NAD⁺ models, such as **2a**, into the 1,4-dihydro products, as shown in Table 1.

Among the reductants used for regioselective reductions of NAD⁺ and model compounds in nonenzymatic systems,^[8a] sodium dithionite has been most often used as a conventional reductant. In the reactions using this reductant, however, the other isomers of the 1,4-dihydro form are produced in significant quantities as by-products. This is probably, at least in part, due to thermal isomerization of the initially formed 1,4-dihydro form to the other isomers,^[8] because the reactions of NAD⁺ models with this reductant require high temperatures and relatively long reaction times. Metal hydrides are also interesting reductants for NAD⁺ and models,^[9] but some of them have serious limitations arising from the isomerization of the 1,4-dihydro form into other isomers, a process catalyzed by intermediates produced from the starting metal complexes,^[9b,c] as well as from the remarkable dependence of the reduction capability on the structures of the NAD⁺

derivatives.^[9d] Compared with such reductants, **1** certainly has an advantage in the efficient regioselective 1,4-reduction of a wide variety of NAD⁺ models and, hence, the present system is potentially useful as a preparative method for various 1,4-NADH model compounds.

With regard to the regioselectivity in the present reactions, it is reasonable to assume that interactions of the Ru^{II} center^[10] or the formyl carbon with the substituents at the 3-position of the NAD⁺ models control the hydrogen transfer from **1** to the 4-position of **2a–e**, as suggested by Fish and co-workers for reductions of NAD⁺ models using [Cp*Rh(bpy)H]⁺ (Cp* = pentamethylcyclopentadienyl).^[9b] This mechanistic assumption is apparently in accord with the much slower reactivity of **2e** which has the bulky diethyl carbamoyl group, since the steric congestion of the carbamoyl group should inhibit interactions of the carbamoyl oxygen and/or nitrogen with the Ru complex. Nevertheless, the reduction of **2e** is regioselective, indicating the essential role of the Ru complex–carbamoyl interactions in the hydrogen transfer. The low yield of **3e** is due to the significant chemical changes in **1** which occur at the reaction temperature (25 °C) to give [Ru(bpy)₂(CO)(CD₃CN)]²⁺, [Ru(bpy)₂(CO)(CH₂OH)]⁺, and other unidentified compounds.^[11] In the case of **2b**, the CF₃ group might interact with the Ru complex through the electronegative fluorine atoms. Such interactions would certainly be weaker than those of the carbamoyl and acetyl groups but would still be effective enough to control the regiochemistry in the hydrogen transfer. Studies into the mechanistic details and synthetic applications of the present reactions are in progress.

Experimental Section

Formyl complex **1** was prepared according to the reported procedure.^[10a] and a similar method was used for the synthesis of [Ru(bpy)₂(CO)(CDO)]⁺ using NaBD₄ (98 atom % D), MeOD (99.5 atom % D), and D₂O (99.8 atom % D); the deuterium purity of the product was confirmed to be > 95 % using ¹H NMR spectroscopy. The NAD⁺ and NADH model compounds were synthesized according to reported procedures^[8,12] or by methods similar to that required for **2a**. The melting points, elemental analysis results, and ¹H NMR and ¹³C NMR spectroscopic data of the new model compounds are as follows:

2b: m.p.: 118–119 °C (decomp); ¹H NMR (400 MHz, CD₃CN): δ = 9.20 (s, 1 H; H-2), 8.91 (d, *J* = 6.2 Hz, 1 H; H-6), 8.80 (d, *J* = 8.2 Hz, 1 H; H-4), 8.21 (dd, *J* = 8.2, 6.2 Hz, 1 H; H-5), 7.49 (m, 5 H; Ph), 5.78 (s, 2 H; CH₂Ph); ¹³C NMR (100 MHz, CD₃CN): δ = 148.9, 144.5, 143.9, 132.9, 131.3, 130.8, 130.6, 130.6, 66.3; elemental analysis: calcd for C₁₃H₁₁F₃NP (383.20): C 40.75, H 2.89, N 3.66; found: C 40.47, H 2.76, N 3.67.

2d: m.p.: 101–102 °C (decomp); ¹H NMR (400 MHz, CD₃CN): δ = 9.12 (s, 1H; H-2), 8.80 (d, *J* = 6.2 Hz, 1H; H-6), 8.75 (d, *J* = 8.1 Hz, 1H; H-4), 8.09 (dd, *J* = 8.1, 6.2 Hz, 1H; H-5), 7.35 (d, *J* = 8.0 Hz, 2H; Ph), 7.29 (d, *J* = 8.0 Hz, 2H; Ph), 7.13 (br. s, 1H; CONH₂), 6.57 (br. s, 1H; CONH₂), 5.70 (s, 2H; CH₂Ph), 2.35 (s, 3H; CH₃); ¹³C NMR (100 MHz, CD₃CN): δ = 163.7, 146.9, 145.4, 145.0, 141.4, 135.5, 131.1, 130.5, 130.4, 129.5, 65.7, 21.3; elemental analysis: calcd for C₁₄H₁₅F₆N₂OP (372.25): C 45.17, H 4.06, N 7.53; found: C 45.33, H 3.93, N 7.52.

Cyclic voltammograms of the NAD⁺ model compounds were measured in acetonitrile solution that contained tetra-*n*-butylammonium tetrafluoroborate (0.1M) as the supporting electrolyte. An ALS/CHI CHI-620 electrochemical analyzer, with a Pt disk working electrode, an Ag/AgNO₃ (0.01M) reference electrode, and a Pt counter electrode was used for the analysis.

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A New Self-Assembling System for Targeted Gene Delivery**

Michael A. W. Eaton,* Terence S. Baker, Catherine F. Catterall, Kenneth Crook, Graham S. Macaulay, Barbara Mason, Timothy J. Norman, David Parker,* Justin J. B. Perry, Richard J. Taylor, Alison Turner, and A. Neil Weir

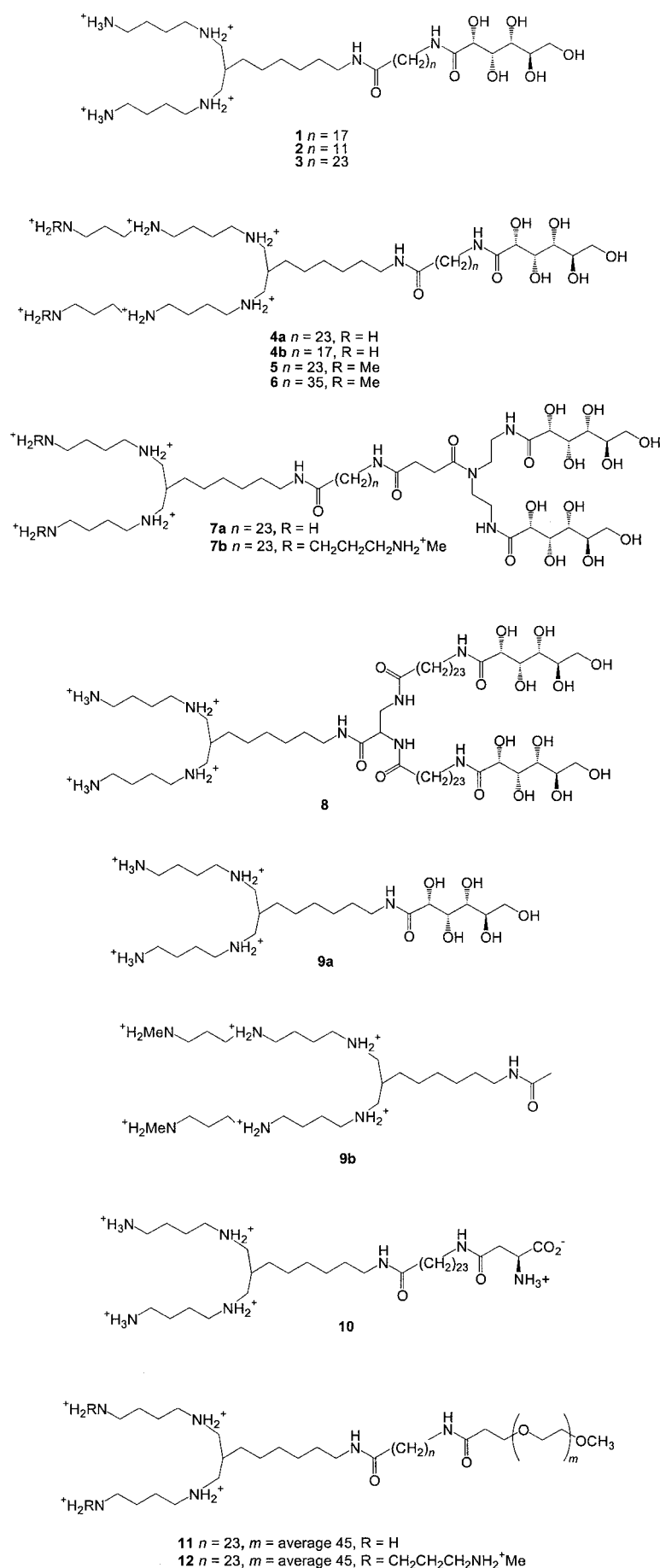
The goal of targeted nonviral gene therapy has aroused considerable interest amongst scientists in recent years.^[1] The ability to deliver recombinant DNA both selectively and efficiently to a given cell-type requires several features working effectively in harmony. Preferably the DNA needs to be compacted to protect it from enzymatic attack,^[2] and tightly bound to a suitable targeting ligand.^[1b] The resultant vector needs to be efficiently delivered to the target cell population with sufficient selectivity to minimize unwanted interaction with other cell types. Finally, once internalized into the desired cell the DNA must escape from the endosome into the cytoplasm^[3] and enter the nucleus while being protected from the action of nucleases en route. Each of the above stages presents a major scientific challenge and different strategies have been formulated. Cationic liposomes, for example, have been studied in detail,^[4] but lack specificity in targeting and generally yield heterogenous complexes with DNA. Cationic polymers, notwithstanding their toxicity in vivo, strongly bind DNA and may afford a pH-dependent release mechanism through a conformational change in the polymer in the more acidic medium of an endosome.^[5] We now report our preliminary work to develop a new modular supramolecular approach to overcome the challenges associated with targeted gene delivery.

Compaction of DNA in nature is achieved through charge neutralization of the polyanion by interaction with the protonated polyamine spermine.^[6] Accordingly, we have prepared a series of amphiphilic tetra- and hexaamines **1**–**12**, which as a consequence of the C₃ or C₄ spacing between each nitrogen site possess 3.6 or 5.2 positive charges at

[*] Dr. M. A. W. Eaton,^[+] T. S. Baker, Dr. C. F. Catterall, Dr. K. Crook, Dr. G. S. Macaulay, B. Mason, Dr. T. J. Norman, Dr. R. J. Taylor, A. Turner, Dr. A. N. Weir
Celltech Chiroscience Ltd.
216 Bath Road, Slough, SL1 4EN (UK)
Fax: (+44) 1753-536-632
E-mail: meaton@celltech.co.uk
Prof. D. Parker, Dr. T. J. Norman, Dr. J. J. B. Perry
Department of Chemistry
University of Durham
South Road, Durham, DH1 3LE (UK)
Fax: (+44) 191-386-1127
E-mail: david.parker@dur.ac.uk

[+] Present address:
Swords Laboratories, Bristol Myers Squibb
Watery Lane, Swords, Co. Dublin (Ireland)

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ambient pH (7.4).^[7a] The DNA-binding polyamine “head” group is separated from a hydrophilic “tail” group by a long-chain hydrocarbon varying in length from C_{12} to C_{36} . The hydrophilic tail groups are either sugars, such as those shown in structures **1–9** (prepared by the reaction of δ -gluconolactone with the appropriate primary amine), zwitterionic amino-acids, for example, **10**, or polyoxyethylene glycol chains (PEG, MW 2000) for example, **11** or **12**. Such non-ionic surfactant tail groups improve water solubility, are biocompatible, and may reduce nonspecific interactions such as protein binding and subsequent uptake by the reticuloendothelial system. The amphiphilic structures **1–12** resemble bolaamphiphiles^[8] and may be expected to self-assemble in solution. Similar aggregates may also be expected to form readily in mixtures where the long-chain hydrocarbon and polyamine groups are held constant but the hydrophilic tail group is varied. Control over DNA compaction and surface-coating characteristics in a supramolecular assembly comprised of a bolaamphiphile and condensed DNA may be achieved by varying the relative proportion of each component.^[7c]

Preliminary DNA-binding experiments were carried out with super-coiled plasmid DNA (pH 7.4, 150 mM NaCl) to rank the relative DNA-binding affinities of bolaamphiphiles (“bolas”) **1–12**. Binding affinity was monitored by a dye-exclusion assay where the titration of the bola (increasing equivalents of the bolaamine versus a constant amount of DNA phosphate, “N:P ratio”) caused a reduction in the intensity of the fluorescence emission from an ethidium bromide/DNA complex.^[9] The following observations were made: a) bolas **8** (with two C_{24} chains) and **10** (with an amino acid tail group) bound comparatively weakly, and b) DNA-binding affinity varied with the length of the hydrocarbon chain for a given polyamine and tail group and followed the order $C_{12} < C_{18} < C_{24} > C_{36}$. Differences in the binding strength between tetraamine- and hexaamine-based bolas were apparent for the C_{18} chain ($N_6 > N_4$), but were not evident for C_{24} -chain bolas, for example, **7a** versus **7b**.

The particles that formed at N:P ratios of 0.5:1 and above were stable over the pH range 3.5 to 7.5, as judged by the dye-exclusion assay and agarose gel electrophoresis. However, the particles formed at N:P ratios of 2:1 and above resisted the attack of added Dnase I or of foetal calf serum when tested for plasmid integrity.^[10] Photon correlation spectroscopy (PCS) suggested that the particles which formed at N:P ratios of about 0.7:1 or at greater than 2:1, without the inclusion of any PEG-bola (**11** or **12**), had a mean diameter of the order of 300 nm. Considerably larger aggregates formed at N:P ratios between 1:1 and 2:1, presumably as a

result of charge neutralization allowing the formation of lattices. More compact particles however were observed to form across the same range of N:P ratios with the inclusion of as little as 2% of a PEG derivative (**11** or **12**). Field-flow fractionation (a separation technique based on size) coupled with multi-angle laser-light scattering allowed time-dependent DNA-compaction studies to be carried out.^[11] Examination of the DNA particles formed from 13 mol% of **12** and 87 mol% of **5** at a N:P ratio of about 1:1 (Figure 1) revealed behavior consistent with the formation of well-defined overall

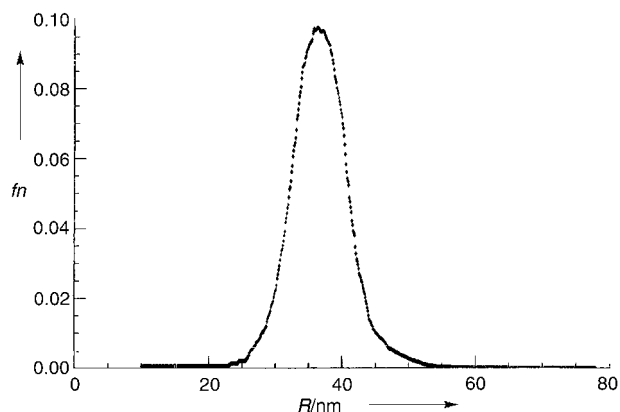


Figure 1. Field-flow fractionation data using multi-angle laser light scattering highlighting the dispersity in particle size for the complex of **5:12** (87:13 mol%) and 50 μ m plasmid DNA (N:P ratio 0.86:1, 293 K, 150 mM NaCl, pH 7.4, 20 mM HEPES), with the differential number fraction f_n expressed as a function of the root-mean-square radius (nm).

spherical particles. A time-independent (over two days) root-mean-square radius (R_{rms}) of 41 nm was observed, a value that compares well with the hydrodynamic radius (R_w) of 48 nm as measured by PCS (theoretical value of $R_w = 1.29 \times R_{\text{rms}}$ assuming a spherical structure^[11, 12]). Measurements of the zeta potential on the same preparations over a N:P ratio of 0.5:1 to 2:1, revealed a surface charge on the particles ranging between -5 and $+10$ mV. In contrast, particles formed from **5** in the absence of PEG-bola exhibited a surface charge ranging from -40 mV to $+20$ mV, which strongly suggests that the PEG tail groups shield the charge on the particle surface.

The nature of the DNA in the particles was examined by circular dichroism (150 mM NaCl, 20 mM *N*-[2-hydroxyethyl]-piperazine-*N'*-[2-ethanesulfonic acid] (HEPES), pH 7.4). As the bolas were added to the B-form DNA the intensity of the longest wavelength (positive) band shifted (from 268 to 290 nm) and reduced in overall intensity to reach a limiting value at an N:P ratio of 1:1 or more. The position of zero-CD (cross-over from negative to positive dichroism) shifted simultaneously from 257 to 268 nm (Figure 2), which is consistent with the retention of the B-form DNA in the bound state.^[13, 14] Taken together, these physicochemical studies are consistent with the formation of spherical or toroidal particles in which the polyamine-bound DNA is compacted inside the supramolecular assembly, with the hydrocarbon chains separating it from the hydrophilic tail groups and bulk water.

The plasmid DNA pEGlacZ,^[12] which codes for the enzyme β -galactosidase, was condensed with the sugar-bola **5** or PEG-

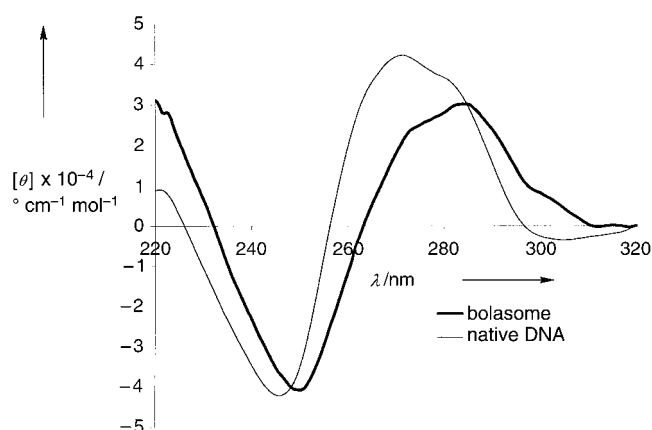


Figure 2. CD spectra of 50 μ m plasmid DNA (293 K, 150 mM NaCl, 20 mM HEPES, pH 7.4) in the absence (thin line) and presence (bold) of the bolaamphiphile **2** (0.6:1 N:P ratio).

bola **12** at N:P ratios of 0.5:1, 1.5:1, and 4:1 and tested for transfection competence in Chinese hamster ovary cells.^[15] The PEG-bola derivative resulted in reporter-gene activity at around the detection limit of the β -galactosidase assay (Figure 3). In contrast the sugar-bola derivative gave a clearly

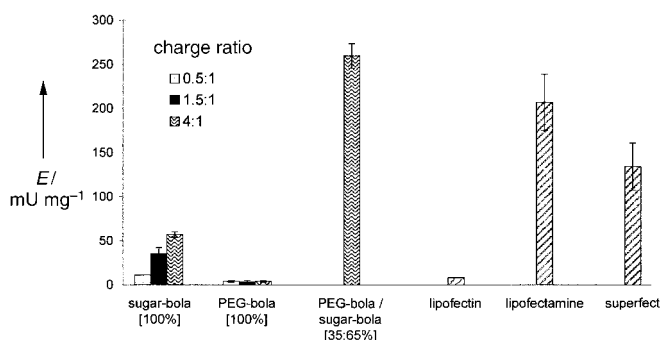
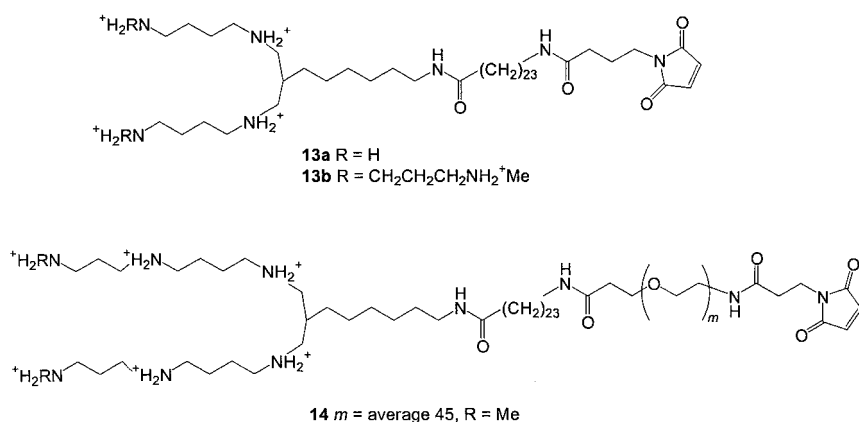


Figure 3. Transfection of Chinese hamster ovary cells with bola/DNA complexes and comparison with commercial transfection reagents.

detectable level of β -galactosidase for particles prepared at the 0.5:1 N:P ratio, and increased by threefold at 1.5:1 and by fivefold at 4:1 N:P ratio. For comparison results are shown for the commercial transfection reagents lipofectin, lipofectamine, and superfect tested with the same plasmid under optimal conditions according to the manufacturer's instructions. The rank order of reporter-gene expression obtained was lipofectamine > superfect > lipofectin, with the sugar-bola at an N:P ratio of 4:1 giving a transfection level intermediate between that of superfect and lipofectin.

When a mixture of sugar-bola and PEG-bola (65:35 mol%) was used to condense plasmid DNA at a 4.1 N:P ratio the resulting reporter-gene expression increased by over fourfold to that obtained with the sugar-bola complex and was comparable to that obtained with lipofectamine. No acute toxicity was found at up to 70 μ g bola/DNA doses in mice.

To demonstrate that antibody-mediated targeting of the bola-condensed DNA particles or "bolasomes" occurred, an antibody Fab' fragment with an exposed hinge cysteine thiol residue was selectively conjugated to the maleimide group of **13** or the PEG-bola derivative **14**. An anti-CD3 antibody was



chosen to direct the targeting to the T cell receptor complex present on human T lymphocytes and on Jurkat cells, a human T-cell line which has been reported to be relatively refractive to transfection.^[16] Accordingly, a mixture of the anti-CD3-Fab'-bola conjugate (0.004 mol %), sugar-bola **5** (67 mol %), and PEG-bola **12** (33 mol %) was used to form anti-CD3 bolasomes at a 3:1 N:P ratio. Control bolasomes containing a Fab' fragment of irrelevant specificity were also prepared.

An AFM image^[17] (Figure 4) of such particles revealed similar overall dimensions to those characterized by PCS, the majority with a diameter of about 80 nm. The transfection competence of both types of particle was tested on Jurkat cells^[18] and resulted in a significant level of reporter-gene

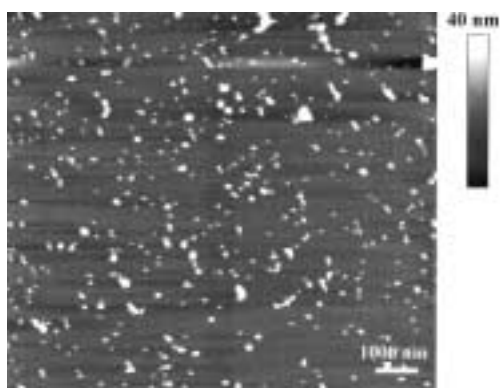


Figure 4. AFM image of particles comprising **5** (67 mol %) and **12** (33 mol %) with 0.004 mol % of the anti-CD3-Fab conjugate of **14**, 3:1 N:P ratio (295 K, 10 mM HEPES).

expression (733 ± 300 pg of β -galactosidase per mg of cytosolic protein of cell extract) for the anti-CD3 bolasomes compared to undetectable expression for the control bolasomes. Furthermore, the specificity of the transfection was confirmed by competitive displacement of the anti-CD3 bolasome in the presence of a large excess ($20 \mu\text{mL}^{-1}$) of

free anti-CD3 IgG, which resulted in complete abolition of transfection. Antibody targeting of the bolasome to the T cell receptor complex has thus allowed uptake and intracellular release of the plasmid DNA, presumably by a receptor-mediated endocytosis pathway.

These studies augur well for the future development of a robust and selectively targeted DNA-delivery system. A new class of water-soluble DNA-compacting agents has been defined which not only induce self-assembly of plasmid DNA into discrete particles of around 100 nm diameter, but also provide the means of regulating surface properties through their hydrophilic tail groups. Importantly, the surface charge of the particles may be controlled by the introduction of PEG groups which serve to minimize certain nonspecific interactions in vivo. Furthermore, the incorporation of a mixture of PEG-bola and sugar-bola, in a defined ratio, improves the transfection competence of the bolasome. Finally, targeted transfection of a chosen cell type can be achieved using a bolasome that includes a bola-conjugated antibody.

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- a) A. E. Martell, R. M. Smith, *Critical Stability Constants, Vol. 1–6*, Academic Press, New York, **1974–1989**; pK_a values for **9a** and **9b** were measured using potentiometric methods (0.1 M NMe₄Cl, 298 K) followed by data analysis using SUPERQUAD: **9a**: 10.76(3), 10.03(2), 9.29(3), 7.59(4); **9b**: 10.49(4), 10.16(3), 9.31(3), 8.61(3), 8.00(2), 7.10(4). Individual data sets typically gave statistical σ and ψ^2 values of 0.27 and 6; b) details of these and additional syntheses are given in: M. A. W. Eaton, T. J. Norman, D. Parker, T. S. Baker, A. N. Weir, C. F. Catterall, WO-A 99/52858, **1999** [*Chem. Abstr.* **1999**, 131(22), 2993 156]; c) syntheses^[7b] were carried out using standard polyamine and peptide coupling procedures and each compound (**1–12**) gave satisfactory ¹H NMR, ES-MS, and microanalytical data in accord with the proposed structures. A representative synthesis for **1a** involved the condensation of diethyl (2-benzyloxyhexyl)malonate with excess diaminobutane, followed by borane reduction (BH₃·THF), Boc protection (Boc₂O/MeOH), hydrogenolysis (Pd(OH)₂/C, H₂-tBuOH), mesylation (MeSO₂Cl/Et₃N/CH₂Cl₂), and amination via the primary

azide (NaN_3 -DMF then H_2 /Pd-C/MeOH) to give a Boc-protected primary amine. This was coupled with 24-(penta-*O*-acetylglucuronyl)amino-tetracosanoic acid (prepared by the opening of δ -gluconolactone with 24-amino-tetracosanoic acid (MeOH, 60 °C, DBU, 10 mins), followed by peracetylation (Ac_2O , pyridine)) using EDC/NHS/MMM- $\text{Et}_3\text{N}/\text{CH}_2\text{Cl}_2$. Deacetylation (aqueous ammonia/MeOH) and removal of the Boc groups ($\text{CF}_3\text{CO}_2\text{H}$ 96%, H_2O 4%, 20 °C, 30 mins) yielded the tetra-trifluoroacetic acid salt of **1** as a colorless lyophilisate. Boc = *tert*-butoxycarbonyl; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; EDC = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide; NHS = *N*-hydroxysuccinimide; MMM = *N*-methylmorpholine.

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- [15] Transfection complexes were prepared in 10 mM HEPES pH 8.0 buffer by adding a plasmid solution dropwise to an equal volume of bola solution to give a final concentration of 30 $\mu\text{g mL}^{-1}$ DNA. N:P ratios were calculated assuming the nucleotide residue had a formula weight of 310. The transfection reagents lipofectin and lipofectamine were obtained from Life Technologies Ltd. and superfect from Qiagen Ltd. Cell culture media and additives were obtained from Life Technologies Ltd. Chinese hamster ovarian cells were plated out in 24-well cell culture plates at 10^5 cells per well in 1 mL culture medium (Iscoves complete medium containing 2 mM glutamine and 10% foetal calf serum). After 24 h the cells were washed with 1 mL of Optimem medium per well. Transfection was carried out over 4 h at 37 °C in the presence of 500 μL of Optimem and 167 μL of condensed DNA (5 μg), then the medium was replaced with 1 mL of culture medium and the incubation continued for a total of 48 h. The levels of the activity of the β -galactosidase reporter gene in the cells were assessed using an enzyme activity kit (Promega Ltd) according to the manufacturer's instructions, where the hydrolysis of the substrate *o*-nitrophenyl- β -D-galactopyranoside was measured spectrophotometrically in washed, lysed-cell supernatants. The assay was calibrated against a β -galactosidase standard (Boehringer Mannheim Ltd.; 0.775 mU enzyme activity per ng of protein). The results were normalized for supernatant protein concentration following measurement of the latter using a BCA protein assay kit (Pierce and Warriner Ltd.)
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- [18] Jurkat E6.1 cells (ECACC) were cultured in Dulbecco's modified eagles medium (DMEM) (Life Technologies Ltd.) that contained 10% foetal calf serum, 2 mM of glutamine, 50 units mL^{-1} of penicillin, and 50 $\mu\text{g mL}^{-1}$ of streptomycin. For transfection, cells were washed and re-suspended in serum-free DMEM at 2×10^6 cells mL^{-1} and 0.5 mL per well added to 24-well culture plates. Bola-condensed DNA (200 μL) was added at 4 $\mu\text{g well}^{-1}$ and the plates incubated for 1 h at

37 °C in 5% CO_2 . Incubation was continued for a total of 24 h following addition of 300 μL of 33% foetal calf serum in DMEM. Cells were harvested by centrifugation and washing with phosphate buffered saline. Cells were lysed and supernatants assayed using a β -galactosidase enzyme-linked immunosorbent assay (ELISA) kit (Roche Diagnostics Ltd). Reporter gene levels were normalized for cytosolic protein.^[15]

A Versatile Periodate-Coupled Fluorogenic Assay for Hydrolytic Enzymes**

Fabrizio Badalassi, Denis Wahler, Gérard Klein, Paolo Crotti,* Jean-Louis Reymond*

The development of new catalysts is being increasingly followed by using combinatorial and evolutionary methods.^[1–3] These approaches require the ability to assay large numbers of samples in parallel. This can be achieved using solid-phase bound assays related to immunoassays^[4] and a variety of spectroscopic methods, such as IR thermography^[5] and mass spectrometry.^[6] One of the most popular methods is the use of chromogenic and fluorogenic substrates as product formation sensors.^[1, 7, 8] Herein, we report a new versatile fluorogenic assay for hydrolytic enzymes. The assay couples product formation to the release of a fluorescent signal, achieved via periodate oxidation and albumin-catalyzed β -elimination, and uses nonactivated, chiral substrates.

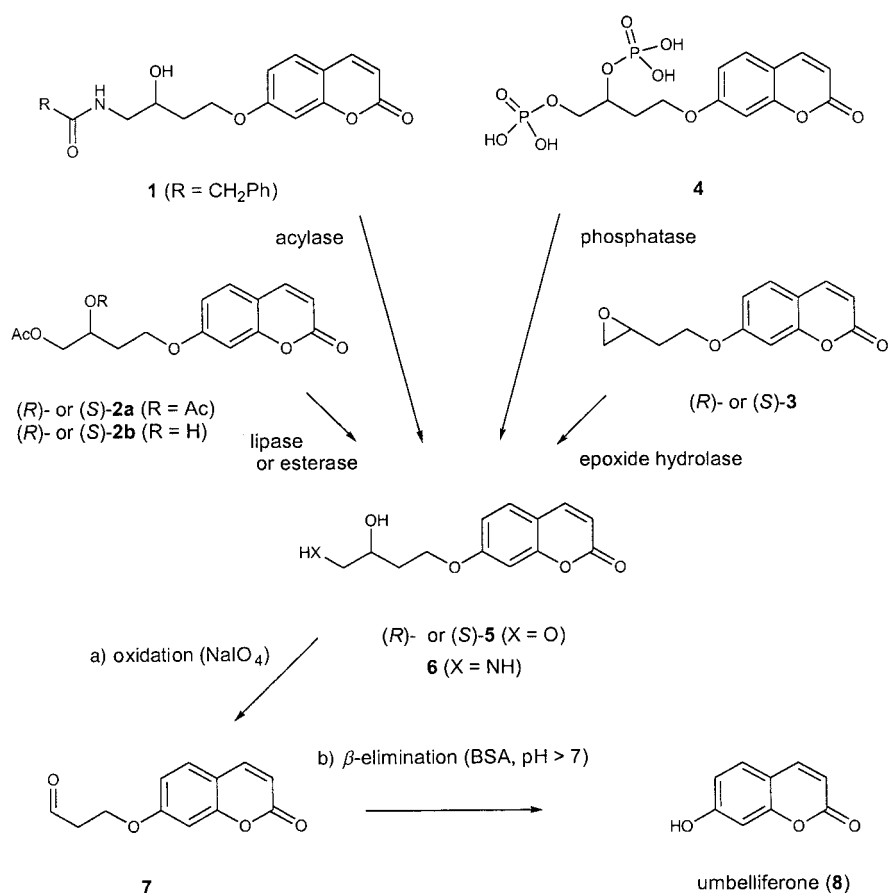
We recently reported an enantioselective fluorogenic assay for alcohol dehydrogenases based on the detection of carbonyl oxidation products, such as **7** (Scheme 1), by β -elimination of the fluorescent product umbelliferone (**8**) catalysed by bovine serum albumin (BSA).^[9, 10] Aldehyde **7** can also be produced from diol **5** or aminoalcohol **6** by oxidation with

[*] Prof. Dr. P. Crotti, F. Badalassi^[+]
Dipartimento di Chimica Bioorganica e Biofarmacia
Università di Pisa
56126 Pisa (Italy)
Fax: (+39)050-43321
E-mail: crotti@farm.unipi.it

Prof. Dr. J.-L. Reymond, D. Wahler,^[+] G. Klein
Department of Chemistry and Biochemistry
University of Bern
Freiestrasse 3, 3012 Bern (Switzerland)
Fax: (+41)31-631-8057
E-mail: jean-louis.reymond@ioc.unibe.ch

[+] These authors contributed equally to the work

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Scheme 1. Periodate-coupled fluorogenic assay for esterases and lipases, epoxide hydrolases, and amidases.

sodium periodate, a reagent known for cleaving diols and aminoalcohols with high chemoselectivity in the presence of other functional groups and used routinely for glycoprotein functionalization.^[11] Therefore, we envisioned that hydrolytic enzymes releasing **5** or **6** from precursors resistant to periodate oxidation would become detectable by fluorescence in the presence of periodate and BSA. Amides such as **1** would react with acylases. Diesters (*R*)-**2a** and (*S*)-**2a** or the monoesters (*R*)-**2b** and (*S*)-**2b** should be excellent substrates for lipases and esterases due to their structural resemblance to glycerides.^[12] Epoxides (*R*)-**3** and (*S*)-**3** would react with epoxide hydrolases and bis-phosphate **4** would react with phosphatases.

Diol **5** and epoxide **3** was prepared by OsO_4 -catalyzed dihydroxylation or *meta*-chloroperbenzoic acid (MCPBA) epoxidation of the parent olefin, respectively. The enantiomers (*S*)-**5** (96.6% enantiomeric excess, *ee*) and (*R*)-**5** (99.2% *ee*) were obtained by dihydroxylation with ADMix α and ADMix β ,^[13] respectively, and converted into epoxides (*S*)-**3** (87% *ee*) and (*R*)-**3** (96% *ee*) by the one-pot Sharpless procedure^[14] or acetylated to (*S*)-**2a** and (*R*)-**2a**. Partial acetylation of either (*S*)-**5** or (*R*)-**5** gave the monoacetates (*S*)-**2b** and (*R*)-**2b**. The racemic aminoalcohol **6** was obtained by aminolysis of the corresponding racemic epoxide **3** with aqueous ammonia^[15] and converted to the phenylacetamide **1** by reaction with the acyl chloride. Bis-phosphate **4** was obtained from the racemic diol **5** by phosphorylation with

dibenzyl *N,N*-diisopropyl phosphoramidite, oxidation with MCPBA, and catalytic hydrogenation (1 atm H_2 , Pd/C, aq EtOH, 1 h).

Substrates **1–4** were found to be stable in the presence of periodate and BSA in aqueous buffers. By contrast, diol **5** and aminoalcohol **6** released umbelliferone (**8**) in 72% and 85% yield, respectively (100 μM substrate, 1 mM NaIO_4 , 2 mg mL^{-1} BSA in pH 8.8 borate buffer). The yield of oxidation was independent of the presence of BSA. The reaction was fast ($t_{1/2} = 15$ min (**5**) and 3 min (**6**)) and would thus reveal any hydrolysis of substrates **1–4** within minutes.

Phenylacetamide **1** was tested with penicillin G acylase, chymotrypsin, and papain, acetates **2a** and **b** with 25 different esterases and lipases, epoxides **3** with epoxide hydrolase (EH) from *Aspergillus niger*^[16] and *Rhodotorula glutinis*,^[17] and bis-phosphate **4** with alkaline phosphatase (AP) from calf intestinal mucosa. A time-dependent increase in fluorescence was observed in the cases where a suitable enzymatic activity was present (Figure 1, Tables 1 and 2; similar results were obtained at pH 7.2). HPLC analysis confirmed that fluorescence originated in umbelliferone (**8**).

The results were similar with or without preincubation with enzyme before addition of periodate and BSA, to show that these reagents did not affect the enzymes. HPLC analysis of the reactions without periodate and BSA showed that **5** and **6**

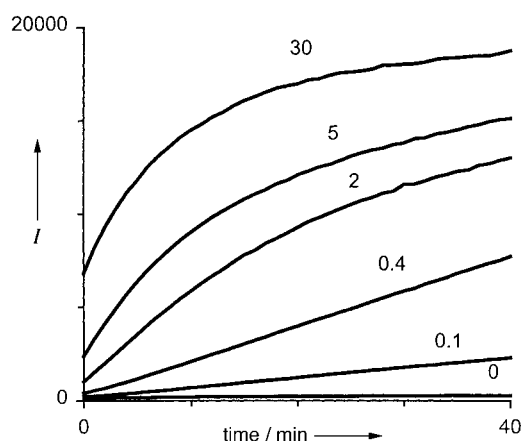


Figure 1. Development of fluorescence intensity (I , at $\lambda = 460$ nm) for the treatment of amide **1** (0.1 mM) at the indicated concentrations of penicillin G acylase (in $\mu\text{g mL}^{-1}$) in the presence of NaIO_4 (1 mM) and BSA (2 mg mL^{-1}) in aq borate buffer (20 mM, pH 8.8, 26 °C). Penicillin G acylase is known to cleave phenylacetamides. There was no reaction in the presence of chymotrypsin or papain (each 0.1 mg mL^{-1}). At high enzyme concentrations, the oxidation/ β -elimination of **6** becomes rate limiting, so that the apparent release rate of **8** is not proportional to enzyme concentration.

Table 1. Apparent reaction rates^[a] of acetates **2a** and **2b** with lipases and esterases.

Lipase source	$V_{(R)-2a}$ [pM s ⁻¹]	$V_{(S)-2a}$ [pM s ⁻¹]	E_{2a} ^[b]	$V_{(R)-2b}$ [pM s ⁻¹]	$V_{(S)-2b}$ [pM s ⁻¹]	E_{2b} ^[b]
<i>Aspergillus niger</i>	40	265	6.5 (S)	3150	3500	1.11 (S)
<i>Aspergillus oryzae</i>	—	—	—	1230	1510	1.23 (S)
<i>Candida antarctica</i>	4230	3940	1.07 (R)	950	1490	1.57 (S)
<i>Candida cylindracea</i>	—	—	—	435	545	1.25 (S)
<i>Chromobacterium visc.</i>	150	510	3.44 (S)	4530	5950	1.31 (S)
Pig pancreas	245	400	1.62 (S)	1490	3190	2.14 (S)
<i>Mucor miehei</i>	—	95	only S	670	720	1.08 (S)
<i>Pseudomonas cepacia</i>	250	580	2.31 (S)	690	1530	2.22 (S)
<i>Pseudomonas fluorescens</i>	3820	4290	1.12 (S)	13200	16700	1.27 (S)
<i>Pseudomonas</i> sp.	2080	1850	1.12 (R)	5870	8860	1.51 (S)
<i>Pseudomonas</i> sp. Type B	4840	5640	1.17 (S)	18100	23600	1.30 (S)
<i>Rhizomucor miehei</i>	—	—	—	640	610	1.05 (R)
<i>Rhizopus niveus</i>	—	—	—	350	540	1.54 (S)
Wheat germ	85	—	only R	1330	2170	1.63 (S)
Esterase source						
<i>Bacillus</i> sp.	—	—	—	245	—	only R
<i>Bac. stearothermophilus</i>	1520	330	4.59 (R)	2010	1580	1.27 (R)
<i>Bac. thermoglucosidasius</i>	170	55	2.82 (R)	640	415	1.54 (R)
Horse liver	1570	3790	2.41 (S)	4460	7530	1.69 (S)
<i>Mucor miehei</i>	—	75	only S	265	405	1.54 (S)
<i>Thermoanaerobium brockii</i>	—	—	—	215	355	1.66 (S)

[a] Apparent rate V as observed in the presence of substrate (0.1 mM), NaIO₄ (1 mM), BSA (2 mg mL⁻¹), and lipase or esterase (0.1 mg mL⁻¹), in aq borate (20 mM, pH 8.8, 26 °C). Fluorescence was converted into umbelliferone concentration according to a calibration curve with pure **8** in the same buffer containing sodium periodate and BSA. The error margin on V is ± 10 pM s⁻¹. The apparent rate of umbelliferone release from diol **5** is 64300 pM s⁻¹. No data are given when the activity was not distinguishable from the fluorescence curve of substrate without enzyme ($V < 20$ pM s⁻¹). [b] E = enantiomeric ratio between $V_{(R)}$ and $V_{(S)}$. Lipases and esterases *Penicillium roqueforti*, *Mucor javanicus*, *Candida lipolytica*, *Saccharomyces cerevisiae*, and *Rhizopus arrhizus* gave no reaction with either **2a** or **2b**.

Table 2. Apparent reaction rates with substrates **1** and **3–6**.^[a]

Substrate	Enzyme	V_{app} [pM s ⁻¹]
6	no enzyme	58400
1	Penicillin G acylase (1 μ g mL ⁻¹)	12820
1	α -Chymotrypsin from bovine pancreas (100 μ g mL ⁻¹)	15
1	Papain (100 μ g mL ⁻¹)	— 10
1	no enzyme	35
5	no enzyme	46300
3	EH from <i>Aspergillus niger</i> (42.0 μ g mL ⁻¹)	20940 (R), 23230 (S)
3	EH from <i>Aspergillus niger</i> (1.3 μ g mL ⁻¹) ^[b]	1785 (R), 2940 (S)
3	EH from <i>Rhodotorula glutinis</i> (100 μ g mL ⁻¹)	2845 (R) ^[c] , 1570 (S) ^[c]
3	no enzyme	720
4	AP from calf intestinal mucosa (100 μ g mL ⁻¹)	565 ^[d]
4	no enzyme	40

[a] Apparent rate of umbelliferone release as observed in the presence of substrate (0.1 mM), NaIO₄ (1 mM), and BSA (2 mg mL⁻¹) in aq borate buffer (20 mM, pH 8.8). Fluorescence was converted into umbelliferone concentration according to a calibration curve with pure **8** in the same buffer containing NaIO₄ and BSA. The error margin on V is ± 20 pM s⁻¹. Similar results were obtained at pH 7.2. [b] Detection limit for the epoxide hydrolase is 1 μ g mL⁻¹. [c] Values at 37 °C, the reported optimal temperature for this enzyme, are $V_{(R)-3}$ = 3500 and $V_{(S)-3}$ = 2000 pM s⁻¹. [d] Apparent rate observed in phosphate (20 mM, pH 7.2) is V_4 = 4380 pM s⁻¹.

were indeed released by the enzymes marked as active in the fluorescence assay. The assay was specific in that no fluorescence signal was observed when using heat-deactivated enzymes.^[18, 19]

The above experiments show that a versatile fluorogenic assay for hydrolytic enzymes is available using the secondary

processing of reaction products **5** or **6** to the fluorescent product **8** by sequential periodate oxidation and BSA-catalyzed β -elimination. This assay is also the first system for fluorescence detection of epoxide hydrolase activity.

Most commercially available fluorogenic enzyme substrates are reactive derivatives of phenols and anilines. Their chemical reactivity makes them completely unstable under certain screening conditions, such as elevated temperature or impure extracts,^[1a] and furthermore risks any high-throughput screening based on these products of isolating catalysts that are not capable of cleaving less-reactive substrates of synthetic interest. Our periodate-coupled assay overcomes these limitations by using completely nonactivated amides, esters, epoxides, and phosphates of aliphatic alcohols and amines.^[20] In addition, and in contrast to our previous assay based on alcohol dehydrogenase,^[21] the periodate oxidation scheme can be readily extended to include a broad variety of substrates related to **1–4** but bearing different substituents and includes further reaction types, that produce diol **5**, aminoalcohol **6**, or related compounds, as products. These advantages, together with the possibility of using optically pure substrates for assaying enantioselectivity, make the present assay very attractive for screening catalysis.

Experimental Section

The *ee* of (R)- and (S)- diols **5** and epoxides **3** were determined by analysis on a chiral HPLC column OD-H (Daicel; 25 cm $\times$ 0.46 cm i.d.) using an hexane/*i*PrOH (8/2) eluent; the *R* isomers eluted first in each case. The absolute configuration of diols (R)- and (S)-**5** were assigned on the basis of the Sharpless mnemonic device applied to the parent olefin. Monoacetates (R)- and (S)-**2b** and diacetates (R)- and (S)-**2a** were prepared from the corresponding (R)-**5** and (S)-**5** diols. The configuration of the diols (R)-**5** and (S)-**5** is maintained in the corresponding epoxides (R)-**3** and (S)-**3**. (R)-**3**: m.p. 60–63 °C; $[\alpha]_D^{20}$ = +21.8 (c 0.45, CHCl₃); elemental analysis for C₁₃H₁₂O₄: calcd: C 67.23, H 5.21; found: C 67.39, H 5.52. (S)-**3**: m.p. 61–64 °C; $[\alpha]_D^{20}$ = –23.0 (c 0.3, CHCl₃); elemental analysis: found: C 66.97, H 5.08; ¹H NMR (CDCl₃): δ = 7.58 (d, 1H, *J* = 9.3 Hz), 7.31 (d, 1H, *J* = 8.3 Hz), 6.75–6.81 (m, 2H), 6.18 (d, 1H, *J* = 9.8 Hz), 4.03–4.19 (m, 2H), 3.05–3.14 (m, 1H), 2.78 (t, 1H, *J* = 4.9 Hz), 2.53 (dd, 1H, *J* = 4.9, 2.4 Hz), 2.04–2.20 (m, 1H), 1.79–1.97 (m, 1H). (R)-**5**: m.p. 94–96 °C; $[\alpha]_D^{20}$ = +17.9 (c 0.55, CH₃OH); elemental analysis for C₁₃H₁₄O₅: calcd: C 62.39, H 5.64; found: C 62.12, H 5.41. (S)-**5**: m.p. 92–93 °C; $[\alpha]_D^{20}$ = –22.4 (c 0.46, CH₃OH); elemental analysis: found: C 62.11, H 5.87; ¹H NMR (CD₃OD): δ = 7.83 (d, 1H, *J* = 9.8 Hz), 7.47 (d, 1H, *J* = 8.3 Hz), 6.85–6.92 (m, 2H), 6.21 (d, 1H, *J* = 9.3 Hz), 4.16–4.23 (m, 2H), 3.81–3.93 (m, 1H), 3.54 (d, 2H, *J* = 6.3 Hz), 1.97–2.13 (m, 1H), 1.74–1.91 (m, 1H). (R)-**2b**: semisolid; $[\alpha]_D^{20}$ = +9.3 (c 0.3, CHCl₃); elemental analysis for C₁₅H₁₆O₆: calcd: C 61.64, H 5.52; found: C 61.91, H 5.79. (S)-**2b**: semisolid; $[\alpha]_D^{20}$ = –9.6 (c 0.31, CHCl₃); elemental analysis: found: C 61.86, H 5.77; ¹H NMR (CDCl₃): δ = 7.61 (d, 1H, *J* = 9.8 Hz), 7.33 (d, 1H, *J* = 7.8 Hz), 6.78–6.83 (m, 2H), 6.21 (d, 1H, *J* = 8.8 Hz), 4.01–4.29 (m, 5H), 2.09 (s, 3H), 1.91–2.00 (m, 2H). (R)-**2a**: m.p. 76–78 °C; $[\alpha]_D^{20}$ = +9.6 (c 0.65, CHCl₃); elemental analysis for C₁₇H₁₈O₇: calcd: C 61.07, H 5.43; found: C 60.84, H 5.21. (S)-**2a**: m.p. 74–76 °C; $[\alpha]_D^{20}$ = –9.8 (c 0.5, CHCl₃); elemental analysis: found: C 61.15, H 5.69; ¹H NMR (CDCl₃): δ = 7.61 (d, 1H, *J* = 9.8 Hz), 7.34 (d, 1H,

$J = 8.8$ Hz), 6.74–6.82 (m, 2H), 6.22 (d, 1H, $J = 9.3$ Hz), 5.24–5.35 (m, 1H), 4.32 (dd, 1H, $J = 11.7, 3.4$ Hz), 3.97–4.15 (m, 2H), 4.10 (dd, 1H, $J = 11.7, 5.9$ Hz), 2.05–2.16 (m, 2H), 2.05 (s, 6H). **4**: ^1H NMR (D_2O , 300 MHz): $\delta = 7.88$ (d, 1H, $J = 9.2$ Hz), 7.50 (d, 1H, $J = 8.5$ Hz), 6.96–6.91 (m, 2H), 6.23 (d, 1H, $J = 9.2$ Hz), 4.53 (m, 1H), 4.20 (m, 2H), 4.00 (m, 2H), 2.12 (m, 2H); ^{13}C NMR ($\text{D}_2\text{O}/\text{CD}_3\text{COCD}_3$, 75 MHz): $\delta = 164.6, 163.2, 156.3, 146.8, 130.8, 114.6, 113.9, 112.9, 102.5, 66.0, 60.7, 58.2, 32.2$; ^{31}P NMR (D_2O , 81 MHz): $\delta = 4.1$ (d, $J = 16.2$ Hz), 3.3 (d, $J = 16.2$ Hz); FAB-MS: 410 $[\text{M}^+]$, 433 $[\text{M} + \text{Na}^+]$. All other new compounds gave satisfactory spectral data.

All substrates and products prepared as 1 mM stock solutions in 50% aq. acetonitrile. All reagents and buffers were prepared in deionized milliQ water. Enzymes were diluted from stock solutions (1 mg mL $^{-1}$) in phosphate buffer saline (PBS; 160 mM NaCl, 10 mM phosphate, pH 7.4), BSA from a stock solution (40 mg mL $^{-1}$) in buffer (20 mM borate, pH 8.8) or PBS. NaIO_4 was diluted from a freshly prepared stock solution in water (10 mM). Assays (0.1–0.2 mL) were followed in individual wells of round-bottom polypropylene 96-well plates (Costar) using a Cytofluor II Fluorescence Plate Reader (PerSeptive Biosystems, filters $\lambda_{\text{ex}} = 360 \pm 20$, $\lambda_{\text{em}} = 460 \pm 20$ nm). Commercial enzyme preparations were purchased from Fluka or Sigma.

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Formation of a Giant Supramolecular Porphyrin Array by Self-Coordination**

Kazuya Ogawa and Yoshiaki Kobuke*

Methodologies for the preparation of multi-porphyrin arrays^[1–5] have been exploited to successfully assemble arrays of more than ten porphyrins.^[6] The formation of extremely long porphyrin arrays by supramolecular coordination^[7] and by covalently linking^[8] porphyrins have recently been reported. We have been trying to extend our previous methodology of self-coordination^[9] and we report here a giant multi-porphyrin array formed by the accumulation of dimeric imidazolyl-substituted Zn porphyrins. These systems should have the excellent photophysical properties of a chlorophyll substitute and enable the transfer of excitation energy to be studied.

Meso-(*N*-methyl)imidazolylporphyrinatozinc compounds such as **1b** afforded quantitatively a dimer of a slipped cofacial orientation **2** by the complementary coordination of an imidazolyl group to the Zn^{II} center (Scheme 1).^[9] The coordination was very strong, but the pentacoordinating Zn^{II} ion prohibited further extension of the coordination structure.

[*] Prof. Y. Kobuke

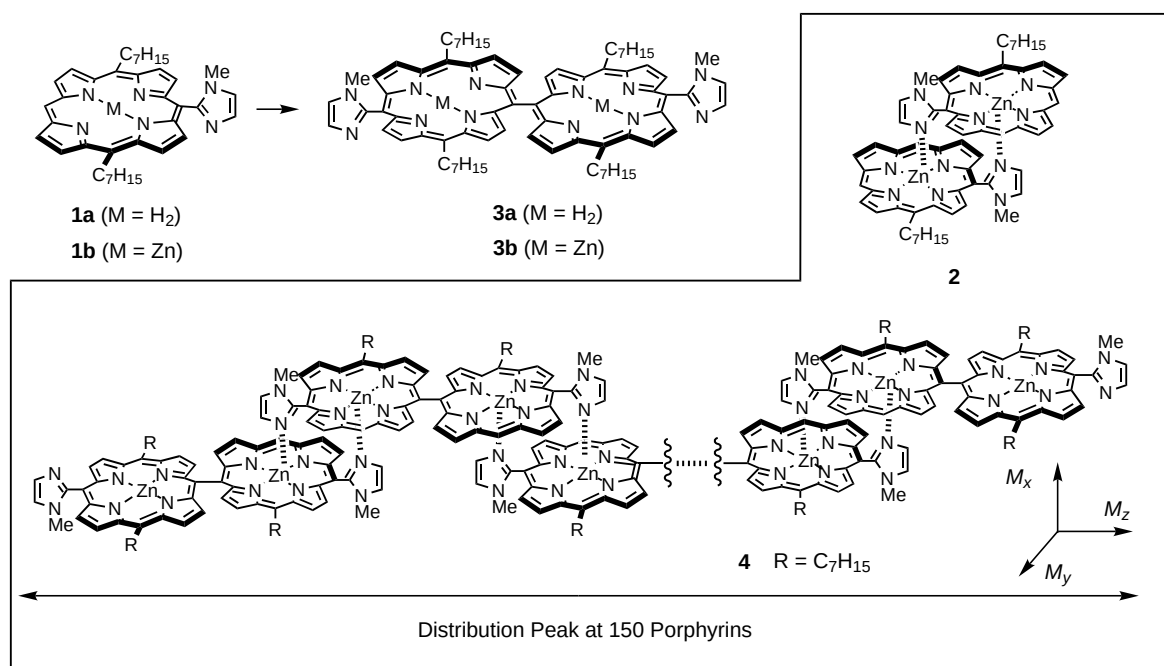
Graduate School of Materials Science
Nara Institute of Science and Technology
8916-5 Takayama, Ikoma, Nara 630-0101 (Japan)
Fax: (+81) 743-72-6119
E-mail: kobuke@ms.aist-nara.ac.jp

Dr. K. Ogawa
CREST, Japan Science and Technology Corporation (Japan)

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



Scheme 1. Synthetic route and structures of self-assembled porphyrins. (Compounds **1b** and **3b** exist in CHCl₃ as **2** and **4**, respectively.)

In order to extend this methodology to obtain higher supramolecules we have prepared mono(*N*-methylimidazolyl)-bis(*n*-heptyl)porphyrin (**1a**) and its Zn complex (**1b**) having one unsubstituted *meso* carbon atom. The coupling of **1b**, which exists as **2** in solution, at the *meso* position afforded the interconnected porphyrinatozinc(II) compound **3b**. Linear multi-porphyrin array **4** was thus obtained by independent complementary coordination at each imidazolylporphyrin unit, with an orthogonal orientation of the π -orbital planes of **3b**.^[10]

Molecular weights of the resulting supramolecular porphyrin complexes were analyzed by using gel-permeation chromatography (GPC; Figure 1). The exclusion limit of the

column was 5×10^5 daltons. Elution of **4** started at 8.4 min and the peak maximum appeared at 11.6 min. The molecular weights at these two points were determined as 5×10^5 and 1×10^5 , respectively, by using polystyrene standards to give a rough estimate. These masses correspond to the link of about 400 and 80 bis(imidazolylporphyrin) units ($M_r = 1298$) through imidazolyl–Zn coordination bonds. The molecular length of the repeating unit has been estimated as 1.43 nm by molecular mechanics calculation using the Cerius 2 program, and hence the length of the porphyrin array reaches 550 nm and 110 nm for the longest and the most abundant species, respectively. It is noteworthy that multi-porphyrin arrays of such huge molecular weights are soluble in chloroform.

Figure 2 compares the absorption spectrum of **4** (7.7×10^{-7} M as **3b**) with those of free base **3a** and Zn complex **2**

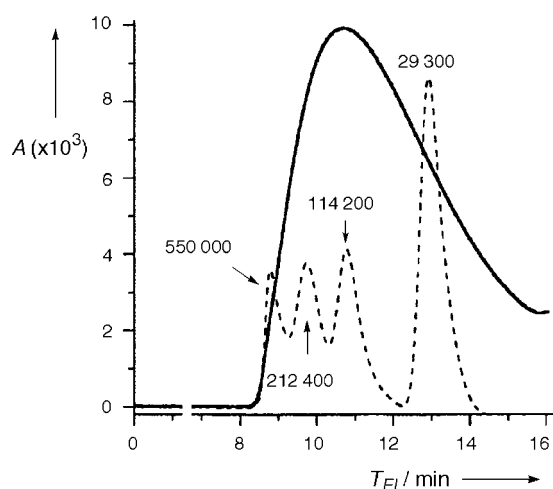


Figure 1. Gel-permeation chromatograms of **4** (bold line) and standard polystyrene mixtures (dotted line, the numbers indicate molecular weights) with a column of exclusion limit 5×10^5 daltons. The eluent is EtOH-free CHCl₃. T_{EL} = elution time.

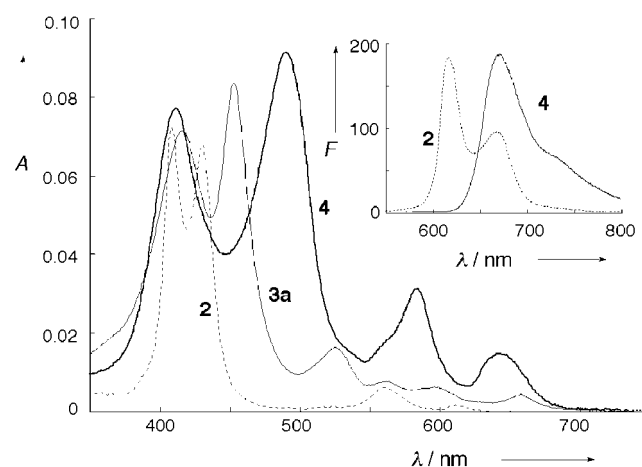


Figure 2. Absorption and fluorescence (inset) spectra in chloroform of imidazolylporphyrinatozinc **2** (dashed line), bis(imidazolylporphyrin) **3a** (thin line), and bis(imidazolylporphyrinatozinc) array **4** (bold line, 7.7×10^{-7} M as **3b**).

in chloroform. The Soret band of the free base **1a** appeared as a single peak at 414 nm and those of the *meso*–*meso*-coupled dimer species were split into twin peaks at 415 and 453 nm for free base **3a** and 410 and 492 nm for the Zn complex **4**. Three in-plane transition moments M_x , M_y , and M_z in the monomeric porphyrin are degenerate when the *meso*-substituent effect is neglected, and show a single Soret band, which appears at 414 nm for **1a**. *Meso*–*meso* coupling to the dimeric free base **3a** induces a red shift of M_z through the interaction of parallel excitons, while no interactions are expected for M_x and M_y as a result of the orthogonality. As a result, the Soret band is split into two: 415 nm for the M_x and M_y components and 453 nm for the M_z component. Coordination to form **4** then gives a blue shift of the transition to 410 nm as a result of the face to face orientation^[9] in M_x and M_y . No M_x – M_x or M_y – M_y interaction is expected because of the presence of an intervening orthogonal porphyrin plane, and therefore the blue shift is small and independent of the degree of organization. In contrast, the M_z component could interact successively with that of the neighboring porphyrin unit, depending on the degree of coordination. These interactions resulted in a splitting of the Soret band by 82 nm. The shift behavior is compatible with the observation that the longer Soret bands of the covalently linked di-, tri-, and tetramer of zinc porphyrin are red-shifted from 410 nm for the monomer in THF to 450, 480, and 490 nm, respectively.^[11] The effect of concentration on the degree of organization was examined in the range 5.0×10^{-9} – 2.2×10^{-4} M. The Soret band at longer wavelength, the most sensitive to the degree of organization, remained constant at 492 nm. The complementary coordination is therefore concluded to be complete at concentrations as low as 5.0×10^{-9} M. The fluorescence emission bands of **4** (shown in the inset of Figure 2) were considerably red-shifted (to 672 nm) by exciton interactions, while the band for the smallest complex **2** is observed at 613 nm. The fluorescence quantum yields of solutions of **2** and **4** in benzene were estimated as 0.043 and 0.053, respectively. It is interesting from a viewpoint of materials capable of light harvesting that the fluorescence intensity of **4** with such a long array structure is not lost at all, but is even higher than that of **2**.

The above-mentioned multi-porphyrin array having such a huge molecular weight was obtained in an EtOH-free chloroform solution. Addition of methanol to a solution of **4** in chloroform (9.9×10^{-7} M) reduced the splitting width of the Soret band as a result of a blue shift of the longer Soret band (M_z transition), which passes through a clear isosbestic point at 478 nm, and a smaller red shift of the shorter Soret band. The addition of 30 vol % of MeOH resulted in the shift almost reaching the saturation point, where the splitting of 41 nm was similar to that of **3a** and shows that the coordinate structure was completely broken. The fluorescence spectra showed similar solvent effects on both the intensity and the emission maximum. These results show that MeOH competes with the imidazolyl group for coordination to the Zn center and can control the degree of organization.

A mixture of **2** and **4** in a 1:5 molar ratio in ethanol-free chloroform was analyzed by GPC by using a column with an exclusion limit of 7×10^4 daltons to separate smaller components (Figure 3, thin line). Only two peaks, one starting at

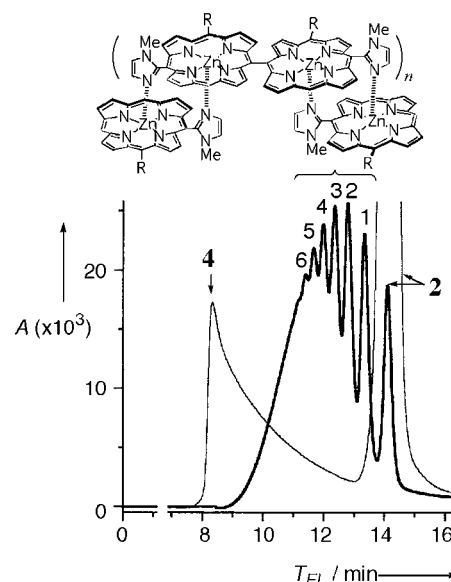


Figure 3. Gel-permeation chromatograms of a mixture of **4** and **2** in a 5:1 molar ratio (thin line) and the same sample mixture first dissolved in CHCl_3 :MeOH (1:1), then evaporated, and re-dissolved in EtOH-free CHCl_3 (bold line). The exclusion limit of the column is 7×10^4 daltons (8 min). The eluent is EtOH-free CHCl_3 . T_{EL} = elution time.

8 min (exclusion limit, **4**) and the other at 14 min (**2**), were detected a few minutes after the mixing. The same sample mixture was dissolved in a chloroform:methanol (1:1) solution, followed by evaporation. The sample was then dissolved in chloroform and subjected to GPC analysis (Figure 3, bold line). Many peaks of intermediate retention times then appeared and a significant shift of the distribution maximum to a lower molecular weight was evident. The peak at the longest retention time (14 min) corresponds to **2**, and peaks at shorter retention times (marked by number n ; $n = 1$ –6) were assigned as a series of compounds in which n units of **3b** are inserted between **1b** terminals. The shift in the longer wavelength part of the split Soret bands was dependent on the intervening number of **3b** units, which is in full accord with progressing exciton interactions (see Supporting Information). The results clearly demonstrate that the coordination-organized supramolecules can scramble in a mixed solvent of chloroform and methanol but cannot in ethanol-free chloroform, which is in accord with the UV titration data, and more importantly that the organization can also be controlled by the addition of an appropriate terminator such as **1b**. The molecular weights of porphyrin oligomers with $n = 1$ –6 (Figure 3) was compared with those calibrated from retention times of polystyrene standards. The calculated molecular weights of porphyrin oligomers were about 1.3 times larger than the estimates and therefore the molecular weights of the porphyrin arrays described as a rough estimate in the earlier part of this paper were found to be even lower estimates.

The one-dimensional propagation of the multi-porphyrin array up to a few hundred nanometers in length with close inter-element distances and in which a reversible adjustment of length equilibrium can be achieved by either changing the media or the addition of a chain terminator are characteristics most desirable for forming molecular photonics and elec-

tronics devices.^[12] Applications toward artificial photosynthesis and nonlinear optics materials are under active investigation.

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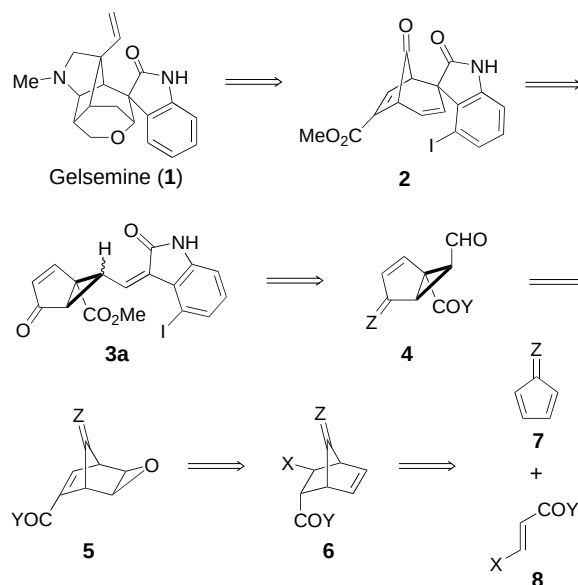
Enantioselective Total Synthesis of (+)-Gelsemine: Determination of Its Absolute Configuration**

Satoshi Yokoshima, Hidetoshi Tokuyama, and Tohru Fukuyama*

Since the structure of gelsemine (**1**) was determined in 1959, many efforts have been directed toward total synthesis of this unique hexacyclic cage-like molecule.^[1] While three groups independently accomplished the total synthesis of

racemic gelsemine in 1994, none of them succeeded in controlling the stereochemistry of the critical spiroindolinone system.^[2] In 1996, our own approach culminated in the completely stereocontrolled total synthesis of (±)-gelsemine featuring a divinylcyclopropane rearrangement to control the stereochemistry of the spiroindolinone system.^[3] Despite these intensive studies, an enantioselective total synthesis of gelsemine has not been reported to date.^[4] Herein, we disclose the first enantioselective total synthesis of (+)-gelsemine.

Our retrosynthetic analysis of optically active gelsemine is illustrated in Scheme 1. According to our racemic synthesis, the stereochemistry of the spiroindolinone system could be



Scheme 1. Retrosynthesis of gelsemine.

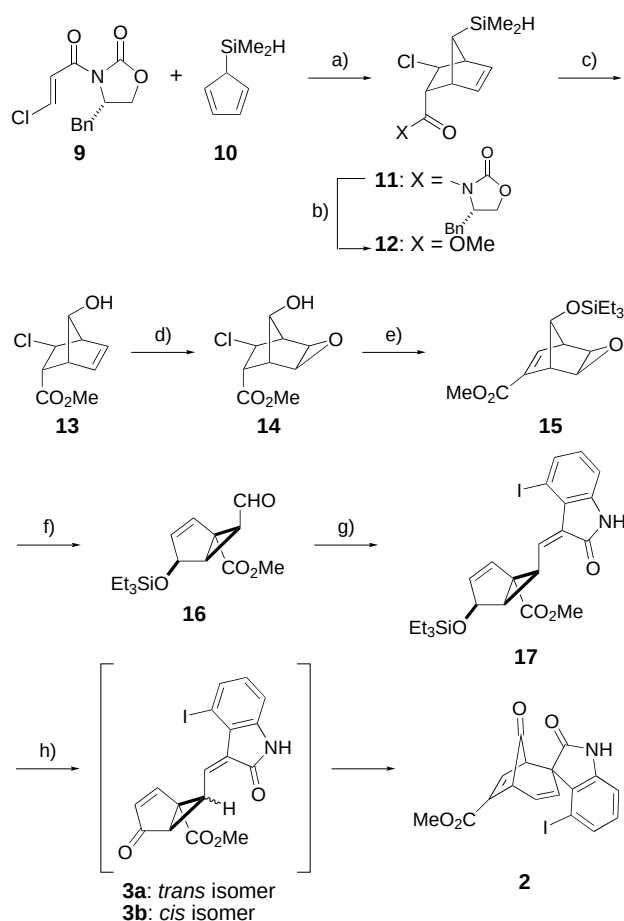
controlled by thermal rearrangement of the key intermediate **3a**, which should be prepared easily by condensation of aldehyde **4** and 4-iodooxindole. The aldehyde **4** could in turn be derived from norbornene epoxide **5** according to the rearrangement reported by Meinwald et al.^[5] It seems quite likely that **5** could be prepared from Diels–Alder adduct **6**.

Our enantioselective total synthesis of gelsemine commenced with a chiral auxiliary controlled asymmetric Diels–Alder reaction. In the presence of Et_2AlCl , the Diels–Alder reaction between dienophile **9** and 5-dimethylsilylcyclopentadiene (**10**)^[6] proceeded smoothly to give adduct **11** as a single isomer (Scheme 2).^[7] The relative and absolute configurations of the adduct were determined by X-ray analysis, based on the configuration of the Evans chiral auxiliary derived from L-phenylalanine.^[8] The chiral auxiliary was removed by treatment with $\text{Sm}(\text{OTf})_3$ in MeOH to afford methyl ester **12**.^[9] Oxidation of the dimethylsilyl group in **12** with H_2O_2 in the presence of KF to provide alcohol **13**,^[6, 10] followed by epoxidation with $t\text{BuOOH}$ and $\text{VO}(\text{acac})_2$, gave epoxide **14**.^[11] After protection of the alcohol as the TES ether, dehydrochlorination by treatment with $t\text{BuOK}$ furnished α,β -unsaturated ester **15**.

After extensive optimization of the acid-catalyzed rearrangement of **15**, we found that the critical rearrangement

[*] Prof. Dr. T. Fukuyama, S. Yokoshima, Dr. H. Tokuyama
Graduate School of Pharmaceutical Sciences
The University of Tokyo, CREST
The Japan Science and Technology Corporation (JST)
7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033 (Japan)
Fax: (+81)3-5802-8694
E-mail: fukuyama@mol.f.u-tokyo.ac.jp

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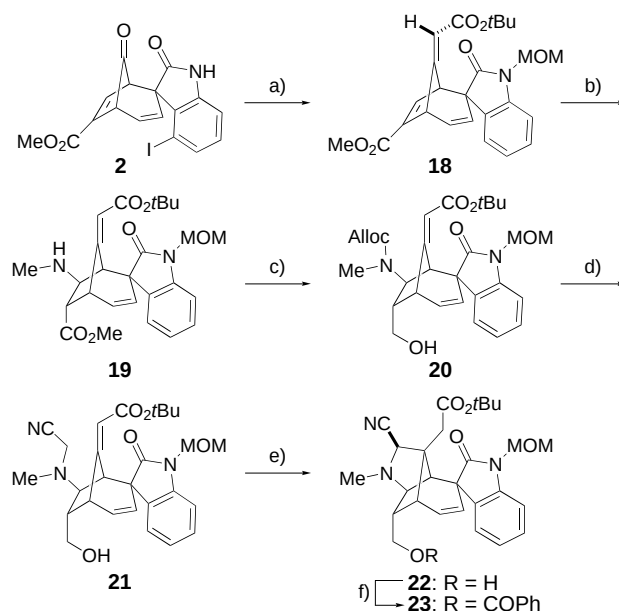
Scheme 2. Synthesis of the bicyclo[3.2.1] system. a) Et_3AlCl , CH_2Cl_2 , -78°C , 88%; b) cat. $\text{Sm}(\text{OTf})_3$, MeOH , 99%; c) 30% H_2O_2 , KF , KHCO_3 , THF/MeOH , 53%; d) $\text{VO}(\text{acac})_2$, $t\text{BuOOH}$, benzene, 100%; e) TESOTf , 2,6-lutidine, CH_2Cl_2 , 97%; f) MAD , toluene, -20°C ; g) 4-iodooxindole, cat. piperidine, MeOH , 60% (2 steps); h) TBAF , THF , 87%; CrO_3 , H_2SO_4 , acetone; toluene/ MeCN , 90°C , 83% (2 steps). Bn = Benzyl, Tf = trifluoromethanesulfonyl, acac = acetylacetonate, TES = triethylsilyl, MAD = methylaluminum bis(2,6-di-*tert*-butyl-4-methylphenoxide), TBAF = tetrabutylammonium fluoride.

could be best carried out by treatment with MAD ^[12] in toluene at -20°C , giving **16** in 65–78% isolated yield. Gratifyingly, the formation of the cyclopropane ring occurred exclusively at the α -position of the methyl ester.^[13] A stereoselective installation of the oxindole moiety according to the procedure developed in our racemic synthesis afforded the desired *Z*-alkylidene indolinone **17** as the exclusive isomer.^[14] After removal of the TES group, the resultant alcohol was subjected to Jones' oxidation to give a mixture of **3a**, **3b**, and **2**. Even at room temperature, the initial product, *cis* isomer **3b**, underwent *cis*–*trans* isomerization to afford *trans* isomer **3a**, or divinylcyclopropane–cycloheptadiene rearrangement to provide bicyclo[3.2.1] system **2**. The formation of *trans* isomer **3a** was of no consequence since it was the key intermediate of our racemic synthesis of gelsemine. Thus, by heating at 90°C , the mixture of three compounds furnished bicyclo[3.2.1] system **2** in 83% yield.

Having established the bicyclo[3.2.1] core of gelsemine in enantiomerically pure form, we next focused our attention on the improved construction of the pyrrolidine ring. Our initial

attempts to construct the ring by means of radical reactions failed, due presumably to the ring strain. After intensive investigations, we found that an intramolecular Michael addition was a viable solution.

Following the procedure established in our racemic synthesis, alcohol **20** was prepared from **2** (Scheme 3).^[3] Elongation of the ketone by Horner–Emmons reaction, one-pot protection of the indolinone nitrogen, and the subsequent

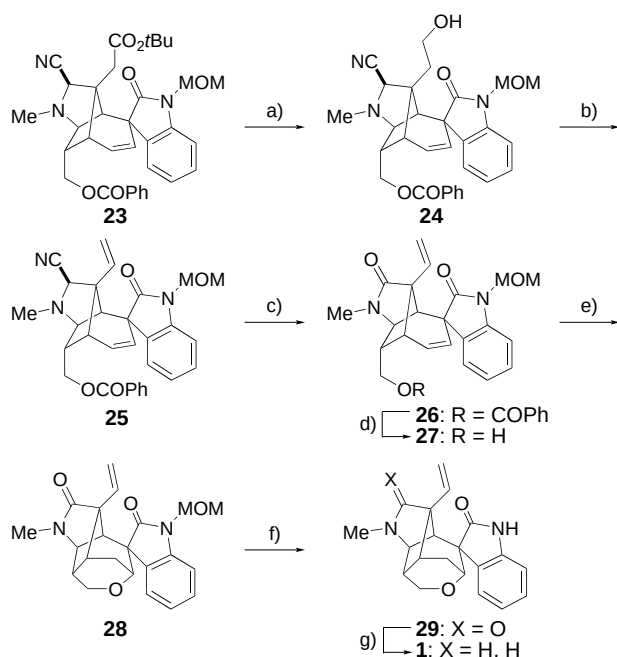


Scheme 3. Construction of the pyrrolidine ring. a) $(\text{EtO})_2\text{POCH}_2\text{CO}_2t\text{Bu}$, $n\text{BuLi}$, THF , 65°C ; MOMCl , $t\text{BuOK}$, 72%; $n\text{Bu}_3\text{SnH}$, AIBN, benzene, reflux, 75%; b) MeNH_2 , MeOH , 100%; c) AllocCl , pyridine, cat. DMAP, CH_2Cl_2 , 94%; LiBH_4 , cat. LiBEt_3H , THF , 94%; d) $[\text{Pd}(\text{PPh}_3)_4]$, pyrrolidine, THF ; ICH_2CN , $i\text{Pr}_2\text{NEt}$, MeCN , 60°C , 78% (2 steps); e) KHMDs , THF , -78 to 0°C , 62%; f) PhCOCl , pyridine, cat. DMAP, CH_2Cl_2 , 92%. MOM = methoxymethyl, AIBN = 2,2'-azobisisobutyronitrile, Alloc = allyloxycarbonyl, DMAP = 4-dimethylaminopyridine, KHMDs = potassium hexamethyldisilazide.

radical deiodination provided **18**. Michael addition of methylamine to the strained α,β -unsaturated ester took place from the less hindered *exo* side to give *trans*-aminoester **19** as a single isomer. After protection of the amine as an allyl carbamate, the methyl ester, which was prone to epimerization, was reduced with LiBH_4 in the presence of a catalytic amount of LiBEt_3H to furnish alcohol **20**.^[15]

At this stage, a cyanomethyl group was installed on the amine to perform the critical intramolecular Michael addition. Thus, removal of the Alloc group, followed by cyanomethylation by treatment with iodoacetone nitrile, gave aminonitrile **21** in 78% overall yield over two steps. Upon deprotonation with KHMDs , **21** smoothly underwent the intramolecular Michael addition, giving pyrrolidine **22** in 62% yield as the sole isomer.

After protection of the alcohol as its benzoate **23**, the *tert*-butoxycarbonylmethyl group was converted into the vinyl side chain as shown in Scheme 4. Firstly, the *tert*-butyl ester was deprotected with formic acid without affecting the *N*-MOM group to give the carboxylic acid, which was subsequently reduced to alcohol **24** via a mixed anhydride. The



Scheme 4. Completion of the total synthesis of (+)-gelsemine (**1**). a) HCO_2H , 96%; ClCO_2Et , NEt_3 , THF, then NaBH_4 , H_2O , 80%; b) $o\text{-NO}_2\text{C}_6\text{H}_4\text{SeCN}$, PBU_3 , THF; MCPBA, then NEt_3 , 97%; c) MCPBA, THF/ H_2O , then NEt_3 , 83%; d) K_2CO_3 , MeOH, 96%; e) $\text{Hg}(\text{OTf})_2 \cdot \text{PhNMe}_2$, MeNO_2 , then aq. NaCl , 97%; NaBH_4 , NaOH , BnNEt_3Cl , $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, 63%; f) TMSCl , NaI , MeCN; NEt_3 , MeOH, 63%; g) DIBAL, toluene, 90%. MCPBA = 3-chloroperoxybenzoic acid, TMS = trimethylsilyl, DIBAL = diisobutylaluminum hydride.

resultant alcohol **24** was converted into vinyl compound **25** in 97% yield according to Grieco's procedure.^[16]

Before construction of the remaining tetrahydropyran ring, it became necessary to transform the aminonitrile into the lactam.^[17] During the course of this investigation, we have developed a facile and direct method for conversion of aminonitriles to lactams, which appears to be generally applicable. Thus, oxidation of **25** with MCPBA, followed by treatment with NEt_3 , afforded **26** in 83% yield.^[18] Methanolysis of **26** provided the alcohol **27**, which was converted into gelsemine following the same procedure used for our racemic synthesis.^[3] The regioselective intramolecular oxymercuration of **27**, followed by reductive demercuration, gave **28**. After removal of the *N*-MOM group, which gave 21-oxogelsemine (**29**), (+)-gelsemine (**1**) was obtained by selective reduction of the *N*-methylactam with DIBAL. The identity of synthetic gelsemine with the natural product was established by direct comparison of spectral and physical properties including optical rotation ($[\alpha]_D^{25} = +16$ ($c = 0.04$ in CHCl_3); ref. [19]; $[\alpha]_D = +15.9$ (CHCl_3)).

In conclusion, the first enantioselective total synthesis of (+)-gelsemine was accomplished, featuring a facile construction of the bicyclo[3.2.1] core of gelsemine by means of the two rearrangement reactions and the efficient formation of the pyrrolidine ring by an intramolecular Michael addition. Moreover, the absolute configuration of gelsemine, which was deduced on the basis of the biogenetic studies, was confirmed for the first time by the total synthesis.

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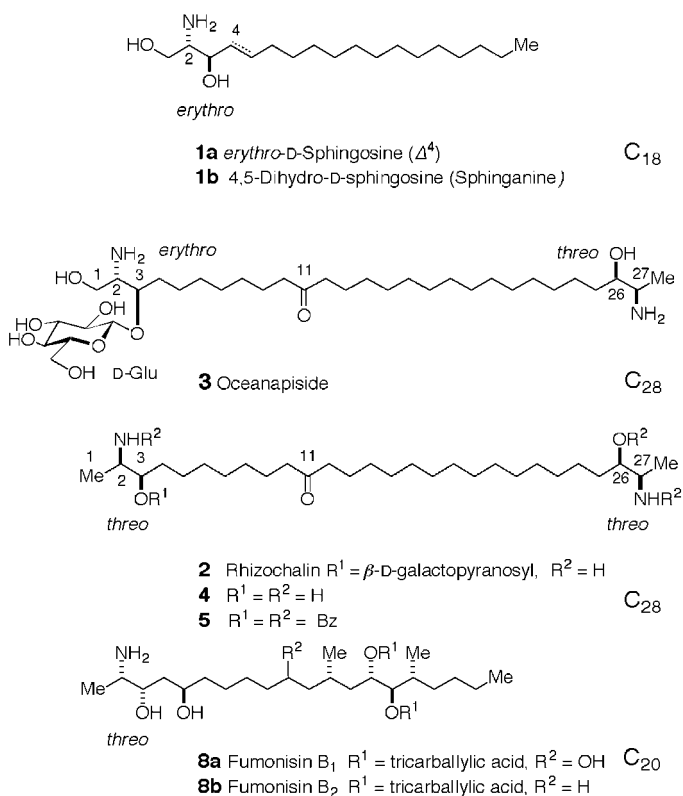
(–)-Rhizochalin is a Dimeric Enantiomorph (2*R*)-Sphingolipid: Absolute Configuration of Pseudo-*C*_{2v}-Symmetric Bis-2-amino-3-alkanols by CD**

Tadeusz F. Molinski,* Tatyana N. Makarieva, and Valentin A. Stonik

Sphingolipids are well-known cellular components of all higher eukaryotes and have attracted renewed interest with the finding that *erythro*-D-sphingosine (**1a**) and its derivatives, such as ceramides (*N*-fatty acyl sphingosines) and sphingosine 1-*O*-phosphate, have important roles in higher mammals as “first messengers”.^[1] Highly modified sphingolipids, such as the sphingofungins,^[2] occur in lower invertebrates and microbes and may also function as inhibitors of various pathways in mammalian sphingolipid biosynthesis. (–)-Rhizochalin (**2**), an antimicrobial galactopyranosyl pseudodimeric α,ω -bipolar sphingolipid, was isolated from the calcareous sponge *Rhizochalina incrustata*,^[3] collected near Madagascar. Although the constitutional structure of **2** was established from spectroscopic and degradative analysis, the stereostructure remained undefined.

Configurational analysis of sphingosine diastereomers by circular dichroism (CD) has been reported.^[4, 5] We have previously devised a CD method for α,ω -bis-sphingoid bases that exploits deconvolution of superposed exciton couplings in the CD spectrum of the corresponding tetrabenzoyl derivatives^[6] and we applied this to the antifungal agent oceanapiside (**3**),^[7] a related α,ω -sphingolipid isolated from a temperate-water marine sponge, *Oceanapia phillipensis* Dendy 1895 (Haplosclerida, Phloeodictyidae). The technique is noteworthy for simplicity, particularly with respect to dimeric amino alkanols, ample sensitivity (≈ 50 nmol), and complementarity with other CD methods for the configurational analysis of sphingolipids.^[4, 5, 8]

Whereas the chain termini in **3** have dissimilar substitution, the pseudosymmetric structure of **2** presents the added



challenge of correlating remote end groups in pseudo-*C*_{2v}-symmetric or *meso* dimeric stereoisomers of α,ω -difunctionalized long chains. Identification of *meso* compounds is trivial (lack of optical activity), but the former stereoisomers present a problem that is refractory to simple end-group analysis by NMR spectroscopy or measurement of optical rotation because the methods do not correlate the chain termini or they lack sensitivity. We show now, by the exciton-coupling CD superposition method, that the complete configuration of (–)-rhizochalin (**2**) is 2*R*,3*R*,26*R*,27*R* and we note a remarkable finding—both ends of **2** are *threo* and enantiomeric to those of known 2-amino-3-alkanols sphingolipids. This suggests that stepwise biosynthesis of bipolar sphingolipids in marine sponges is a highly ordered process that may self-regulate cognate precursor selection for chain assembly.

A suitable chromophoric derivative of **2** was prepared as follows (Scheme 1). The aglycone, **4**, obtained by methanolysis of **2** (MeOH, 1M HCl, 75 °C, 24 h), was transformed into the corresponding tetrabenzoyl derivative **5** (*N*-benzoylimidazole, CH₃CN, catalytic DBU, 70 °C, 24 h),^[9] which was subsequently purified by HPLC (≈ 50 nmol). Benzoylation of **4** with BzCl/pyridine (60 or 100 °C) was less suitable and resulted in extensive formation of the bis-oxazoline **6**.

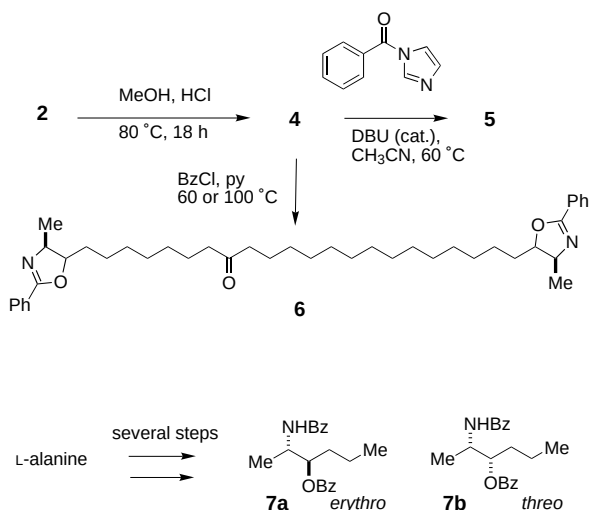
The CD spectra of **5** in MeOH (Figure 1, dotted line) revealed Cotton effects (CEs) of moderate magnitude [$\Delta\epsilon = +7.3$ (221) and -5.0 (237 nm)] with the bisignate antiphase characteristics indicative of exciton coupling between vicinal benzoate–benzamide pairs. Ten of the sixteen stereoisomers of **4** are expected to show unique CD spectra and are depicted in Scheme 2. Several stereoisomers can be considered as almost pseudo-*C*_{2v}-symmetric or *meso* dimers, in which the

[*] Prof. T. F. Molinski
 Department of Chemistry
 University of California
 Davis, CA 95616 (USA)
 Fax: (+1) 530-752-8995
 E-mail: tfmolinski@ucdavis.edu

T. N. Makarieva, V. A. Stonik
 Pacific Institute of Bioorganic Chemistry
 Far East Branch of the Russian Academy of Science
 Vladivostok-22 (Russian Federation)

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



Scheme 1. Above: Synthesis of the tetrabenzoyl derivative **5** from (–)-rhizochalin (**2**). Below: Synthesis of stereodefined compounds **7a, b** from L-alanine.^[6] DBU = 1,8-diazabicyclo[5.4.0]undecane, Bz = benzoyl, py = pyridine.

symmetry of the unbranched chain is broken only by the presence of a midchain keto group.^[10] Inspection of the ten structures reveals that these include four “*C*_{2v}” isomers **4a, c, e, g** and two “*meso*” isomers **4i, j**. The isomers not depicted here correspond to structures obtained by interchange of configurations at the chain termini in **4b** and **4d** (and their enantiomers) along with enantiomers of the “*meso*” compounds **4i, j**. The former non-“*meso*” isomers arise when the end groups have different relative configurations—they can also be considered as the isomers resulting from placement of the midchain keto group at a position either proximal or distal to a given terminus.^[11]

The observation of optical activity in the derivative **5** immediately eliminated the possibility of “*meso*” isomers, such as **4i, j**. Stereochemical correlation of the positive long-wavelength CE observed in **5** was not possible by inspection alone. Instead, the CD spectrum of **5** was compared to hybrid spectra of dimeric tetrabenzoyl “virtual molecules” constructed by linear combinations of spectra measured from stereodefined compounds **7a, b**, each synthesized from L-alanine, as

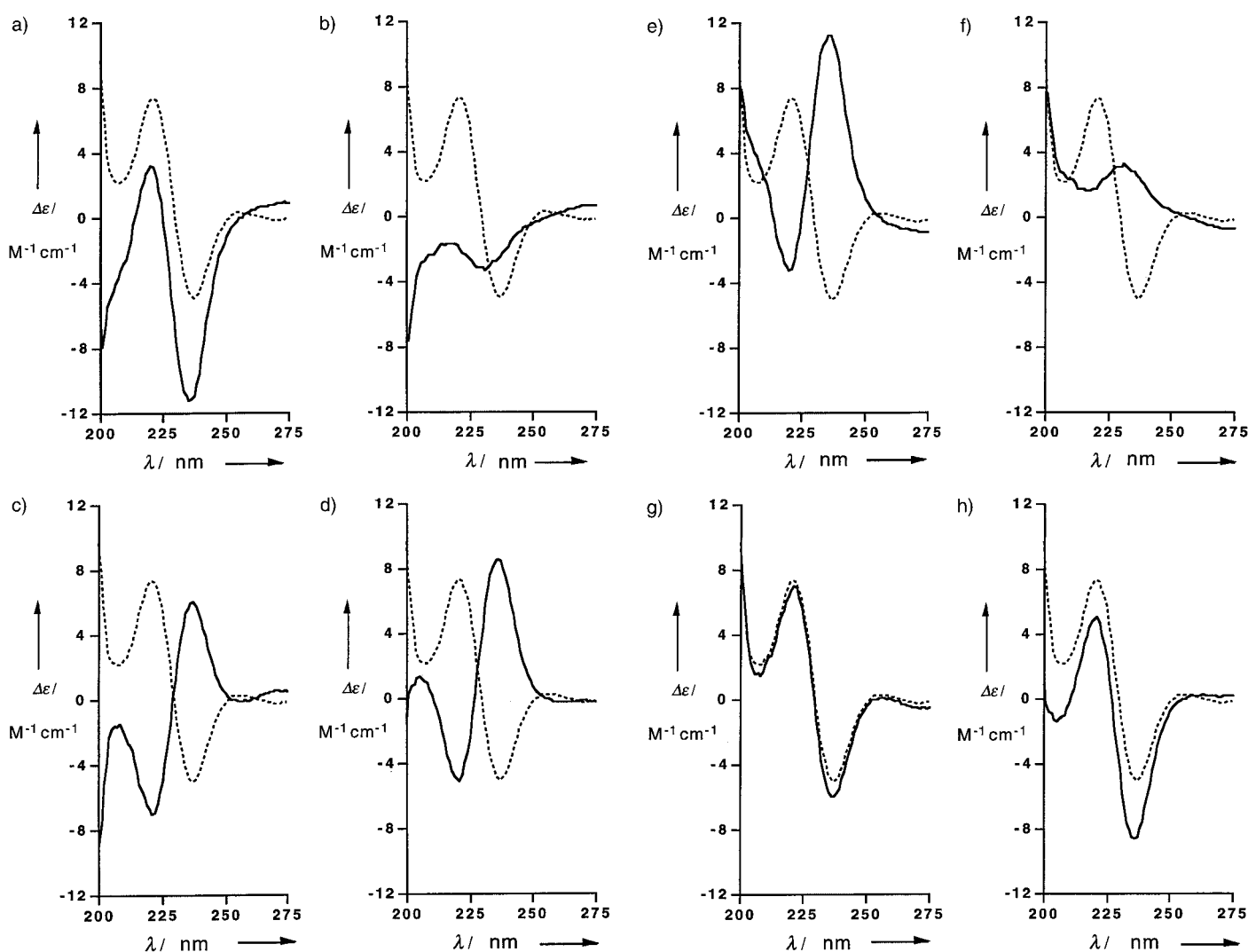
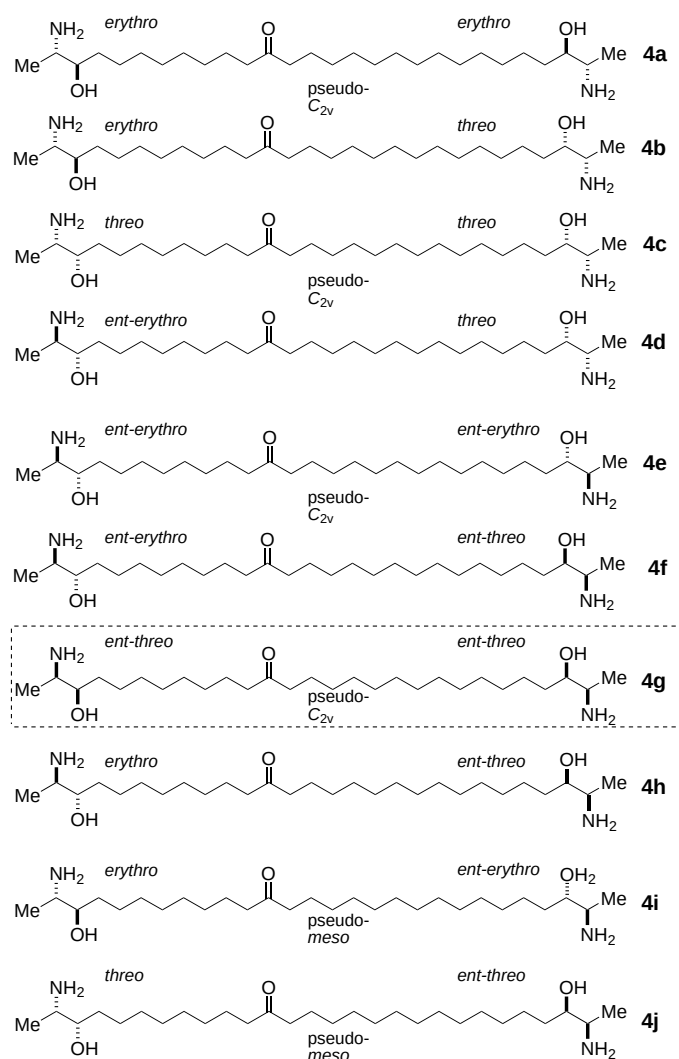


Figure 1. Hybrid CD spectra (solid lines) of eight diastereomers of “virtual molecules” related to **5a–h**, respectively (Scheme 2). These hybrid spectra are generated by linear combinations of the CD spectra of **7a** and **7b**. Spectra of pseudo-*meso* compounds are not shown. The overlaid CD spectrum (dashed line) is of the *N,N',O,O'*-tetrabenzoyl derivative **5** (*c* = 2.2 × 10^{–4} M) of rhizochalin aglycone (**4**) derived from the natural (–)-rhizochalin (**2**). All spectra were recorded in MeOH at 25 °C.



Scheme 2. Structures of the unique stereoisomers of (–)-rhizochalin aglycone **4a–h** that could be distinguished by CD of the corresponding tetrabenzoyl derivatives **5a–h**. Another six stereoisomers (total = 16) are not shown—these correspond to interchange of the end-group configurations of **4b, d**, their enantiomers, and two additional pseudo-*meso* isomers which are enantiomers of **4i** and **4j**. The box indicates that **4g** is the compound derived from natural (–)-rhizochalin (**2**).

previously described.^[6] The required spectra for *ent*-**7a, b** were not measured, but were obtained by simple inversion of the CD spectra of **7a, b**. Hybrid CD spectra corresponding to the combinations of end groups found in the tetrabenzoyl derivatives of the eight optically active stereoisomers are shown in Figure 1 a–h (solid lines).

An excellent match was obtained for the CD spectrum of the tetrabenzoyl derivative **5** and the hybrid spectrum of the virtual molecule in Figure 1 g. Both the relative and absolute configuration are provided by the CD spectrum, however, we confirmed the *threo,threo* configuration of **5** by ¹H NMR spectroscopic single-frequency decoupling of the tetrabenzoyl derivative. The pair of spin systems H-2/H-3 and H-26/H-27 were not resolved, even at 600 MHz, but COSY analysis and decoupling of the methyl signals (H-1/H-28) revealed a single, relatively large vicinal coupling constant ($J = 5.5$ Hz) for the H-2/H-3 and H-26/H-27 pairs that indicated a *threo* config-

uration (compared with *threo*-**7b**, $J = 5.2$ Hz and *erythro*-**7a**, $J = 2.6–2.7$ Hz).^[6] The coupling-constant assignment was further corroborated by the characteristic upfield chemical shift ($\delta = 6.38$ (2 × br. d, 1H, $J = 9$ Hz)) of the signal assigned to NH for *N,O*-dibenzoyl derivatives of *threo*-2-amino-3-alkanols (the signal is shifted downfield in *erythro* compounds, $\Delta\delta \sim 0.6$).^[6] Thus, the configurations of **2, 4**, and **5** are *2R,3R,26R,27R*.

The established configuration of (–)-rhizochalin (**2**) corresponds to a diastereomer with *threo,threo* termini, which is the opposite absolute configuration to known methyl-terminated sphingolipids, such as the fumonisins B1 and B2 (**8a, 8b**)^[12, 13] and C₁₄ marine sphingolipids.^[14] The configuration of other α,ω -bipolar sphingolipids are unknown. These include compound BRS1 from an unidentified sponge,^[15] the coriacenins from the Mediterranean sponge *Clathrina coriacea*,^[16] rhapsamine from the Antarctic sponge *Leucetta leptorhaphis*,^[17] and leucettamols A and B,^[18] although there is evidence that leucettamol A may possess *erythro, erythro* stereodiads.^[18] Plants and higher organisms appear to select more conservatively for the *2S* configuration at the committed step in the biosynthesis of sphinganine (**1b**)—the pyridoxal 5'-phosphate dependent condensation of palmitoyl CoA with (*S*)-serine or (*S*)-alanine, which proceeds with loss of CO₂ but retention of C-2 configuration.^[19] The enzymes that catalyze the analogous biosynthesis of **1a** are a serine palmitoyl transferase^[20] of the α -oxoamine synthase family,^[21] followed by an NADPH-dependent ketoreductase. It is likely that the biosynthesis of **2**, like that of the fumonisins, is catalyzed by homologous enzymatic activity. We propose that at least two independent amino acid fatty acyl transferases, or homologous subunits in the same enzyme, are operative in the biosynthesis of dimeric sphingolipids in marine sponges—one incorporates alanine with *2R,3R* stereoselectivity and a second incorporates serine with *2S,3R* stereoselectivity. Comparing **2** with **3** suggests that commitment to a particular diastereomer is determined by selection of the amino acid and subsequent reduction.

Careful analysis of sponge extracts shows that only the single stereoisomers of **2** or **3** are likely to be present in each of the respective organisms. Indeed, **2** and **3** are the dominant polar lipids in extracts obtained from the sponges (0.42^[7] and 0.11 %^[3] dry weight, respectively)—no stereoisomers or regioisomers were detected.^[7] Consequently, the enzymes controlling the bipolar biosyntheses of **2** and **3** must exhibit stringent control over the selection of cognate amino acids, the ordering of assembly at opposing chain termini by internal recognition, and the stereochemical outcome of each condensation reaction. It is clear that all-*threo* stereochemistry arises in **2** by enzyme selection for alanine with obligate *R* stereochemistry at C-2 and C-27 in the product, but it is not apparent whether D-alanine is employed as a precursor for **2**, with retention of configuration at the C-2 center, or L-alanine is used with an unorthodox inversion at C-2.

Both **2** and **3** have potent antifungal activity against the human pathogen *Candida glabrata* (minimum inhibition concentration (MIC) for **3** $\approx 3 \mu\text{g mL}^{-1}$).^[7] Recent findings show that an endogenous α,ω -diamine acts as a suppressor of sphinganine biosynthesis in J77A4.1-transformed murine cell lines; this has implications for the regulation of sphingolipid

biosynthesis.^[22] A detailed understanding of marine α,ω -bipolar sphingolipids may reveal mechanistic details that pertain to D-sphinganine biosynthesis in other organisms.

Experimental Section

All new compounds were purified by HPLC and gave satisfactory high-resolution mass spectra and ^1H NMR. See the Supporting Information for general procedures, CD measurements, and selected NMR spectra and MS results for compounds **5** and **6**.

Compound 4: A solution of (–)-rhizochalin (**2**, 2 mg)^[3] in 2% HCl/anhydrous MeOH (0.5 mL) was heated at 75 °C for 24 h in a sealed tube, after which the solution was cooled and concentrated under a stream of N_2 . The residue was subjected to microcolumn chromatography (7 × 65 mm, silica) with elution in two stages: a) MeOH/ CHCl_3 (1/4) to obtain 1-*O*-methylgalactopyranosides; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ (9/4/1) which provided the ninhydrin-positive product **4** (1.3 mg). DCI-MS (NH_3): m/z : found, 471.4514 [$M+\text{H}$]⁺; calcd for $\text{C}_{28}\text{H}_{59}\text{N}_2\text{O}_3$, 471.4526.

Compound 5: A solution of **4** (100 μg , 0.11 μmol) in dry CH_3CN (0.30 mL) was treated with *N*-benzoylimidazole (2.1 μmol) and DBU (0.5 mg, see ref. [9]). The solution was heated at 70 °C for 24 h and allowed to cool to 25 °C over 24 h. Volatiles were removed under a stream of N_2 and the residue subjected to microcolumn chromatography (pipette, silica, EtOAc/*n*-hexane (2/7)) to obtain a nonpolar UV-active fraction (0.3 mg). Final purification was achieved by HPLC (Dynamax, 5 μ silica, 10 × 300 mm, EtOAc/*n*-hexane (3/7), 3.3 mL min^{−1}) to give pure **5** (retention time = 22.0 min). ^1H NMR (600 MHz, CDCl_3): δ = 8.02 (d, ^3J = 8.3 Hz, 4H; *ortho*-PhCOO), 7.72 (d, ^3J = 8.3 Hz, 4H; *ortho*-PhCONH), 7.58–7.38 (m, 12H; aryl H), 6.39, 6.38 (2 × br. d, ^3J = 8.5 Hz, 2H; NH), 5.21 (2 × m, 2H; H-3/H-26), 4.525 (2 × qdd, ^3J = 8.5, 7.2, 5.5 Hz, 2H; H-2/H-27), 2.34, 2.33 (2 × t, ^3J = 7.2 Hz, 4H; H-10/H-12), 1.75 (m, 38H), 1.28 (2 × d, ^3J = 7.2 Hz, 6H; H-1/H-28); matrix-assisted laser desorption/ionization Fourier transform MS: m/z : found, 909.5388 [$M+\text{H}$]⁺; calcd for $\text{C}_{56}\text{H}_{74}\text{N}_2\text{O}_7\text{Na}$, 909.5394.

Attempted perbenzoylation of **4** (1 mg) using excess BzCl /pyridine (60 or 100 °C) resulted almost exclusively in formation of the bis-oxazoline **6** ($\approx$ 0.5 mg after HPLC purification (silica, EtOAc/*n*-hexane (1/4), 3.3 mL min^{−1}, retention time = 22 min)). Selected data: ^1H NMR (400 MHz, CDCl_3): δ = 6.04 (2 × m, 2H; H-3/H-26), 5.22 (2 × m, 2H; H-2/H-27), 2.36, 2.35 (2 × t, ^3J = 7.4 Hz, 4H; H-10/H-12), 1.591, 1.594 (2 × d, ^3J = 6.8 Hz, 6H; H-1/H-28); DCI-MS (NH_3): m/z (%): found, 643 (4) [$M+\text{H}$]⁺, 642.4780 (6) [M^+], 105 (100); calcd for $\text{C}_{42}\text{H}_{62}\text{N}_2\text{O}_3$, 642.47604.

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- [10] Strictly, compound **4** lacks an axis of rotation due to the presence of the ketone group in an even-numbered carbon chain. Mislow and co-workers comment on the natural tendency for chemists to ascribe a quantitative meaning to asymmetry; for example, molecules such as 1-stearoyl-2,3-dipalmitoylglycerin are described as having “very slight” asymmetry (A. B. Buda, T. Auf der Heyde, K. Mislow, *Angew. Chem.* **1992**, 104, 1012–1031; *Angew. Chem. Int. Ed. Engl.* **1992**, 31, 989–1007). In the present context, one can think of **4** as possessing “very slight” C_{2v} symmetry because, for all intents, the molecule behaves spectroscopically and chemically as a C_{2v} entity, even though improper rotations are absent.
- [11] Herein lies a limitation to the CD method as applied to dimeric sphingolipids with the same constitution at each chain terminus and midchain substitution. We can uniquely distinguish all isomers with the same local relative configuration at each end group (for example, all C_{2v} isomers), but cannot distinguish those stereoisomers with different local relative configurations at each end from their counterparts obtained by interchanging end-group configurations.
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Amphoteric Character of 2-Vinyloxiranes: Synthetic Equivalents of β,γ -Unsaturated Aldehydes and a Vinylogous Enolate**

Mark Lautens,* Stéphane G. Ouellet, and Stéphane Raeppe

Oxiranes and 2-vinyloxiranes undergo a number of useful transformations including reactions with nucleophiles^[1] and Lewis acid mediated rearrangements.^[2] In contrast, the utility of vinyloxiranes as precursors to nucleophilic species has not been established. In this report we describe two reactions of

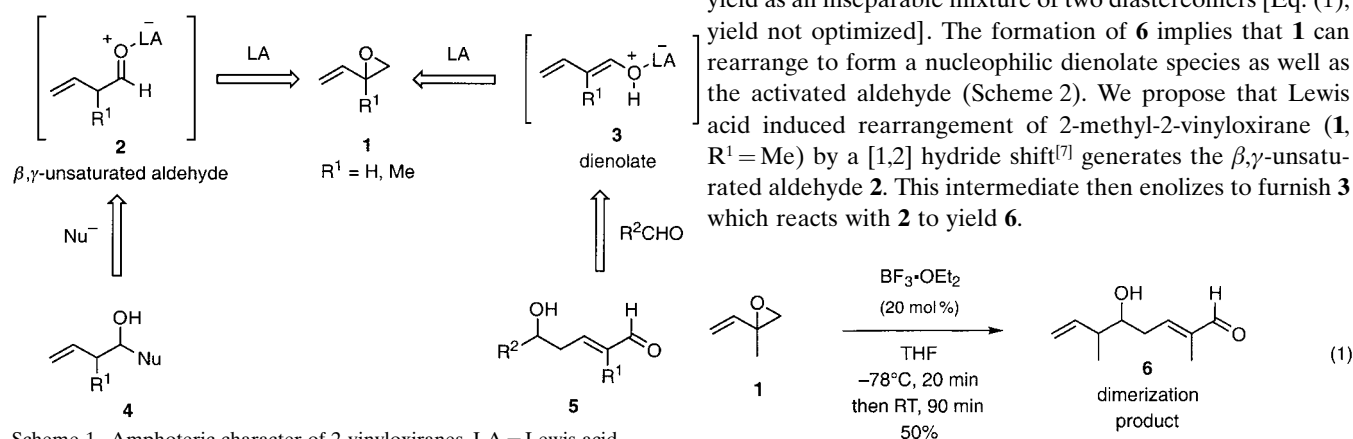
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[*] Prof. Dr. M. Lautens, S. G. Ouellet, Dr. S. Raeppe
Department of Chemistry
Lash Miller Chemical Laboratories
University of Toronto
80 St. George Street, Toronto, M5S 3H6, ON (Canada)
Fax: (+1) 416-978-6083
E-mail: mlautens@alchemy.chem.utoronto.ca

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2-vinyloxiranes that reveal their electrophilic and nucleophilic character when treated with a Lewis acid.

The Lewis acid induced formation of β,γ -unsaturated aldehydes from 2-vinyloxiranes **1** has been reported but very little is known about the utility of the product in subsequent reactions, perhaps due to its instability upon attempted isolation.^[3, 4] Our goal was to explore the reactivity of the in situ generated aldehyde **2** (Scheme 1) by selecting a nucleophile that was compatible with the Lewis acid.^[2b] The



Scheme 1. Amphoteric character of 2-vinyloxiranes. LA = Lewis acid.

potassium salts of allyl and crotyl trifluoroborate developed by Batey and co-workers^[5] served this purpose since $\text{BF}_3 \cdot \text{OEt}_2$ is a typical additive with these nucleophiles and it is also commonly used in the rearrangement of epoxides.^[2a] Our results are summarized in Table 1. We found that allyl, as well as crotyl, trifluoroborates react with various 2-vinyloxiranes.

Table 1. Addition of potassium allyl and crotyl trifluoroborates to 2-vinyloxiranes.^[a]

Entry	Substrate	Nucleophile	Product(s) ^[6]	Yield [%] ^[b]
1				88
2				92
3				72 ^[c]
4				65 ^[d]
5				70 ^[d]
6				74 ^[e]
7				78 ^[e]

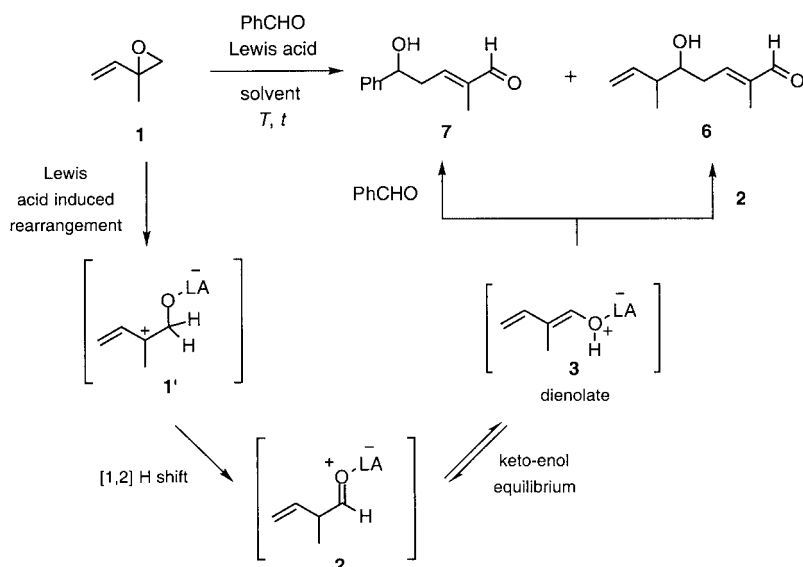
[a] For typical conditions see the Experimental Section. [b] Yields of isolated product(s). [c] 1:1 Mixture of the *syn* and *anti* cross-coupling products. [d] Only one diastereoisomer was observed. [e] Combined yields.

Both *syn* and *anti* products can be obtained in good yields and with excellent diastereoselectivity by varying the geometry of the nucleophile (entries 4–5). When *E*- and *Z*-crotyl trifluoroborates were reacted with the aldehyde arising from rearrangement of the 2-methyl-2-vinyloxirane, moderate facial selectivity was observed (entries 6–7).

A potentially more important discovery was noted in the absence of an external nucleophile. Upon treatment with $\text{BF}_3 \cdot \text{OEt}_2$, 2-methyl-2-vinyloxirane (**1**, $\text{R}^1 = \text{Me}$) dimerized to furnish the δ -hydroxy- α,β -unsaturated aldehyde **6** in 50% yield as an inseparable mixture of two diastereomers [Eq. (1), yield not optimized]. The formation of **6** implies that **1** can rearrange to form a nucleophilic dienolate species as well as the activated aldehyde (Scheme 2). We propose that Lewis acid induced rearrangement of 2-methyl-2-vinyloxirane (**1**, $\text{R}^1 = \text{Me}$) by a [1,2] hydride shift^[7] generates the β,γ -unsaturated aldehyde **2**. This intermediate then enolizes to furnish **3** which reacts with **2** to yield **6**.

If this proposal is correct, addition of a different aldehyde would give a cross-aldol adduct. Indeed, when we added one equivalent of benzaldehyde to **1** in the presence of 20 mol % of $\text{BF}_3 \cdot \text{OEt}_2$, we obtained, after 15 h at room temperature, a mixture composed of dimerized and cross-aldol products **6** and **7** (1:4) in 71% combined yield.^[8] With this preliminary result in hand we carried out a study of the parameters that influence this new reaction (nature and amount of Lewis acid, solvent, temperature, etc.) and we have obtained highly selective cross-coupling.

We first examined the effect of the solvent on the coupling between 2-methyl-2-vinyloxirane (**1**, $\text{R}^1 = \text{Me}$) and benzaldehyde as a model reaction and found that THF gave higher yields and fewer side-products as compared to acetonitrile or diethyl ether. In order to increase the yield and favor the formation of the cross-aldol product **7**, we studied the influence of the Lewis acid (Table 2). The reactions were carried out using an equimolar solution of 2-methyl-2-vinyloxirane and benzaldehyde in THF to which the Lewis acid was added.^[9] The results clearly show that aluminum-, boron-, magnesium-, tin-, and scandium-based Lewis acids can catalyze this reaction. The best total yield of **6** and **7** was obtained using either $\text{BF}_3 \cdot \text{OEt}_2$ or SnCl_4 (71% and 73%, respectively) but lower cross-aldol selectivity was observed. The best ratio of **7** to **6** was obtained using 10 mol % of $\text{Sc}(\text{OTf})_3$ (OTf = triflate, trifluoromethanesulfonate). The optimal reaction conditions were obtained by premixing the aldehyde (1.0 equiv) and $\text{Sc}(\text{OTf})_3$ (10–15 mol %) in THF at -15°C (cryobath), and



Scheme 2. Proposed mechanism for the formation of **6** and **7** from **1**. The temperature T and time t values correspond to those of the reactions described in Table 2.

Table 2. Effect of the Lewis acid.^[a]

Entry	Lewis acid ^[b]	t [h]	Combined yield [%] ^[c]	Ratio (7 : 6) ^[d]
1	Me_2AlCl in hexanes	2	44	6.7:1
2	$\text{BF}_3 \cdot \text{OEt}_2$	16	71	4.1:1
3	$\text{MgBr}_2 \cdot \text{OEt}_2$	15	14	1:0
4	$\text{Sc}(\text{OTf})_3$	1	49	12.4:1
5	SnCl_4	3	73	2.9:1

[a] All reactions were run at 0.23 M concentrations using distilled PhCHO (1.0 equiv) in dry THF under nitrogen at 0 °C for 10 min and then warmed to room temperature, except for entry 4 which was kept at 0 °C throughout the reaction. [b] 0.2 equivalents of the Lewis acid was used in each case, except in entry 4 where 0.1 equivalents were used. [c] Combined yield of isolated products.

by adding a slight excess of 2-methyl-2-vinyloxirane (**1**, $\text{R}^1 = \text{Me}$; 1.3 equiv) in THF with a syringe pump over 1 h. Slow addition of the 2-vinyloxirane significantly reduced the amount of dimerization product. We did not observe the formation of compound **7** below -20°C or with a catalyst loading lower than 10 mol %.

We tried different aryl aldehydes under the optimized conditions (Table 3) and obtained the cross-coupled products

Table 3. Effect of different electrophiles in the reaction shown in Equation (2).^[a]

Entry	Electrophiles	Products ^[a]	Yield [%] ^[b]
1	4-nitrobenzaldehyde	8	88
2	2-nitrobenzaldehyde	9	73
3	4-cyanobenzaldehyde	10	90
4	4-bromobenzaldehyde	11	78
5	4-methoxybenzaldehyde	12	55
6	2-furaldehyde	13	97
7	cinnamaldehyde	14	91 ^[8]

[a] For typical conditions see the Experimental Section. [b] Yield of isolated product.

in moderate to excellent yields. The mild reaction conditions are compatible with a large range of substituents on the aromatic ring (entries 1–5). Moreover, a heteroaromatic ring (entry 6) and conjugated aldehyde (entry 7) worked well. On the other hand, benzaldehyde derivatives bearing an acidic proton (such as carboxylic acid and hydroxy moieties) did not afford the desired cross-aldol products. Blocking these sites (as the methyl ester and silyl ether, respectively) resulted in an efficient reaction. Likewise, easily enolizable aliphatic aldehydes led to cross-aldol products, albeit in lower yield.^[10]

For the first time, the amphoteric character of 2-vinyloxiranes has been disclosed. We found that 2-vinyloxiranes are a synthetic equivalent of β,γ -unsaturated aldehydes,^[11] and that the potassium salts of allyl and crotyl trifluoroborates were suitable nucleophiles for in situ addition reactions. We also demonstrated that 2-methyl-2-vinyloxirane reacts with *E*- and *Z*-crotylborates with moderate selectivity. We are currently investigating the scope of this transformation with a variety of different nucleophiles.^[12] We also found that 2-methyl-2-vinyloxirane, upon treatment with an appropriate Lewis acid, can act as a nucleophile and add to various aldehydes. This transformation constitutes a convenient and efficient alternative to the use of a silyl dienol ether, prepared from tiglic aldehyde,^[13] in the vinylogous Mukaiyama aldol reaction.^[14]

Experimental Section

($\pm$)-(*E*)-1-Phenyl-1,6-heptadien-4-ol: Potassium allyltrifluoroborate salt (232 mg, 1.568 mmol, 1.5 equiv) and $\text{BF}_3 \cdot \text{OEt}_2$ (13 μL , 0.105 mmol, 10 mol %) were successively added to a solution of (*E*)-2-phenyl-2-vinyloxirane (153 mg, 1.047 mmol, 1.0 equiv) in anhydrous THF (10 mL) at 0 °C, and the suspension was stirred at 0 °C for 30 min. The reaction mixture was quenched with an aqueous saturated solution of NH_4Cl , diluted with AcOEt, and extracted three times (3×50 mL AcOEt). The combined organic layers were successively washed with H_2O and brine, dried over anhydrous Na_2SO_4 , and concentrated. The crude residue was purified by flash chromatography on silica gel (AcOEt/hexanes 10/90) to give the desired product (174 mg, 0.924 mmol, 88 % yield) as a colorless oil. $R_f = 0.30$ (AcOEt/hexanes 20/80); IR (neat): $\tilde{\nu} = 3386$ cm^{-1} (br.), 3026, 2928, 1641, 1494, 1433, 966, 744, 693; ^1H NMR (400 MHz, CDCl_3): $\delta = 7.38$ –7.19 (m, 5H; phenyl), 6.49 (d, 1H, $J = 15.9$ Hz; H-1), 6.25 (dt, 1H, $J = 15.7$, 7.3 Hz; H-2), 5.93–5.80 (m, 1H; H-6), 5.16 (d, 1H, $J = 17.5$ Hz; H-7), 5.15 (d, 1H, $J = 11.1$ Hz; H-7), 3.84–3.75 (m, 1H; H-4), 2.50–2.19 (m, 4H; H-3 and H-5), 1.75 (d, 1H, $J = 3.6$ Hz; OH); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 137.3$, 134.6, 133.1, 128.5, 127.2, 126.1, 126.0, 118.0, 70.2, 41.3, 40.4; MS: m/z (%) = 188 (10) [M^+], 170 (15), 147 (20), 129 (30), 118 (100), 104 (25), 91 (40); HR-MS: calcd for $\text{C}_{13}\text{H}_{16}\text{O} = 188.1201$, found = 188.1207; molecular formula: $\text{C}_{13}\text{H}_{16}\text{O}$ (188.26 g mol^{-1}).

(*E*)-5-Hydroxy-2-methyl-5-(4-nitrophenyl)-2-penten-1-al (**8**): A solution of $\text{Sc}(\text{OTf})_3$ (73 mg, 0.149 mmol, 15 mol %) and 4-nitrobenzaldehyde (150 mg, 0.993 mmol, 1.0 equiv) in anhydrous THF (5 mL) was stirred at -15°C (cryobath) under argon, and a solution of 2-methyl-2-vinyloxirane (127 μL , 1.290 mmol, 1.3 equiv) in anhydrous THF (2 mL) was slowly added with a syringe pump over 70 min. The reaction mixture was stirred for a further 2 h at -15°C , quenched with an aqueous saturated solution of NaHCO_3 and diluted with Et_2O . The organic layer was successively washed with H_2O and brine, dried over anhydrous Na_2SO_4 , decolorized over charcoal, filtered through celite, and concentrated. The crude residue was

purified by flash chromatography on silica gel (Et₂O/hexanes 50/50 → 100/0) to give the desired product (205 mg, 0.871 mmol, 88% yield) as a pale yellow oil. R_f = 0.27 (Et₂O/hexanes 90/10); IR (neat): $\tilde{\nu}$ = 3506.1 cm⁻¹ (br.), 3115.9, 2921.6, 2710.9, 1692.9, 1648.3, 1605.4, 1512.0, 1404.1, 1345.1, 1237.9, 1106.8, 1063.1, 1019.7, 850.8; ¹H NMR (400 MHz, CDCl₃): δ = 9.43 (s, 1H; H-1), AB signals (4H, δ = 8.25, 7.58, J_{AB} = 8.7 Hz; aromatic H), 6.56 (tq, 1H, J = 7.2, 1.2 Hz; H-3), 5.07 (dd, 1H, J = 6.2 Hz; H-5), 2.89–2.77 (m, 2H; H-4), 2.20 (br.s, 1H; OH), 1.71 (s, 3H; CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 195.17, 150.94, 148.99, 147.25, 141.35, 126.46, 123.73, 71.90, 38.37, 9.31; MS: m/z (%) = 218.22 (3.5) [M^+ – OH], 152.15 (57.5), 84.13 (100.0); HR-MS: calcd for C₁₂H₁₄NO₄ = 236.0923, found = 236.0921; molecular formula: C₁₂H₁₃NO₄ (235.23 g mol⁻¹).

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Total Synthesis of (+)-Eurylene and (+)-14-Deacetylene*^{**}

Yoshiki Morimoto,* Koji Muragaki, Toshiyuki Iwai, Yukio Morishita, and Takamasa Kinoshita

Recently biologically active and structurally novel triterpene polyethers, natural products which are thought to be biogenetically derived from squalene, have been isolated from both marine and terrestrial plants. Among the polyethers are cytotoxic eurylene (**1**) and 14-deacetylene (**2**; see Scheme 1), isolated from the wood of *Eurycoma longifolia* by Itokawa et al.^[1] The stereostructures and conformations of **1** and **2** have been elucidated by X-ray crystallographic analysis and spectroscopic methods.^[2] The mechanism of action for the cytotoxic activities of these natural polyethers, however, remains to be clarified, because these molecules are available only in restricted amounts from natural sources. Therefore, the development of an efficient synthesis was desired for these polyethers. The novel structures, the cytotoxic activities, and the conformation–activity relationships^[2] of **1** and **2** have attracted the attention of many synthetic organic chemists; however, the total synthesis of **2** with potent cytotoxic activity has never been accomplished.^[3] Here we report the efficient and stereoselective total synthesis of (+)-eurylene (**1**) and (+)-14-deacetyl eurylene (**2**), featuring monool- and diol-differentiated chemoselective oxidative cyclizations promoted by rhenium(VII) and chromium(VI) oxo species, respectively.

Our retrosynthetic analysis of **1** and **2** is depicted in Scheme 1. The key events for the total synthesis are the stereoselective construction of the *trans* and *cis* tetrahydrofuran (THF) rings and the differentiation of the 14-hydroxy group. To solve this problem, we focused on the hydroxy-directed *syn* oxidative cyclization of acyclic bishomoallylic alcohols promoted by rhenium(VII)^[4] and chromium(VI) oxides.^[5] Thus, the *trans* THF ring will be constructed by applying our Re^{VII} protocol^[6] to the bishomoallylic monool moiety in triol **3**, while the *cis* THF ring is constructed by the Cr^{VI}-induced *cis*-selective cyclization of the bishomoallylic vicinal diol. The pseudo-*meso* triol **3** will, in turn, be derived

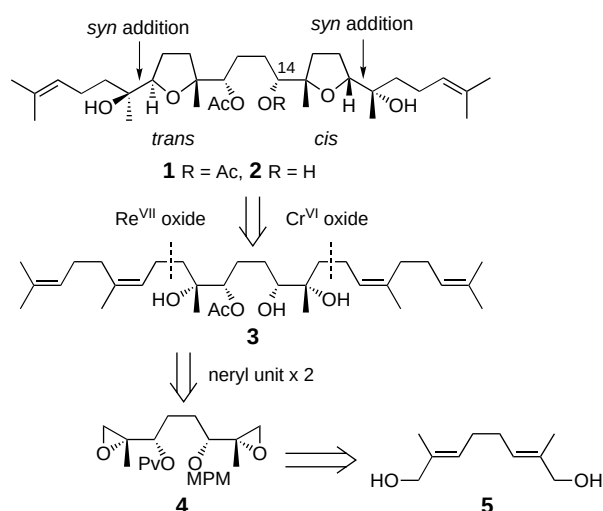
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[*] Prof. Dr. Y. Morimoto, K. Muragaki, T. Iwai, Y. Morishita, Prof. Dr. T. Kinoshita
Department of Chemistry, Graduate School of Science
Osaka City University
Sumiyoshiku, Osaka 558-8585 (Japan)
Fax: (+81) 6-6605-2522
E-mail: morimoto@sci.osaka-cu.ac.jp

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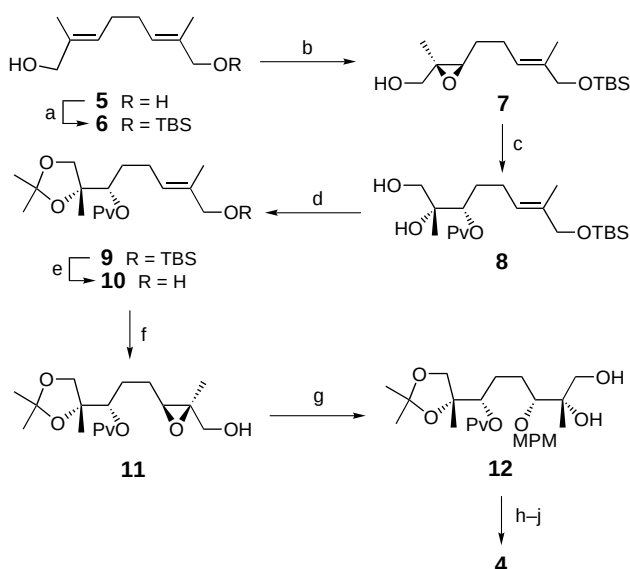
Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



Scheme 1. Retrosynthetic analysis of **1** and **2**. Pv = pivaloyl; MPM = 4-methoxyphenylmethyl.

from the appropriately protected diepoxide **4** by extending the side chains with two neryl units in a bidirectional manner. It was envisaged that the diepoxide **4** can be prepared by Sharpless asymmetric epoxidation of the readily available diol **5** and subsequent regioselective ring-opening reactions.

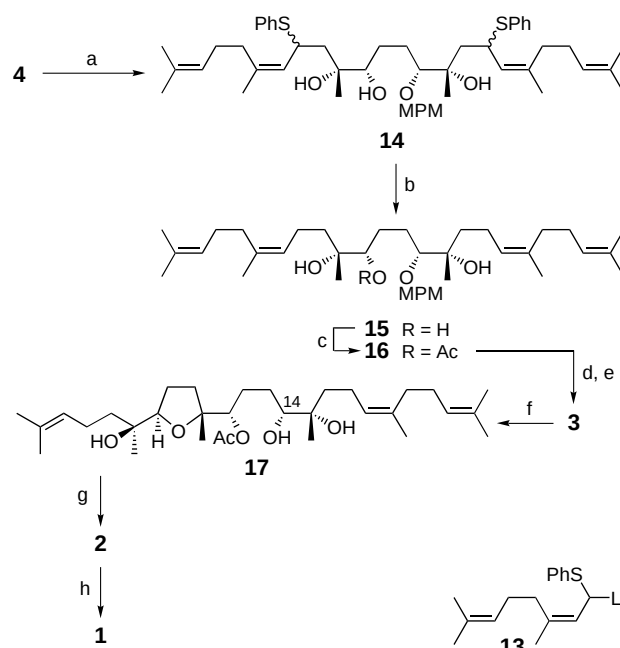
The stereoselective preparation of the diepoxide **4** began with monoprotection of the known diol **5**^[7] as a *tert*-butyldimethylsilyl (TBS) ether ($\rightarrow$ **6**; Scheme 2). Sharpless asymmetric epoxidation^[8] of the allylic alcohol **6**^[9] using (–)-diethyl D-tartrate (D-(–)-DET) afforded the epoxy alcohol **7** in high optical purity.^[10] The pivalate group was introduced regioselectively into **7** by a titanium-assisted epoxide-opening



Scheme 2. a) TBSCl, imidazole, CH_2Cl_2 , RT, 1 h, 55%; b) TBHP, $\text{Ti}(\text{O}i\text{Pr})_4$, D-(–)-DET, MS 4 Å, CH_2Cl_2 , -20°C , 2 h, 83% (98% ee); c) $\text{Ti}(\text{O}i\text{Pr})_4$, PvOH, benzene, 0°C , 2 h, 83%; d) 2,2-dimethoxypropane, camphorsulfonic acid, CH_2Cl_2 , 0°C , 2 h, 88%; e) Bu_4NF , THF, RT, 3 h, 99%; f) TBHP, $\text{Ti}(\text{O}i\text{Pr})_4$, L-(+)-DET, MS 4 Å, CH_2Cl_2 , -20°C , 3 h, 89%; g) $\text{Ti}(\text{OMPM})_4$, MPMOH, benzene, 60°C , 12 h; h) $\text{AcOH}/\text{H}_2\text{O}$ (4/1), RT, 5 h; i) MsCl , Py, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{RT}$, 5 h; j) K_2CO_3 , MeOH, RT, 1 h, 41% (4 steps). Ms = methanesulfonyl, Py = pyridine, TBHP = *tert*-butyl hydroperoxide.

reaction^[11] to yield 1,2-diol **8** as a single diastereomer; subsequent acetone protection and desilylation furnished the allylic alcohol **10** in good overall yield. The asymmetric epoxidation of **10** using L-(+)-DET provided the epoxy alcohol **11**, which was subjected to a $\text{Ti}(\text{OMPM})_4$ -mediated epoxide-opening reaction^[11, 12] with *p*-anis alcohol to produce the desired 1,2-diol **12** and a 1,3-diol derivative in a ratio of approximately 3:1. Deprotection of the acetonide in 1,2-diol **12**, mesylation of both primary hydroxy groups in the resultant tetraol, and subsequent basic treatment of the dimesylate finally gave the requisite diepoxide **4** in 41% yield (based on four steps from **11**).

The bidirectional chain extension proceeded smoothly by alkylation of **13**, the lithio derivative of neryl phenyl sulfide,^[6b] with the diepoxide **4** in the presence of *N,N,N',N'*-tetramethylethylenediamine (TMEDA), yielding the bisulfide **14** as a mixture of diastereomers (Scheme 3).^[13] Compound **14** was



Scheme 3. a) Compound **13**, TMEDA, THF, -78°C , 30 min, then 0°C , 2 h; b) Na, THF/*i*PrOH (2/1), reflux, 15 h, 86% (2 steps); c) Ac_2O , Py, RT, 12 h, 91%; d) DDQ, MS 4 Å, CH_2Cl_2 , 0°C , 2 h, 68%; e) $\text{AcOH}/\text{H}_2\text{O}$ (4/1), RT, 16 h, 89%; f) $[(\text{CF}_3\text{CO}_2)\text{ReO}_3 \cdot 2\text{CH}_3\text{CN}]$, TFAA, $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (9/1), -40°C , 1.5 h, 84%; g) PCC, CH_2Cl_2 , RT, 30 min, 47%; h) Ac_2O , Py, RT, 40 h, 80%.

desulfurized under Bouvault–Blanc conditions^[6b, 14] to provide the triol **15**, and selective acetylation of the *sec*-alcohol afforded the diol **16** in good overall yield. The MPM protecting group in **16** was converted into the 4-methoxybenzylidene acetal through interaction of the neighboring hydroxy groups with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).^[15] Subsequent acidic hydrolysis furnished the key substrate **3** required for the oxidative cyclizations.

The optimal conditions were determined, and the treatment of triol **3** with eight equivalents of the oxorhenium(VII) complex $[(\text{CF}_3\text{CO}_2)\text{ReO}_3 \cdot 2\text{CH}_3\text{CN}]$ ^[16] and ten equivalents of trifluoroacetic anhydride (TFAA) in a mixed solvent system ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ 9/1)^[6] at -40°C for 1.5 h diastereoselectively

gave the expected *trans* THF product **17** in 84% yield; the right-hand part of the molecule was intact. On the other hand, when **17** was treated with a stoichiometric amount of the oxochromium(vi) complex pyridinium chlorochromate (PCC),^[17] in CH₂Cl₂ at room temperature for 30 min,^[5] the bishomoallylic vicinal diol moiety was oxidatively cyclized, probably via a chelated dialkoxochromium intermediate,^[5a, 18] with complete *cis* diastereoselectivity to produce (+)-14-deacetylerylene (**2**).^[19] The spectral characteristics (¹H and ¹³C NMR, IR, MS, and HRMS) of synthetic **2** were identical to those reported for the natural product.^[1b] Finally, selective acetylation of the 14-hydroxy group in **2** afforded another objective, (+)-eurylene (**1**),^[19] whose spectral data were also consistent with the ¹H NMR spectrum of an authentic sample^[3a] and with the data reported for the natural product.^[1]

In conclusion, we have accomplished the first total synthesis of (+)-14-deacetylerylene (**2**), featuring chemoselective THF ring formations stereocontrolled by *syn*-oxidative cyclizations of the bishomoallylic monool and diol induced by oxorhenium(vii) and -chromium(vi) species, respectively. The reason for the intriguing relationship^[2] between the conformations and cytotoxicities of **1** and **2** is under investigation.

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Cuprophilicity: Spectroscopic and Structural Evidence for Cu–Cu Bonding Interactions in Luminescent Dinuclear Copper(II) Complexes with Bridging Diphosphane Ligands*

Chi-Ming Che,* Zhong Mao, Vincent M. Miskowski, Man-Chung Tse, Chi-Keung Chan, Kung-Kai Cheung, David Lee Phillips,* and King-Hung Leung

Copper(i)–copper(i) bonding interactions have widely been invoked to be a driving force for the self-assembly of copper(i) aggregates and to play an important role with regard to the photoluminescence of polynuclear copper(i) complexes con-

[*] Prof. Dr. C.-M. Che, Dr. D. L. Phillips, Z. Mao, Dr. V. M. Miskowski, Dr. M.-C. Tse, Dr. C.-K. Chan, Dr. K.-K. Cheung, Dr. K.-H. Leung
Department of Chemistry, The University of Hong Kong
Pokfulam Road, Hong Kong SAR (P.R. China)
Fax: (+852) 2857-1586
E-mail: cmche@hku.hk
phillips@hku.hk

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taining phosphane or pyridine ligands.^[1–6] Weak but real $d^{10}-d^{10}$ metal–metal bonding is well established for 2nd and 3rd transition-series metal complexes,^[7–10] but the important case of Cu^I centers has remained unresolved. A recent electron diffraction study on Cu_2O found evidence for Cu–Cu bonding,^[11] and this result is of possible relevance to copper oxide superconductors. There is also biological relevance with regard to the binuclear “ Cu_A ” site of cytochrome oxidases and the corresponding model compounds.^[12] However, several studies on binuclear Cu^I complexes have denied the existence of Cu–Cu bonding,^[13] so the existence of “cuprophilicity”, analogous to “aurophilicity” has remained unsubstantiated. While $\text{Cu}^I \cdots \text{Cu}^I$ separations as short as 2.412(1) Å are known,^[14] spectroscopic evidence for $\text{Cu} \cdots \text{Cu}$ interaction has been notably lacking, which can be principally attributed to the ubiquity of ligands incorporating organic unsaturated units that greatly complicate interpretation.

We prepared $[\text{Cu}_2(\text{dcpm})_2]\text{X}_2$ (dcpm = bis(dicyclohexylphosphanyl)methane, $\text{X} = \text{ClO}_4^-$ (**1**), PF_6^- (**2**), and I^- (**3**)) and $[\text{Cu}_2(\text{dcpm})_2(\text{CH}_3\text{CN})_2]\text{X}_2$ ($\text{X} = \text{ClO}_4^-$ (**4**), PF_6^- (**5**)) complexes by reacting the appropriate Cu^I salts, $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{X}$ or CuI , with dcpm in acetone solutions. The absence of CH_3CN in (**1**) and (**2**) requires recrystallization from dichloromethane. Their crystal structures have been determined.^[15] Complexes **4** and **5** recrystallized from CH_3CN and have CH_3CN ligands coordinated to each copper atom, resulting in Y-shaped trigonal P_2CuN configurations. In $[\text{Cu}_2(\text{dcpm})_2]\text{X}_2$ (**1–3**), counteranions weakly interact with the Cu atoms in a similar trigonal fashion ($\text{X} = \text{ClO}_4$, $\text{Cu} \cdots \text{O}$, av. 2.687; $\text{X} = \text{PF}_6$, $\text{Cu} \cdots \text{F}$ 2.79(1); $\text{X} = \text{I}$, $\text{Cu} \cdots \text{I}$, av. 2.622 Å). The intramolecular $\text{Cu} \cdots \text{Cu}$ separations fall in the range 2.639(2)–2.938(2) Å, which are shorter than or close to the sum of the Van der Waals radii of Cu^I centers and increase in the order: **1**(average separations 2.685), **2**(2.790(5)) < **4**(2.8096(9)), **5**(2.810(2)) < **3**(av. 2.905 Å). This is consistent with the idea that a stronger Cu^I -anion/solvent interaction such as that in **3** would lead to weaker intramolecular metal–metal interaction. These $\text{Cu} \cdots \text{Cu}$ separations are all substantially shorter than that of the previously reported complex $[\text{Cu}_2(\text{dppm})_2(\text{CH}_3\text{CN})_4](\text{ClO}_4)_2$ ($\text{Cu} \cdots \text{Cu} = 3.426(3)$ Å; dppm = bis(diphenylphosphanyl)methane) in which the Cu atoms adopt pseudo-tetrahedral geometry with four donor ligands^[16a] and of $[(\text{Cu}_2)(i\text{Pr})_2\text{PCH}_2\text{P}(i\text{Pr})\text{H}_2](\text{CH}_3\text{CN})_2]$ ($\text{Cu} \cdots \text{Cu} = 2.973(1)$ Å) in which the Cu atoms are three coordinate.^[16b] The mononuclear compound $[\text{Cu}(\text{PCy}_3)_2]\text{ClO}_4$ (**6**; PCy_3 = tricyclohexylphosphine) has also been prepared by reacting $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{ClO}_4$ with PCy_3 its crystal structure reveals a trigonal $\text{P}_2\text{CuO}-\text{ClO}_3$ geometry with no intermolecular Cu–Cu contacts in the crystal lattice.^[15]

In the compounds of the present study there is a clear propensity for the coordination of ligands, in addition to the bridging diphosphanes, which exceeds that of the analogous binuclear Au^I dcpm complexes.^[17] However, the steric hindrance of dcpm appears to limit the number of additional ligands to a single one per Cu^I center, so a close metal–metal contact can be maintained, albeit with trigonally coordinated geometry at each Cu^I center (Figure 1). Indeed, the ^{31}P NMR spectroscopy data in CD_2Cl_2 solution clearly indicate a Cu^I -anion interaction in that the chemical shifts depend on the

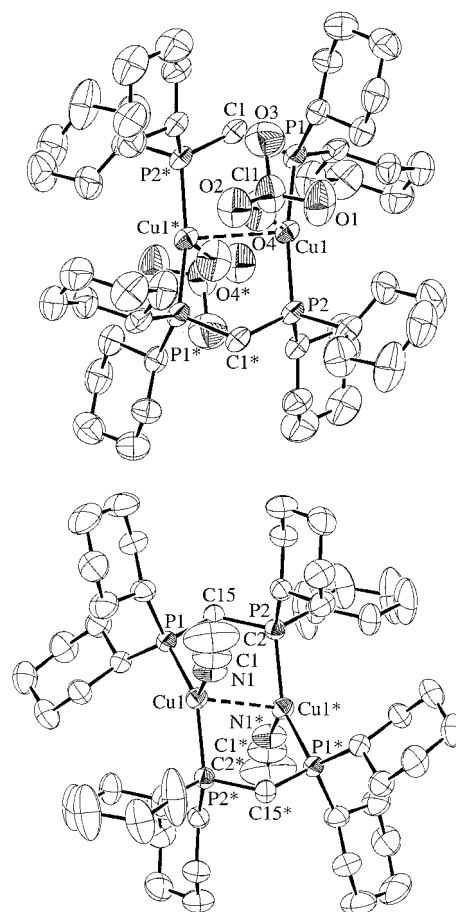


Figure 1. ORTEP plots of a) **1** and b) **4**; thermal ellipsoids set at the 40% probability level. Selected distances [Å] and angles [°] for one independent molecule of **1**: $\text{Cu1} \cdots \text{Cu1}^*$ 2.731(2), $\text{Cu1} \cdots \text{O4}$ 2.558(6), $\text{Cu1}-\text{P1}$ 2.228(2), $\text{Cu1}-\text{P2}$ 2.229(2); $\text{P1}-\text{Cu1}-\text{P2}$ 162.48(8), $\text{P1}-\text{Cu1}-\text{P2}^*$ 113.5(3); for **4**: $\text{Cu1} \cdots \text{Cu1}^*$ 2.8096(9), $\text{Cu1}-\text{N1}$ 2.029(4), $\text{Cu1}-\text{P1}$ 2.248(1), $\text{Cu1}-\text{P2}^*$ 2.249(1); $\text{P1}-\text{Cu1}-\text{P2}^*$ 143.86(4), $\text{P1}-\text{Cu1}-\text{N1}$ 108.8(1), $\text{P2}^*-\text{Cu1}-\text{N1}$ 105.7(1), $\text{N1}-\text{C1}-\text{C2}$ 176.4(9), $\text{P1}-\text{C15}-\text{P2}$ 111.5(2).

anion: $\delta = 15.9$ for **1** and **4**; $\delta = 18.4$ for **2** and **5**; $\delta = 4.5$ for **3**. In contrast, **1**, **2**, **4**, and **5** have identical ^{31}P chemical shifts in CD_3CN , ($\delta = 9.8-9.9$), which indicate anion displacement by the solvent.

Spectroscopic and photophysical data are summarized in Table 1. In dichloromethane solution, the UV/Vis spectra of complexes **1** and **2** show intense absorption at 311 and 307 nm respectively, with extinction coefficients greater than $10^4 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$ similar to the assigned $^1(d_{\sigma^*} \rightarrow p_{\sigma})$ transitions of $[\text{Au}_2(\text{dcpm})_2]^{2+}$ ^[17] and $[\text{Au}_2(\text{dmpm})_3]^{2+}$ ^[18] (dmpm = bis(dimethylphosphanyl)methane). As mononuclear Cu^I phosphane complexes such as **6** do not have any significant absorption at wavelengths greater than 280 nm, we assign this absorption at 311 or 307 nm to a metal–metal $3d \rightarrow 4p$ transition. This is strongly supported by resonance Raman spectroscopy. Figure 2 shows the resonance Raman spectrum obtained with 299.1 nm excitation and the absorption spectrum of complex **1** in dichloromethane. As with $[\text{Au}_2(\text{dcpm})_2]^{2+}$, Raman intensity appears only in a single low-frequency mode and its overtone, which we assign to the Cu^I-Cu^I stretch; this reveals that the excited-state distortion is concentrated in the Cu–Cu bond. The assigned $\tilde{\nu}(\text{Cu}_2)$ of

Table 1. Spectroscopic and photophysical properties of complexes **1–6**.

Complex	Medium (<i>T</i> [K])	λ_{abs} [nm] (ϵ [dm ³ mol ⁻¹ cm ⁻¹])	λ_{em} [nm]/ τ [μ s] ^[a]	ϕ_{em}
[Cu ₂ (μ -dcpm) ₂](ClO ₄) ₂ (1)	CH ₃ CN (298)	269 (4830), 319 (4820)	480/4.2	0.048
	CH ₂ Cl ₂ (298) ^[c]	278 (5840), 311 (14090)	~480 (w, br)	< 10 ⁻⁴
	glass (77) ^[b] solid (298)		420 475/44	
[Cu ₂ (μ -dcpm) ₂](PF ₆) ₂ (2)	CH ₃ CN (298)	269 (4850), 319 (4830)	480/4.2	0.045
	CH ₂ Cl ₂ (298) ^[c]	307 (16660)	~440 (w, br)	< 10 ⁻⁴
	glass (77) ^[b] solid (298)		417/29 475/63 ^[d]	
[Cu ₂ (μ -dcpm) ₂]I ₂ (3)	CH ₂ Cl ₂ (298)	280 (7410), 323 (3450)	485/2.4	5.7 × 10 ⁻⁴
	glass (77) ^[b] solid (298)		456/17 460/8.3	
[Cu ₂ (μ -dcpm) ₂ (CH ₃ CN) ₂](ClO ₄) ₂ (4)	CH ₃ CN (298)	269 (4880), 319 (4850)	480/4.4	0.046
	CH ₂ Cl ₂ (298) ^[c]	278 (5590), 311 (13510)	~480 (w, br)	< 10 ⁻⁴
	glass (77) ^[b] solid (298)		420/43 418/1.9	
[Cu ₂ (μ -dcpm) ₂ (CH ₃ CN) ₂](PF ₆) ₂ (5)	CH ₃ CN (298)	269 (4890), 319 (4850)	480/4.4	0.044
	CH ₂ Cl ₂ (298) ^[c]	307 (16550)	~440 (w, br)	< 10 ⁻⁴
	glass (77) ^[b] solid (298)		420 411/8.2	
[Cu(PCy ₃) ₂]ClO ₄ (6) ^[e]	CH ₃ CN (298)	210 (23400), 230 (sh) (12500), 272 (900)	nonemissive	
	CH ₂ Cl ₂ (298) ^[c]	238 (11500), 248 (9700), 275 (sh) (270)	nonemissive	
	glass (77) ^[b] solid (298)		416/182 491 ^[f]	

[a] For emission spectra measurements excitation wavelength: 330 nm; for luminescence lifetime measurements excitation wavelength: 355 nm. [b] Solvent: EtOH/MeOH (1/4). [c] In dilute CH₂Cl₂ solution slow decomposition occurred. [d] A weak emission maxima at 380 nm was also observed. [e] For emission spectra measurements excitation wavelength: 280 nm; for luminescence lifetime measurements excitation wavelength: 266 nm. [f] Biexponential decay, τ_1 : 3.2 μ s; τ_2 : 14.3 μ s.

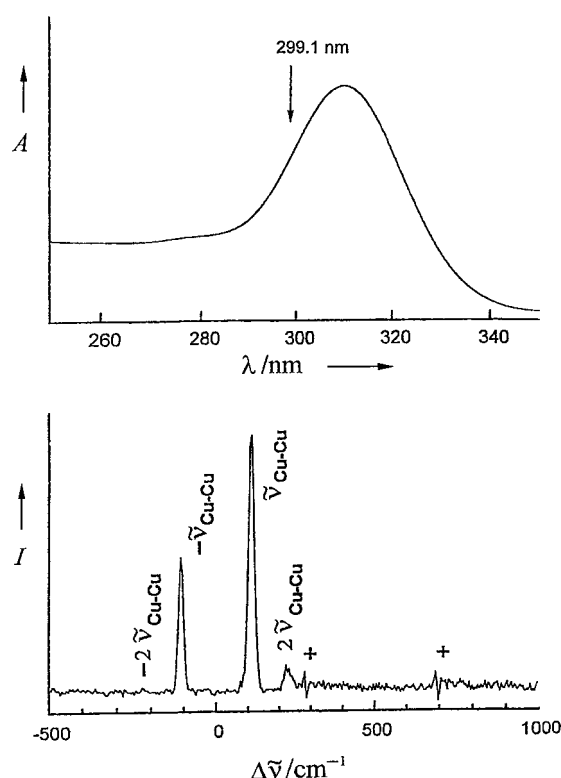


Figure 2. UV/Vis absorption spectrum of **1** (top) and resonance Raman spectrum (bottom) of **1** with excitation at 299.1 nm in dichloromethane (solvent subtraction artifacts marked by +).

104 cm⁻¹ can be compared to a $\tilde{\nu}(\text{Au}_2)$ of 88 cm⁻¹ for [Au₂(dcpm)₂]²⁺.^[7e] Clearly the [Cu]₂ bond must be much weaker than that of [Au]₂, since a simple mass calculation

predicts that the diatomic M₂ stretching frequency should be larger for Cu₂ than Au₂ by a factor of 1.76 for equivalent force constants. Using previously described calculation methods,^[7e] the excited state Cu–Cu stretching vibration is estimated to occur at 150 cm⁻¹, consistent with the electronic assignment of a transition to an excited state with increased metal–metal bonding. In acetonitrile, the binuclear Cu^I complexes show an absorption at 315–320 nm with a substantially lower extinction coefficient (Figure 3). We suggest that coordination of the acetonitrile solvent molecules to the Cu^I centers may greatly weaken the Cu^I...Cu^I interaction. The persistence of the weak absorption at 315–320 nm in CH₃CN solution may indicate equilibrium among complexes with differing numbers of bound acetonitrile molecules.

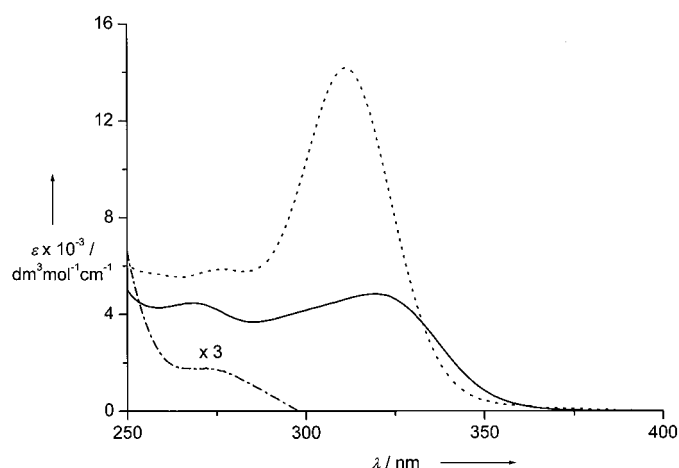


Figure 3. Electronic absorption spectra of **1** (---), **6** (-.-.-), and **4** (—) in dichloromethane and **4** (—) in acetonitrile at room temperature.

In the solid state, the binuclear Cu^I complexes show intense phosphorescence with emission maxima that are sensitive to the anion and solvent (Table 1). Remarkably, the change in the solid-state emission maxima is large, 411 to 475 nm (complexes **4** and **1** in Table 1), simply by coordination of CH₃CN to the [Cu₂(dcpm)₂]²⁺ core. Because the mononuclear Cu^I compound **6** shows an intense room-temperature solid-state emission at 491 nm, assignment of the lower-energy emission at 475–480 nm of the binuclear Cu^I complexes to a Cu–Cu bonded excited state is not favored. We suggest that increased interaction between neighboring solvent molecules or anions with Cu^I centers in the Cu–Cu bonded excited state in the crystal lattice may lower the energy of essentially single-center excited states and give rise to lower-energy photoluminescence. While the binuclear Cu^I complexes are weakly emissive in noncoordinating dichloromethane (quantum yield < 10^{−4}, broad emission at ~440 nm for compounds **2** and **5**, ~480 nm for **1** and **4**), they show an intense emission at around 480 nm in acetonitrile solution.

The Cu···Cu distances of the dinuclear Cu^I complexes described herein are comparable with those of most of the reported polynuclear Cu^I aggregates that show visible photoluminescence at wavelengths greater than 500 nm.^[3, 4] The large Stokes shift in emission energies suggests that metal–ligand influenced states may dominate the Cu^I luminescence, as has been concluded from recent studies of Au^{II} and Ag^{II} systems.

Experimental Section

Details of solvent purification for the photophysical studies, and resonance Raman measurements have been described elsewhere.^[20, 21] [Cu(CH₃CN)₄]⁺X[−] (X = ClO₄[−] or PF₆[−]) were prepared according to the published procedures.^[22] All reactions and manipulations were carried out under an argon atmosphere unless otherwise indicated.

[Cu₂(μ-dcpm)₂]₂X₂ (X = ClO₄[−] **1** or PF₆[−] **2**): A mixture of [Cu(CH₃CN)₄]⁺X[−] and dcpm (1:1 molar ratio) in acetone (20 mL) was stirred for 1 h at room temperature. Upon removal of solvent and subsequent addition of diethyl ether, a white solid was obtained, which was recrystallized from CH₂Cl₂/diethyl ether. For **1**, yield 89%. ³¹P{¹H} NMR δ = 9.9 (in CD₃CN), 15.9 (in CD₂Cl₂); FT-IR (Nujol, NaCl): $\tilde{\nu}$ = 1110.9(s), 1033.8(s, ClO₄[−]) cm^{−1}; elemental analysis calcd (%) for C₃₀H₆₂O₈P₄Cl₂Cu₂: C 52.53, H 8.11; found C 52.59, H 8.26. For **2**, yield 86%; ³¹P{¹H} NMR δ = 9.8 (in CD₃CN), 18.4 (in CD₂Cl₂); FT-IR (Nujol, NaCl): $\tilde{\nu}$ = 821.6(s), 844.8(s, PF₆[−]) cm^{−1}; elemental analysis calcd (%) for C₃₀H₆₀P₄F₁₂Cu₂: C 48.66, H 7.51; found: C 48.56, H 7.65.

[Cu₂(μ-dcpm)₂]₂I₂ **3**: A mixture of CuI and dcpm (1:1 molar ratio) in acetone (15 mL) was stirred for 2 h at room temperature. The white suspension was filtered and the solid recrystallized from CH₂Cl₂/diethyl ether, yield 95%; ³¹P{¹H} NMR δ = 4.5 (in CD₂Cl₂); elemental analysis calcd (%) for C₃₀H₆₂P₄I₂Cu₂: C 50.13, H 7.74; found: C 49.86, H 7.92.

[Cu₂(μ-dcpm)₂](CH₃CN)₂X₂ (X = ClO₄[−] **4** or PF₆[−] **5**): Recrystallization of compound **1** or **2** in CH₃CN/diethyl ether gave **4** or **5**. Their structures were determined by X-ray crystallography. The coordinated CH₃CN molecule is easily lost upon prolonged exposure to air and satisfactory elemental analyses have not been obtained. For **4**: ³¹P{¹H} NMR δ = 9.8 (in CD₃CN), 15.9 (in CD₂Cl₂), FT-IR (Nujol, NaCl): $\tilde{\nu}$ = 2304.8(w), 2268.1(m, C≡N), 1093.6(vs), 1039.6(s, ClO₄[−]) cm^{−1}. For **5**: ³¹P{¹H} NMR δ = 9.9 (in CD₃CN), 18.4 (in CD₂Cl₂); FT-IR (Nujol, NaCl): $\tilde{\nu}$ = 2310.6(w), 2268.1(m, C≡N), 837(vs, PF₆[−]) cm^{−1}.

[Cu(PCy₃)₂]₂ClO₄ **6**: A mixture of [Cu(CH₃CN)₄]⁺ClO₄[−] and PCy₃ (1:2 molar ratio) in acetone (15 mL) was stirred for 2 h at room temperature. The solvent was removed and the white solid was recrystallized from CH₂Cl₂/diethyl ether, yield 43%; FAB-MS (+ve, *m/z*): 624[M⁺ − ClO₄]; ³¹P{¹H}

NMR δ = 18.2 (in CDCl₃); elemental analysis calcd (%) for C₃₆H₆₆P₂ClO₄·Cu: C 59.74, H 9.19; found: C 60.12, H 9.28.

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- Crystal data for **1**: C₃₀H₆₂P₄Cu₂·2ClO₄, formula weight = 1143.17, monoclinic, space group *P2₁/a* (No. 14), *a* = 17.591(5), *b* = 14.553(3), *c* = 23.174(6) Å, β = 106.69(2)°, *V* = 5682(2) Å³, *Z* = 4, *D_c* = 1.336 g cm^{−3}, *F*(000) = 2432, *T* = 301 K, 595 parameters; *R₁* = 0.062, *R_w* = 0.089, GOF = 2.78 for 5115 data (*I* > 3σ(*I*)), max./min. residual electron density 1.10/−1.15 e Å^{−3}. Diffractometer: Rigaku AFC7R; μ(MoKα) = 10.03 cm^{−1}; one crystallographic asymmetric unit comprises two independent halves of the complex cation and two perchlorate anions; Crystal data for **4**: C₃₄H₉₈N₂P₄Cu₂·2ClO₄, formula weight = 1225.27, orthorhombic, space group *Pbca* (No. 61), *a* = 15.295(3), *b* = 20.000(3), *c* = 20.419(3) Å, *V* = 6246(1) Å³, *Z* = 4, *D_c* = 1.303 g cm^{−3}, *F*(000) = 2608, *T* = 301 K, 317 parameters; *R₁* = 0.067, *R_w* = 0.095, GOF = 2.98 for 4855 data (*I* > 3σ(*I*)), max./min. residual electron density 1.09/−1.01 e Å^{−3}. MAR diffractometer; μ(MoKα) = 9.18 cm^{−1}. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-142369 **1**, -142370 **2**, -142371 **3**, -142609 **4**, -142372 **5**, -142373 **6**. Copies of the data can be obtained free of charge on

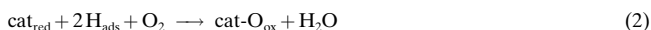
application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Low-Temperature Activation of Dioxygen and Hydrocarbon Oxidation Catalyzed by a Phosphovanadomolybdate: Evidence for a Mars–van Krevelen Type Mechanism in a Homogeneous Liquid Phase**

Alexander M. Khenkin and Ronny Neumann*

Aerobic heterogeneous catalytic oxygenation of hydrocarbons with transition metal oxides often proceeds by a Mars–van Krevelen (M-vK) type mechanism [Eqs. (1) and (2)].^[1]



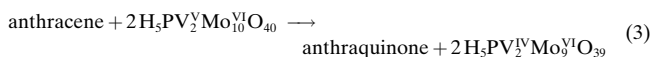
This mechanism involves activation of the C–H bond and transfer of lattice oxygen from the oxide to yield the product and the reduced catalyst. Re-oxidation of the catalyst by dioxygen and formation of water then follows. Heterogenization of homogeneous catalysis seeks to combine the selectivity and mild conditions typical of homogeneous catalysis with the handling advantages inherent in heterogeneous catalysis.^[2] Here, we consider the application of the inverse concept, that is, homogenization of heterogeneous catalysis, to M-vK type oxygenations. Since archetypal M-vK oxygenation reactions can both activate C–H bonds in hydrocarbons and utilize dioxygen as a terminal oxidant, such catalysis in

homogeneous media could lead to viable, selective low-temperature aerobic oxidation of hydrocarbons.

Polyoxometalates (POMs)^[3] are discrete, anionic and soluble (mixed) metal oxides. They have the potential to activate dioxygen^[4] and to function as oxidation catalysts.^[5] Oxygen transfer in the liquid phase has been demonstrated using chromium(v)-oxo-containing POMs^[6] and activation of a C–H bond has been observed using phosphovanadomolybdates.^[7] Thus, hydrocarbons such as cyclic dienes are oxydehydrogenated but to our knowledge there are no reports of combined hydrocarbon activation, oxygen transfer, and dioxygen activation through a M-vK type mechanism in homogeneous media.^[8]

We now report that the phosphovanadomolybdate $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ can activate both aromatic and benzylic C–H bonds in hydrocarbons, for example, anthracene and xanthene, in homogeneous liquid phases by electron transfer. Subsequent oxygen transfer from the POM leads to the formation of the quinone/ketone. The reaction occurs by a M-vK type mechanism.

Hydrocarbons (60 mM) and $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40} \cdot 34\text{H}_2\text{O}$ ^[9] (1 mM) were treated with one atmosphere of O_2 in CH_3CN at 60 °C for 18 h. Analysis of the reaction mixtures showed that selective oxidation (> 99 %) of anthracene to anthraquinone, xanthene to xanthone, fluorene to fluorenone, and diphenylmethane to benzophenone occurred with turnover numbers of 24, 30, 13, and 1, respectively. Initial strong support for a M-vK type mechanism, namely, that the source of the oxygen in the product was from the POM lattice and not from atmospheric O_2 , was obtained by a classic $^{18}\text{O}_2$ -labeling experiment. In the oxidation of anthracene or xanthene (20 mM) catalyzed by $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40} \cdot 34\text{H}_2\text{O}$ (1 mM) in CH_3CN under $^{18}\text{O}_2$ (1 atm, 96.1 % purity) at 60 °C, the amount of ^{18}O found in the product by GC-MS increased as the reaction progressed. Incorporation of ^{18}O was 0, 14.8, and 22.5 mol % after 1, 5.4, and 10.2 turnovers for anthraquinone and 0, 12.3, and 19.6 mol % after 1, 4.9, and 11.4 turnovers for xanthone. Full incorporation of ^{18}O is expected from the outset for autooxidation since the initial radical formed will always react with $^{18}\text{O}_2$. Second, a reaction (60 °C, 24 h) of equimolar amounts of anthracene or xanthene and $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ under Ar in an NMR tube also yielded 48 % of anthraquinone and 71 % of xanthone, as evidenced by ^1H NMR spectroscopy. Since each POM molecule can be formally considered as a three-electron oxidant [Eqs. (3) and (4)], the maximum conversions into anthraquinone and xanthone are 50 and 75 %, respectively.



Third, similar anaerobic reactions of equimolar amounts of anthracene and $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ enriched with ^{17}O or ^{18}O yielded labeled anthraquinone as measured by ^{17}O NMR spectroscopy ($\delta = 515$) and GC-MS (^{18}O), respectively. An approximately 75 % ^{18}O -enriched $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ yielded

[*] Prof. Dr. R. Neumann, Dr. A. M. Khenkin
 Department of Organic Chemistry
 Weizmann Institute of Science
 Rehovot 76100 (Israel)
 Fax: (+972) 8-9344142
 E-mail: Ronny.Neumann@weizmann.ac.il

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

anthraquinone labeled with 70 % ^{18}O . Fourth, the reaction stoichiometry that conforms with a M-vK mechanism [Eqs. (3) and (4)] indicates that 1.5 equivalents of O_2 are consumed per catalytic cycle for the oxidation of anthracene, while a 3:1 O_2 :anthracene stoichiometry is expected for autooxidation with a significant number of propagation chains. Indeed, oxygen consumption was 1.45 ± 0.2 equivalents per reaction cycle.

Since $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ is a hydrate one needs to exclude the possibility of water as the source of oxygen leading to the formation of products by its nucleophilic attack on the radical cation intermediate (see also below).^[10] Thus, an excess of AcOH was added to the reaction mixture (anthracene or xanthene (20 mM), $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40} \cdot 34\text{H}_2\text{O}$ (1 mM), AcOH (160 mM), 60°C , 1 atm O_2 , 16 h). Only anthraquinone and xanthone at 90 and 95 % conversion were obtained; there was no acetylation by the nucleophilic AcOH. In a reaction carried out in an anhydrous medium (anthracene (20 mM), $\text{Q}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ (1 mM, $\text{Q} = (\text{C}_4\text{H}_9)_4\text{N}$), hexafluoro-2-propanol (1 mL), 55°C , 1 atm O_2 , 16 h) anthraquinone was the only product. The addition of water had no significant effect.

The experiments described above indicated that oxygenation occurred by oxygen transfer from a lattice oxygen atom of the POM to the hydrocarbon substrate. Further aspects of the oxidation mechanism and catalytic cycle were studied. Of initial interest was the mode of activation of the C–H bond, which can occur by the formation of a donor–acceptor complex, electron transfer,^[11] or hydrogen abstraction.^[12] The interaction of hydrocarbons with $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ was studied by measuring the appearance of the absorbance corresponding to the reduced POM at 750 nm. Kinetic profiles obtained from the reaction of $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40} \cdot 34\text{H}_2\text{O}$ (1 μmol) and the hydrocarbon (10 μmol) in CH_3CN (3 mL) under Ar at 60°C showed that the reactions were pseudo-first-order in $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$. The rate constants correlated very well ($r^2 = 0.89$) with the gas-phase ionization potential^[13] of the hydrocarbon (Figure 1). Hydrocarbons with both weaker benzylic C–H bonds (80–85 kcal/mol) found in xanthene and stronger aromatic C–H (ca. 105 kcal/mol) bonds found in

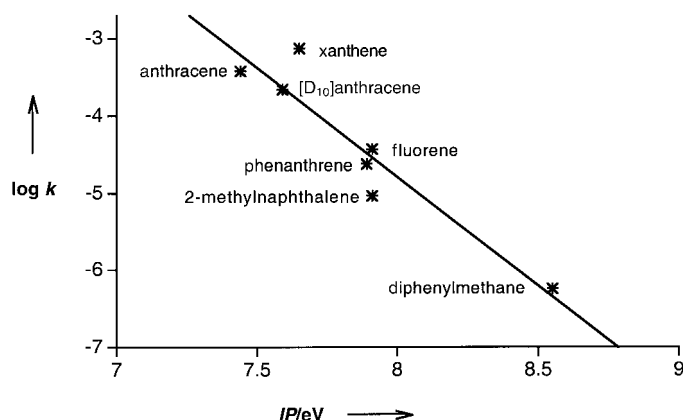


Figure 1. The reaction of $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ with hydrocarbons as a function of the gas-phase ionization potential of the hydrocarbon. The reduction of $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ was followed by a diode-array UV/Vis spectrometer at 750 nm. Conditions: $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ (1 μmol) and hydrocarbon (10 μmol) in CH_3CN (3 mL) under Ar at 60°C . Pseudo-first-order rate constants (s^{-1}) were obtained from the initial rates for up to 20 % conversion.

anthracene were purposely used in the comparison. Clearly, the initial interaction is by formation of a donor–acceptor complex between the hydrocarbon and the POM, or electron transfer to form a radical cation and the mono-reduced $\text{PV}^{\text{IV}}\text{V}^{\text{V}}\text{Mo}_{10}\text{O}_{40}^{6-}$ species. ESR experiments (see Supporting Information) clearly showed significant formation the radical cation intermediate.

Although the reduction of the POM with $[\text{D}_{10}]$ anthracene as the substrate was about twice as slow as when anthracene was used, an inverse kinetic isotope effect, $k_{\text{H}}/k_{\text{D}} = 0.85 \pm 0.05$ for the formation of anthraquinone, was measured in a competitive reaction (anthracene (10 mM), $[\text{D}_{10}]$ anthracene (10 mM), $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40} \cdot 34\text{H}_2\text{O}$ (1 mM), 60°C , 1 atm O_2). This result is consistent with a change of hybridization from sp^2 to sp^3 at the 9(10) aromatic carbon atom of anthracene in the rate-determining step.^[14] Therefore, formation of a C–O bond must occur in the rate-determining step. More insight into the oxygen-transfer step was obtained by measurement of the IR spectra of ^{18}O -enriched $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ after catalytic reaction with anthracene under $^{16}\text{O}_2$ (Figure 2). In ^{18}O -labeled

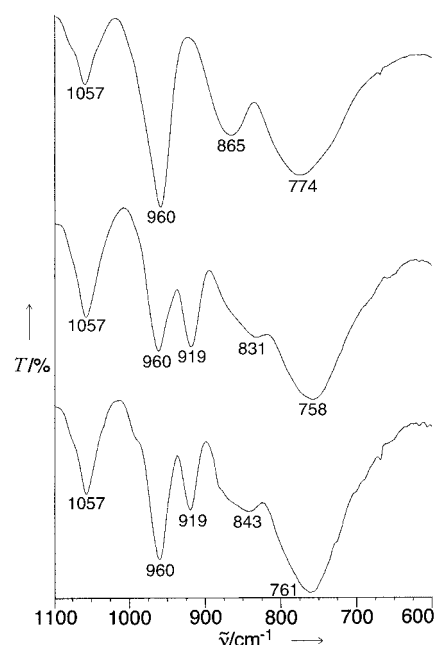
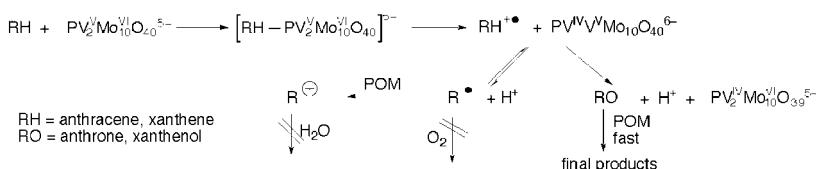


Figure 2. The IR spectrum of ^{18}O -labeled $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ before and after reaction with anthracene and $^{16}\text{O}_2$. Top: unlabeled $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$; middle: ^{18}O -labeled $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$; bottom: ^{18}O -labeled $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ after reaction with anthracene and $^{16}\text{O}_2$ after ten turnovers.

$\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ (middle) there is a shift relative to unlabeled $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ of the $\text{M}=\text{O}_t$ (terminal) absorption (two distinct peaks) from 960 to 919 cm^{-1} . Additionally, there are absorption peaks shifts from 865 to 831 cm^{-1} and 774 to 758 cm^{-1} for corner and edge-shared oxygen atoms, respectively. A 69 % incorporation of ^{18}O into anthraquinone was observed along with an increase in the peak ratio of 960 (^{16}O) to 919 cm^{-1} (^{18}O) after ten turnovers (bottom; anthracene (60 mM), $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ (1 mM), CH_3CN , 60°C , $^{16}\text{O}_2$). Shifts from 831 to 843 cm^{-1} and 761 to 758 cm^{-1} were also observed in the spectra. Clearly, there is re-incorporation of ^{16}O into the POM from $^{16}\text{O}_2$. The results show that hydrocarbon oxygenation



Scheme 1. Activation and oxygenation of aromatic hydrocarbons by $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$.

through a M-vK type mechanism is possible in a homogeneous liquid phase using POMs as catalysts (Scheme 1).

The reaction is initiated by the relatively fast formation of a donor–acceptor complex and then a radical cation. Subsequent formation of a free radical and oxygenation is discounted from the isotope incorporation experiments and anaerobic reactions. Homolytic cleavage of the C–H bond (not shown) does not occur because compounds that easily react in this way (for example, cyclohexene) did not react in this system. Nucleophilic attack of water was also discounted. The rate-determining step appears to be the oxygen transfer from the POM^[15] to the activated hydrocarbon as indicated by the inverse deuterium isotope effect. Consequent reaction steps are fast, since no intermediates such as anthrone are observed, and benzylic alcohols react much faster.

Experimental Section

Reactions were carried out in 15-mL glass pressure tubes. Typically, appropriate amounts of hydrocarbon substrate (all highest purity commercial products) and $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40} \cdot 34\text{H}_2\text{O}$ ^[9] were dissolved in CH_3CN . The gases $^{18}\text{O}_2$ (96.1%) or $^{16}\text{O}_2$ were added by four consecutive pump/thaw cycles on a Schlenk line. Conversions were measured by gas-liquid chromatography (HP 6890) on a 5% phenylmethylsilicone (30 m, 0.32 mm ID, 0.25 μm coating) column. Isotope incorporation was quantified by GC-MS (HP 5973 (same column)). Reactions under anaerobic conditions in CD_3CN , similarly carried out in high-pressure NMR tubes, were quantified by ^1H NMR spectroscopy. Labeled $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ was prepared by drying the POM at 120°C for 24 h and then adding 50 equivalents of H_2^{17}O (10.2%) or H_2^{18}O (94.3%) in dry CH_3CN and mixing for 18 h. The drying/exchange cycle was repeated three times. ^{18}O enrichment (see Figure 2, middle) is estimated as about 50% at the terminal oxygen atoms and to be greater than 90% (by deconvolution by assuming a 40 cm^{-1} shift, and from the equal intensities of the peaks) at the edge and corner-shared oxygen atoms. Total enrichment for potentially transferable oxygen atoms is therefore about 75%. Anhydrous reactions were carried out using $((\text{C}_4\text{H}_9)_4\text{N})_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ in dried solvents. The isotope effect in the oxidation of $[\text{H}_{10}]$ - and $[\text{D}_{10}]$ anthracene in a competitive reaction was measured by mixing anthracene (10 mm), $[\text{D}_{10}]$ anthracene (10 mm), $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40} \cdot 34\text{H}_2\text{O}$ (1 mm) at 60°C , in O_2 (1 atm). The isotope ratio was computed by comparing the ratio of $[\text{H}_{10}]$ - and $[\text{D}_{10}]$ anthracene remaining under pseudo-first-order conditions at up to 35% conversion. Samples for IR spectra (Nicolet Protégé 460) were prepared by placing a few drops of the POM-containing solution on a NaCl plate and evaporating off the solvent. ESR spectra were measured in 1-mm tubes at ambient temperature. UV/Vis kinetic measurements were performed on a diode-array instrument (HP 5842) equipped with a magnetic stirrer and a temperature bath ($60 \pm 0.1^\circ\text{C}$). ^{17}O NMR spectra (Bruker DMX 400) were measured at 54.244 MHz and referenced to H_2^{17}O .

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Oxidosqualene Cyclase Residues that Promote Formation of Cycloartenol, Lanosterol, and Parkeol**

Michelle M. Meyer, Michael J. R. Segura, William K. Wilson, and Seiichi P. T. Matsuda*

Eukaryotes make sterols from three different cyclic intermediates.^[1] Lanosterol (**7**) is the initial carbocyclic sterol precursor in animals^[2] and fungi.^[3] Cycloartenol (**5**) and parkeol (**8**) are isomeric sterol biosynthetic intermediates in plants^[4,5] and sea cucumbers,^[6] respectively. Lanosterol, cycloartenol, and parkeol synthases cyclize (*S*)-2,3-oxidosqualene ((*S*)-**1**)^[7] to the intermediate protosteryl cation **2**^[8] and form different products by promoting cationic cyclization

[*] Prof. Dr. S. P. T. Matsuda, M. M. Meyer, M. J. R. Segura, Dr. W. K. Wilson
Department of Chemistry and
Department of Biochemistry and Cell Biology
Rice University
6100 South Main Street, Houston, TX 77005 (USA)
Fax: (+1) 713-348-5154
E-mail: matsuda@rice.edu

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and rearrangement reactions terminated by multiple deprotonation options (Scheme 1). A conserved active-site valine in lanosterol synthase participates in tetracycle formation,^[9] and a conserved isoleucine at the corresponding position in cycloartenol synthase influences both cyclization and deprotonation.^[10, 11] In search of other catalytic residues, we mutated conserved lanosterol-synthase residues where cycloartenol synthase maintains a different residue, and thereby generated an enzyme that makes parkeol as its major product.

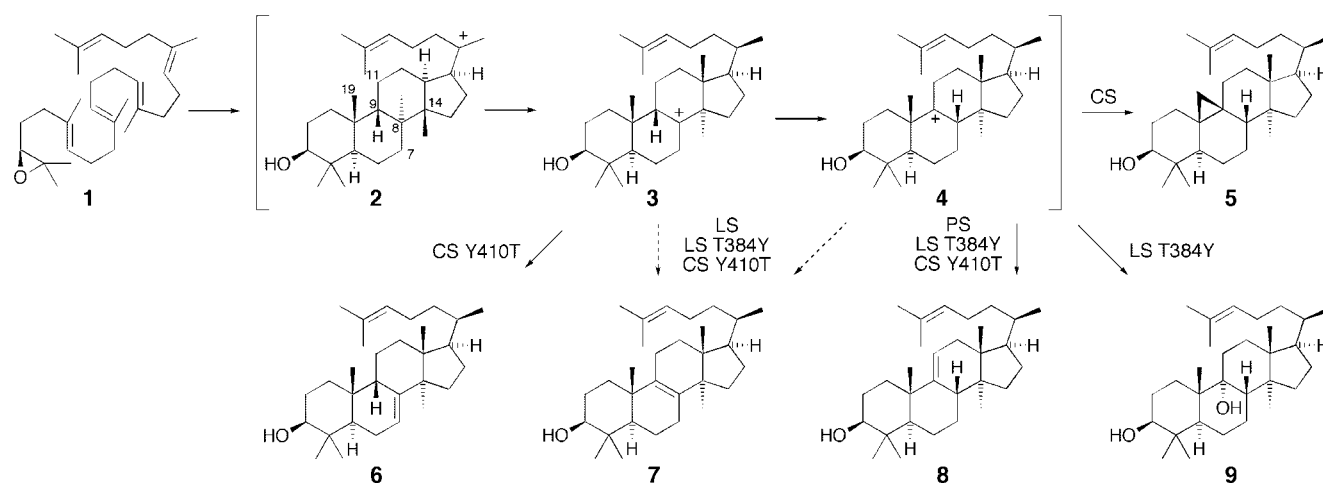
The Thr384 residue is conserved in lanosterol synthase. All known cycloartenol synthases have Tyr at this position, which corresponds^[12] to an active-site residue in the squalene–hopene cyclase^[13] crystal structure.^[14, 15] The conservation pattern and the predicted active-site location are consistent with a role in the catalytic differences between these enzymes. We mutated Thr384 in *Saccharomyces cerevisiae* lanosterol synthase into Tyr, and expressed the mutant gene with the vector pRS305GAL in the lanosterol-synthase/squalene-synthase double-mutant yeast strain LHY3.^[10] Incubation with exogenous racemic 2,3-oxidosqualene (**1**) as previously described^[9] produced a triterpene alcohol fraction (which comigrates with lanosterol during thin layer chromatography) and a more polar product. The triterpene alcohol fraction exhibited ¹H NMR spectroscopic signals characteristic of a lanosterol (**7**) and parkeol (**8**) mixture, and GC-MS after acetylation confirmed these structures. The more polar product was shown to be lanost-24-ene-3 β ,9 α -diol (**9**; Table 1, Scheme 1) by NMR spectroscopy (¹H, ¹³C, DEPT, COSY-DEC, and HSQC); this is a known synthetic compound^[16] that has not been isolated from nature or from an enzymatic reaction. Parkeol (**8**) results from C-11 deprotonation, and the diol **9** is formed by quenching an intermediate C-9 carbocation with water, a process that is preceded in the biosyntheses of diplopterol,^[17] dammarenediol,^[18] and lupanediol.^[19] The 9 α stereochemistry is consistent with water attack concerted with hydride migration from C-9 to C-8.

Table 1. Product composition of *S. cerevisiae* lanosterol-synthase mutants.

Mutation	7 [%]	8 [%]	9 [%]
none	100	0	0
Val454 Ile ^[9]	100	0	0
Val454 Leu ^[9]	100	0	0
Thr384 Tyr	79	11	10
Thr384 Tyr/Val454 Ile	13	64	23
Thr384 Tyr/Val454 Leu	20	60	20

Mutating the lanosterol-synthase residue Val454 has also been shown to alter the product structure.^[9] To determine how these two catalytically relevant positions interact, we made a lanosterol-synthase mutant with both Thr384 and Val454 converted into their cycloartenol-synthase counterparts. The Thr384 Tyr/Val454 Ile derivative produced primarily parkeol (**8**), with lanosterol (**7**) and 9 α -diol (**9**) by-products (Table 1). The methylene added by mutating valine to isoleucine shifted production dramatically from lanosterol to parkeol, and doubled the amount of water-quenched material. This is a synergistic effect; the Val454 Ile mutation alone did not change the product profile.^[9] The Tyr384 derivative allowed parkeol formation, and greater bulk at position 454 amplified this effect and also increased diol formation. The larger residue may displace the substrate or basic residue to expose the C-9 carbocation to water. We examined the importance of shape at position 454 by replacing the *sec*-butyl side chain of Ile with the isobutyl side chain of Leu. The Thr384 Tyr/Val454 Leu mutant is also a parkeol synthase (albeit slightly less accurate than the Thr384 Tyr/Val454 Ile derivative; see Table 1) and generated the same two by-products.

These mutants are particularly informative when compared to complimentary experiments in which *A. thaliana* cycloartenol synthase was mutated to resemble lanosterol synthase at the residues Tyr410 and Ile481, which correspond to Thr384 and Val454, respectively.^[20] The Tyr410 Thr mutation abolished cycloartenol formation completely; the resultant enzyme converted 2,3-oxidosqualene (**1**) into 65 % lanosterol



Scheme 1. Lanosterol synthase (LS), cycloartenol synthase (CS), and parkeol synthase (PS) cyclize 2,3-oxidosqualene (**1**) to the protosteryl cation **2** and catalyze rearrangements and deprotonations to form their respective triterpene alcohol products. It is not established whether the intermediate C-8 or C-9 cations, **3** and **4**, actually occur but these are drawn to facilitate visualization of which protons depart. Cycloartenol (**5**) and parkeol (**8**) result from C-19 and C-11 deprotonation, respectively. Lanost-24-ene-3 β ,9 α -diol (**9**) is easily envisioned as a water-quenched C-9 cation. Losing the proton originally at C-9 (possibly after shifting to C-8) yields lanosterol (**7**). 9 β -Lanosta-7,24-dien-3 β -ol (9 β - Δ 7 lanosterol, **6**) could result from deprotonation of the C-8 cation or from deprotonation concerted with a methyl shift from C-8 to C-14. Y = Tyr, T = Thr.

(7), 33 % 9 β -lanosta-7,24-dien-3 β -ol (9 β - Δ 7-lanosterol, 6), and 2 % parkeol (8; Scheme 1).^[20] Adding the Ile 481 Val mutation increased lanosterol formation to 75 % and lowered 9 β - Δ 7-lanosterol and parkeol production to 24 % and 0.6 %, respectively.^[20]

Both lanosterol-synthase and cycloartenol-synthase product specificity is markedly altered by mutating a hydroxylated residue to its counterpart in the other enzyme (exchanging threonine for tyrosine, and vice versa). Exchanging the differentially conserved aliphatic residues (valine and isoleucine) amplifies the effect. A striking difference between the two studies is that although a cycloartenol synthase with lanosterol-synthase residues produces mostly lanosterol, introducing cycloartenol-synthase residues into lanosterol synthase generates a parkeol synthase that does not form cycloartenol. Thus, cycloartenol synthase promotes a kinetically favored route to cyclopropane ring formation that can be modified to produce thermodynamically favored products, but modifying lanosterol synthase to form a cyclopropyl ring requires an additional motif that remains unidentified.

Cycloartenol (5), parkeol (8), and the 9 α -diol 9 are probably derived from the C-9 cation 4, and are formed by enzymes with Tyr at the position studied (lanosterol-synthase Thr 384 Tyr mutants, native cycloartenol synthase, and cycloartenol synthase Ile 481 Val mutants^[11]). 9 β - Δ 7-Lanosterol (6) is most readily rationalized as deriving from the C-8 cation 3, and is formed exclusively by an enzyme bearing Thr in place of Tyr 410. Lanosterol (7) could be derived from either cation, and is produced by enzymes with either Tyr or Thr. The residues in question may stabilize different intermediate cations or facilitate the formally *syn* hydride shift which is essential to form cycloartenol (5) but not lanosterol or parkeol (7 and 8, respectively).

As no natural parkeol synthase has been cloned, sequence information is unavailable. This enzyme may resemble the mutant enzymes developed in this study, with tyrosine and isoleucine (or possibly leucine) at positions corresponding to residues 384 and 454 in *S. cerevisiae* lanosterol synthase, respectively. Experiments to test this hypothesis by cloning and characterizing parkeol synthase are in progress.

Experimental Section

DNA manipulation, microorganisms, and culture conditions: Oligo-directed mutagenesis, transformation, and DNA amplification techniques followed established protocols.^[21] Yeast transformation and protein expression were performed as described previously.^[9]

Enzymatic reaction, product identification, and quantitation: Recombinant yeast was grown on a 4-L scale in inducing medium, and in vitro reactions with racemic 2,3-oxidosqualene (1; 100 mg) were performed as described.^[9] Reactions generally approached completion (as judged by thin layer chromatography), and isolated yields were generally $\approx$ 80 %. Lanosterol (7) and parkeol (8) were identified by comparison of their ¹H NMR spectra to those of authentic standards. Although NMR spectra for lanost-24-ene-3 β ,9 α -diol (9) have not been reported previously, the 24,25-dihydro analogue (lanostane-3 β ,9 α -diol) has been characterized.^[22] NMR spectroscopic signals in the ring system of 9 matched very closely those of the dihydro derivative, and the side chain provided signals corresponding to those of lanosterol (7).^[22] Selected data for compound 9: ¹H NMR (500 MHz, CDCl₃, 25 °C, referenced to Si(CH₃)₄; atom numbering as in ref. [22]): δ = 0.786 (s, H-18), 0.808 (s, H-29), 0.924 (s, H-30), 0.996 (s, H-28), 1.035 (s, H-19), 1.602 (s, H-27), 1.681 (s, H-26), 3.201 (m, H-3); ¹³C NMR

(125 MHz, CDCl₃, 25 °C, referenced to CHCl₃ at 77.0 ppm): δ = 14.59, 15.40, 16.73, 17.62, 18.27, 18.58, 21.42, 23.74, 24.85, 25.71, 27.36, 27.95, 27.95, 28.28, 29.13, 29.52, 33.82, 35.80, 36.39, 38.80, 40.54, 42.64, 45.18, 45.69, 47.45, 50.36, 77.17, 78.64, 125.16, 130.95. Ratios of products were determined by ¹H NMR quantitation using well-resolved signals.

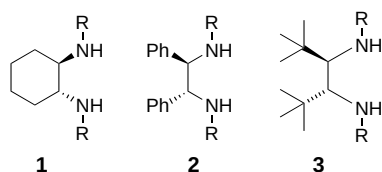
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A New Efficient Synthesis of (*R,R*)-2,2'-Bipyrrolidine: An Interesting Chiral 1,2-Diamine with *C*₂ Symmetry**

Alexandre Alexakis,* Axel Tomassini, Cyril Chouillet, Sylvain Roland, Pierre Mangeney, and Gerald Bernardinelli

Chiral *C*₂-symmetrical diamines emerged recently as versatile auxiliaries or ligands in many asymmetric transformations.^[1] For example, *N,N'*-dialkyl-1,2-cyclohexanediamine (**1**), *N,N'*-dialkyl-1,2-diphenyl-1,2-ethanediamine (**2**), *N,N'*-dialkyl-1,2-di(*tert*-butyl)-1,2-ethanediamine (**3**)^[2] have given excellent results in many reactions.^[1a]



To understand why the corresponding chiral amins are such efficient auxiliaries, one must first examine the particular conformation under which they react. Most studies show that in such cyclic amins (imidazolidine in this case), the preferred conformation is one with the N substituent located *trans* to the substituent on the adjacent carbon. In this conformation, each nitrogen atom becomes a stereogenic center with different stereodirecting and/or chelation ability; one lone pair participates in an anomeric effect, whereas the other one does not, making the nitrogen center more basic. This is easily seen from the X-ray structure of **4**, an amina derived from the monohydrazone of glyoxal (Figure 1).^[3]

However, is this conformation necessary? To answer this question we needed to prepare an imidazolidine where the N substituent and the substituent on the adjacent carbon are *cis* to each other. This may be attained by taking advantage of the preferential *cis* ring junction of the bicyclo[3.3.0]azaoctane ring system (see analogous tricyclic system in Scheme 1). Amins of (*R,R*)-2,2'-bipyrrolidine, which contains two nitrogen atoms in five-membered rings, are good examples of such a conformational restriction.

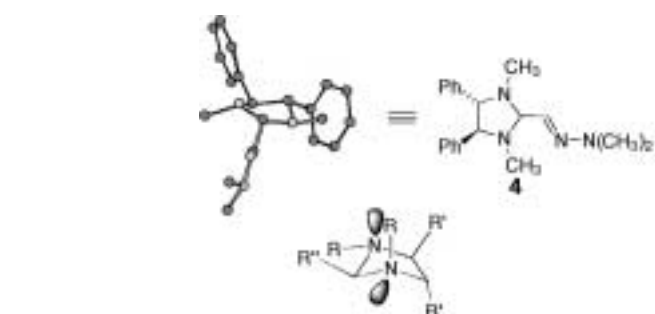
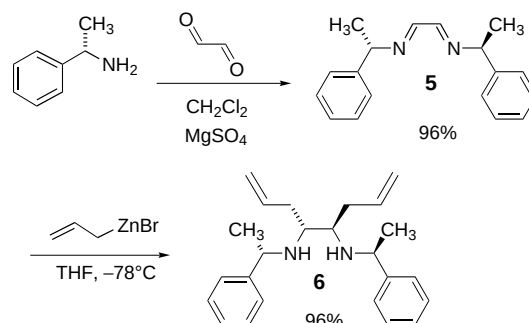


Figure 1. Conformation of chiral imidazolidine **4** and, below, a general representation of the conformation of these compounds.



Scheme 1. Comparison of the conformations of monocyclic and tricyclic imidazolidines.

(*R,R*)-2,2'-bipyrrolidine (**9**) was first prepared by Hirama and co-workers, by resolution with tartaric acid.^[5] Another synthesis has been reported by Kotsuki et al., starting from mannitol or tartaric acid.^[6] We have developed an alternative shorter strategy, which is feasible on a large scale (no chromatographic purifications are required). The first steps are based on the chiral diamine synthesis reported by Neumann et al.,^[7] latterly improved by Savoia and co-workers,^[8] which starts from *N,N*-bis[(*S*)-1-phenylethyl]ethanediamine (**5**; Scheme 2).^[9]

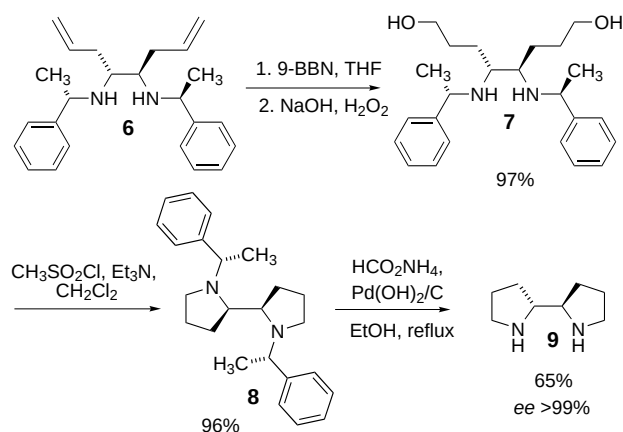


Scheme 2. Preparation of starting material **6**.

By this method, *N,N*-bis[(*S*)-1-phenylethyl]-(*R,R*)-4,5-diamino-1,7-octadiene (**6**) is obtained in good yield (60 %) and excellent diastereoselectivity (>99 %) when isolated and purified. Alternatively, a simple acid–base washing procedure afforded a quantitative yield of material suitable for further transformation. Double hydroboration of this diamine using 9-BBN in tetrahydrofuran,^[10] gave **7** in good yield with complete regiocontrol (Scheme 3). The cyclisation was carried out on the crude material by formation of the bis-mesylate (=bis-methane sulfonate) with methanesulfonyl chloride and subsequent treatment with triethylamine in dichloromethane.^[11] The resulting cyclic compound **8** (*N,N*-bis[(*S*)-1-phenylethyl]-(*R,R*)-2,2'-bipyrrolidine) was isolated

[*] Prof. A. Alexakis, A. Tomassini
Université de Genève
Département de Chimie Organique
30, quai Ernest-Ansermet, 1211 Genève 4 (Switzerland)
Fax: (+41)22-328-7396
E-mail: alexandre.alexakis@chiorg.unige.ch
C. Chouillet, S. Roland, Dr. P. Mangeney
Université P. et M. Curie
Laboratoire de Chimie des Organo-Eléments
4 place Jussieu, 75252 Paris Cedex 05 (France)
Dr. G. Bernardinelli
Université de Genève
Laboratoire de Cristallographie
24, quai Ernest-Ansermet, 1211 Genève 4 (Switzerland)

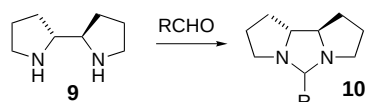
[**] The authors thank BASF for a generous gift of enantiopure phenylethylamine and the Swiss National Science Foundation (grant no. 20-53967.98) for financial support.



Scheme 3. Preparation of 2,2'-bipyrrolidine (9) starting from 6. 9-BBN = 9-borabicyclo[3.3.1]nonane.

in 60 % yield or simply purified by acid–base washing to give 95 % yield. The final step is the debenzoylation of the nitrogen groups with Pearlmann's catalyst, using ammonium formate as a hydrogen source, in refluxing ethanol (Scheme 3). This synthesis of (*R,R*)-2,2'-bipyrrolidine (9) could be carried out on a large scale (0.4 mol) in 61 % overall yield, starting from 6 and using only acid–base wash purifications and a final distillation, with an *ee* value of >99 % (the *ee* value was determined by ³¹P NMR spectroscopy).^[12]

With (*R,R*)-2,2'-bipyrrolidine (9) in hand, we easily prepared the corresponding amins 10 with several aldehydes,^[13] by simple stirring of the diamine with the aldehyde, in diethyl ether at room temperature (Scheme 4). None of the reactions however, gave products suitable for X-ray crystallography.



Scheme 4. Formation of amins 10. R = Ph, *p*-FC₆H₄, *p*NO₂C₆H₄.

In order to prove that the preferred conformation is as shown in Scheme 4, we relied on the coordination of the nitrogens to a metal salt. Thus, we were able to form two solid complexes 11 and 12, obtained from reactions of 8 and 9, respectively, with zinc chloride in diethyl ether. Compounds 11 and 12 were recrystallized from ethanol to obtain crystals suitable for analysis.

As can be seen from the X-ray structure (Figure 2) of complex 11, the 2,2'-bipyrrolidine framework adopts a stair-like conformation, perfectly fitting our hypothesis. In particular, the hybridization of the nitrogens remains clearly sp³, as may be found by the sum of the bond angles, 322°. The mean N–Zn bond is 2.125(3) Å. We may assume that amins 10 also adopt such a stair-like conformation.

Compound 12 displayed a more complex crystal structure, where two monomeric forms (diamine:zinc = 1:1) 12a and a dimeric form (diamine:zinc = 2:1) 12b alternate (Figure 3). This behavior reflects, in fact, the strong basicity of the pyrrolidine unit, which allows for the replacement of the chlorine atom by a nitrogen. It should be noted, however, that

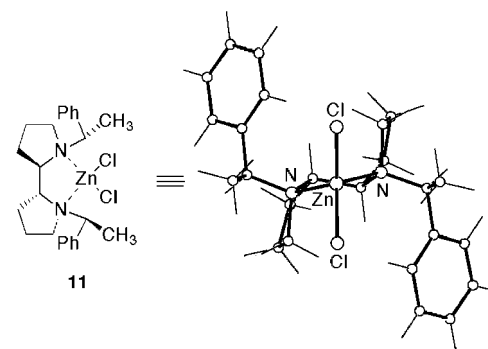


Figure 2. X-ray structure of 11, the complex formed between *N,N*-bis[(*S*)-1-phenylethyl]-(*R,R*)-2,2'-bipyrrolidine (8) and ZnCl₂.

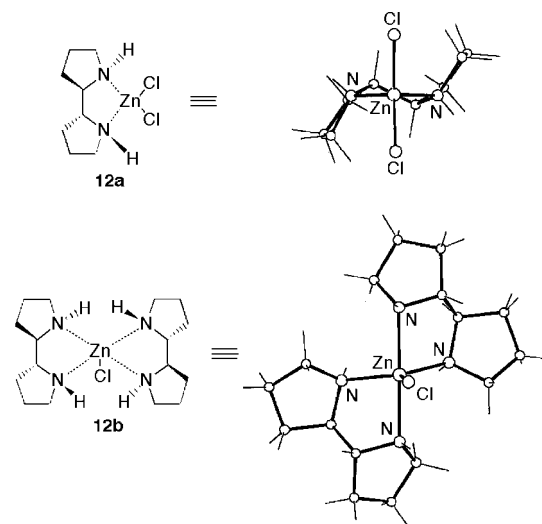


Figure 3. X-ray structures of 12a and 12b, the complexes formed between 2,2'-bipyrrolidine (9) and ZnCl₂.

both the monomeric and dimeric structures adopt the same stair-like conformation, exactly as in complex 11. The angle formed by the two adjacent five-membered rings is lower, that is, the molecule is less bent and shows a slightly lower degree of pyramidalization of the nitrogen atoms. The monomeric form has quasi perfect C₂ symmetry, again with a tetrahedral zinc atom. The dimeric form is a remarkable trigonal bipyramid, with two nitrogens in the apical positions, and two nitrogens and the chlorine atom in equatorial positions. The N–Zn apical bonds are longer than the N–Zn equatorial bonds (2.203(4) Å and 2.095(4) Å, respectively). The dimeric complex is located on a crystallographic C₂ axis passing through the Zn⁺, Cl, and Cl[−] atoms. The Cl[−] atom is located 3.941(1) Å from the Zn⁺ atom and is involved in hydrogen bonds with two nitrogen atoms of the pyrrolidine rings: N–H = 0.93(4) Å, H...Cl = 3.388(4) Å, N–H...Cl = 152(4)°.

The behavior of this diamine is being actively studied in asymmetric synthesis and the results will be reported in due course.

Experimental Section

Compound 6 was prepared as reported by Savoia and co-workers^[8] on a large scale (0.4 mol).

Compound 7: *cis,cis*-1,5-Cyclooctadiene (0.178 L, 1.45 mol) was added dropwise to a stirred solution of BH₃·Me₂S complex (95 %, 0.144 L,

1.45 mol) in dry THF (1.45 L) at -5°C under N_2 . The mixture was stirred for 45 min at -5°C (a white solid was formed). The resulting mixture was heated at reflux for 1 h, and then cooled to room temperature. A solution of **6** (127 g, 0.36 mol) in anhydrous THF (1.00 L) was added under N_2 . The mixture was stirred for 1 h and cooled to 0°C . An aqueous solution of 3 M NaOH (0.40 L) was added dropwise followed by an aqueous solution of 30% H_2O_2 (0.56 L). The mixture was stirred for 30 min at 0°C and NaOH (74 g) was added. The mixture was extracted 3 times with Et_2O (3×1.00 L) and the combined organic layers were dried over K_2CO_3 , filtered, and concentrated to give an orange oil. The crude product was purified by acid–base washing to give 135 g (97%) of *N,N'*-bis[(*S*)-1-phenylethyl]-(*R,R*)-4,5-diamino-1,8-octanediol (**7**) with a good purity (a small sample was purified on silicagel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 90/10) with 83% yield for spectroscopic analysis). ^1H NMR (200 MHz, CDCl_3 , 25°C): δ = 1.30 (d, 6H, J = 6.6 Hz), 1.38–1.24 (m, 12H), 2.41–2.25 (m, 2H), 3.56–3.49 (m, 4H), 3.77 (q, 2H, J = 6.6 Hz), 7.40–7.28 (m, 10H); ^{13}C NMR (50 MHz, CDCl_3 , 25°C): δ = 26.6, 28.2, 29.6, 55.6, 62.4, 126.8, 127.8, 128.1, 145.2; IR: $\tilde{\nu}$ = 3331, 3026, 2926, 2859, 1492, 1452, 1370, 1300, 1270, 1215, 1110, 1059, 1009, 907, 763, 701 cm^{-1} ; GC-MS (electron ionization (EI)) m/z (%): 192 (100) [$\text{C}_{12}\text{H}_{18}\text{NO}$], 174 (12) [$\text{C}_{12}\text{H}_{16}\text{N}$], 105 (79) [C_8H_6], 70 (12) [$\text{C}_4\text{H}_8\text{N}$].

Compound **8**: Et_3N (0.15 L, 1.05 mol) and $\text{CH}_3\text{SO}_2\text{Cl}$ solution (0.08 L, 1.05 mol) were added to a stirred solution of **7** (135 g, 0.35 mol) in anhydrous CH_2Cl_2 (1.00 L) under nitrogen cooled to 0°C . The mixture was warmed to room temperature and stirred for 10 min. The solvent was removed and the crude product was purified by acid–base washing to give 118 g (96%) of *N,N*-bis[(*S*)-1-phenylethyl]-(*R,R*)-2,2'-bipyrrrolidine **8** (a small sample was purified on silicagel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 90/10) with 60% yield, for spectroscopic analysis). ^1H NMR: (200 MHz, CDCl_3 , 25°C): δ = 1.20 (d, 6H, J = 6.6 Hz), 1.68–1.45 (m, 4H), 1.90–1.70 (m, 4H), 2.55–2.35 (m, 2H), 2.83–2.65 (m, 2H), 3.10–2.85 (m, 2H), 3.54 (q, 2H, J = 6.6 Hz), 7.40–7.15 (m, 10H); ^{13}C NMR (50 MHz, CDCl_3 , 25°C): δ = 16.9, 24.3, 26.4, 50.6, 61.4, 63.8, 127.2, 127.4, 128.3, 144.0; IR: $\tilde{\nu}$ = 2968, 2199, 1493, 1451, 1372, 905, 732 cm^{-1} ; GC-MS (EI) m/z (%): 174 (100) [$\text{C}_{12}\text{H}_{16}\text{N}$], 105 (60) [C_8H_6], 70 (49) [$\text{C}_4\text{H}_8\text{N}$]; MS (electron spectroscopy imaging) m/z (%): 349 (100) [$\text{M}^+ + 1$], 245 (45) [$\text{C}_{16}\text{H}_{23}\text{N}_2^{+}$], 174 (23) [$\text{C}_{12}\text{H}_{16}\text{N}$], 142 (40) [$\text{C}_8\text{H}_{16}\text{N}_2^{+}$], 106 (69) [C_8H_6^{+}].

In the synthesis of compounds **7** and **8**, 5–10% of 1,5-cyclooctanediol contaminated the product but it can be removed in the final distillation.

Compound **9**: Pd(OH) $_2$ /C (20%, 16 g) and anhydrous HCO_2NH_4 (128 g, 2.03 mol) were added to a solution of **8** (118 g, 0.34 mol) in EtOH (2.00 L). The mixture was refluxed 1.5 h with vigorous stirring under N_2 . After cooling to room temperature the mixture was filtered through celite, the residue was washed with EtOH, and the filtrate was concentrated. The residue was dissolved in Et_2O (1.00 L) and stirred for 15 min over K_2CO_3 , before being filtered and concentrated to give the crude diamine as a pale yellow oil. The crude product was purified by distillation under reduced pressure to yield 31 g (65% overall based on **7**) of **9**. ^1H NMR: (200 MHz, CDCl_3 , 25°C): δ = 1.2–2.3 (m, 8H), 2.6–3.3 (m, 6H), 4.0 (br.s, 2H); ^{13}C NMR (50 MHz, CDCl_3 , 25°C): δ = 25.9, 29.7, 46.9, 64.2; IR: $\tilde{\nu}$ = 3278, 2957, 2868, 1560, 1457, 1398, 1368, 1336, 1074, 908, 814, 763, 700, 600 cm^{-1} ; GC-MS (EI) m/z (%): 70 (100) [$\text{C}_4\text{H}_8\text{N}$], 43 (6) [$\text{C}_2\text{H}_5\text{N}$], 18 (6) [C_2H_4]; ^{31}P NMR (162 MHz, CDCl_3 , 25°C): δ = 146.7 [(N) $_2$ P-(–)menthol], 147.4 [(N) $_2$ P-(+)menthol].

General procedure for the synthesis of amins **10**: Aldehyde (1 mmol) was added to a stirred solution of 2,2'-bipyrrrolidine (140 mg, 1 mmol) in Et_2O (10 mL). The mixture was stirred at room temperature (1–5 h) until none of starting material remained (determined by GC). The solvent was removed under reduced pressure to give the crude amina.

Compound **11**: Anhydrous ZnCl_2 (50 mg, 0.37 mmol) was added to a stirred solution of **8** (130 mg, 0.37 mmol) in Et_2O (10 mL). After solubilization, a white precipitate was formed and collected by filtration. This solid was recrystallized from EtOH.

12a and **12b**: Anhydrous ZnCl_2 (135 mg, 1 mmol) was added to a stirred solution of **9** (140 mg, 1 mmol) in Et_2O (10 mL). After solubilization, a white precipitate was formed and collected by filtration. This solid was recrystallized from EtOH.

Crystal structure determinations: Cell dimensions and intensities were measured at 200 K on a Stoe IPDS diffractometer with graphite-monochromated MoK_{α} radiation (μ = 0.71069 $\text{\AA}$). Data were corrected for linear

prediction and for absorption. The structures were solved by direct methods using MULTAN87^[14], all other calculations used the XTAL system.^[15] [$\text{ZnCl}_2(\text{C}_{24}\text{H}_{32}\text{N}_2)_2$]·(CH_3OH) $_{0.25}$ (**11**): M_r = 492.8, μ = 1.25 mm^{-1} , T_{min} , T_{max} = 0.7363, 0.7735, ρ_x = 1.35 g cm^{-3} , orthorhombic, $P2_12_12_1$, Z = 4, a = 10.4081(7), b = 12.8932(8), c = 18.0095(11) $\text{\AA}$, U = 2416.8(3) $\text{\AA}^3$. 30376 measured reflections, 4719 unique reflections of which 4253 were observable [$|F_o| > 4\sigma(F_o)$]; R_{int} for equivalent reflections = 0.026. Full-matrix least-squares refinement (on F) using weight of $1/[\sigma^2(F_o) + 0.0003(F_o^2)]$ gave final values R = 0.025, wR = 0.030, for 271 variables and 4253 contributing reflections. Flack parameter:^[16] x = $-0.01(1)$. Hydrogen atoms were placed in calculated positions.

[$\text{ZnCl}(\text{C}_8\text{H}_{16}\text{N}_2)_2$] $^+ \text{Cl}^-$ [$\text{ZnCl}_2(\text{C}_8\text{H}_{16}\text{N}_2)_2$] (**12**): M_r = 969.9, μ = 2.11 mm^{-1} ; T_{min} , T_{max} = 0.6732, 0.7366, δ_x = 1.53 g cm^{-3} , tetragonal, $P4_22_2$, Z = 4, a = 13.8279(6), c = 22.0358(12) $\text{\AA}$, U = 4213.5(4) $\text{\AA}^3$. 52049 measured reflections, 4110 unique reflections of which 3231 were observable [$|F_o| > 4\sigma(F_o)$]; R_{int} for equivalent reflections = 0.043. Full-matrix least-squares refinement (on F) using weight of $1/[\sigma^2(F_o) + 0.0002(F_o^2)]$ gave final values R = 0.027, wR = 0.030, for 230 variables and 3231 contributing reflections. Flack parameter:^[16] x = 0.00(2). Hydrogen atoms were placed in calculated positions except for hydrogens bound to the nitrogen atoms which were observed and refined with a fixed value of U_{iso} . The Zn and Cl atoms of the dimer moiety (and the Cl^-) are located on twofold axes in special positions 4a. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-143123 (for **11**) and CCDC-143124 (for **12**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Isolation of a Pyridinium Alkoxy Ion Bridged Dimeric Zinc Complex for the Coupling Reactions of CO₂ and Epoxides**

Hoon Sik Kim,* Jai Jun Kim, Byung Gwon Lee, Ok Sang Jung, Ho Gyeom Jang, and Sang Ook Kang*

The synthesis of cyclic carbonates by metal-catalyzed coupling reactions of carbon dioxide and epoxides has attracted much attention with regard to the utilization of carbon dioxide, a gas responsible for global warming.^[1, 2] Among the various catalysts and catalyst systems so far reported,^[3–5] the use of transition metal based catalysts in conjunction with Lewis bases such as amines or phosphanes has proved to be a highly active combination for promoting the coupling reactions.^[6] Zinc(II) complexes have also been applied as catalysts, owing to their high activity in the cyclization and copolymerization of carbon dioxide and epoxides.^[7] However, despite extensive studies on the reaction mechanism,^[8] potential intermediates, which could clarify the reaction pathway to the formation of cyclic carbonates, have never been structurally characterized or isolated. Accordingly, discussion about these intermediates has usually been speculative.

To understand the reaction pathway more definitely and to elucidate the promoting role of the Lewis base, we have conducted mechanistic studies on the coupling reactions in the presence of a well-defined, soluble catalyst, [L₂ZnBr₂]^[9] (**1a**: L = pyridine; **1b**: L = 2-methylpyridine; **1c**: L = 2,6-dimethylpyridine; **1d**: L = 2-chloropyridine). Here we disclose the first isolation and characterization of the pyridinium alkoxy ion bridged zinc complexes [Zn₂Br₄(μ-OCHRCH₂–NC₅H₅)₂] (**2a**: R = H, **2b**: R = CH₃), which clearly demonstrate the role of the Lewis base in the coupling reactions of carbon dioxide and epoxides.

Treatment of [(C₅H₅N)₂ZnBr₂] (**1a**) with ten equivalents of ethylene oxide or propylene oxide in CH₂Cl₂ for 10 h at room temperature resulted in a relatively air-stable white precipitate, **2a** or **2b**, respectively, which was characterized by elemental analysis as well as by ¹H and ¹³C NMR spectroscopy.

The ambiguous spectroscopic data of the white precipitates led us to carry out a single-crystal X-ray diffraction study to elucidate the structure of **2**. To our surprise, the X-ray study reveals **2b** to be an addition product with two pyridinium adducts bridging two electrophilic metal centers (Figure 1).^[10]

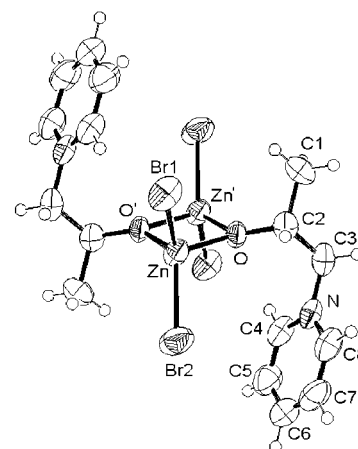


Figure 1. X-ray crystal structure of **2b**. Selected bond lengths [Å] and angles [°]: Zn–O' 1.956(5), Zn–O 1.971(5), Zn–Zn' 2.9420(19), N–C3 1.477(12), C2–C3 1.493(13); O'–Zn–O 83.0(2), O'–Zn–Br2 121.94(16), O–Zn–Br2 112.57(16), O'–Zn–Br1 113.08(16), O–Zn–Br1 112.52(16), Br2–Zn–Br1 110.86(6), C2–O–Zn' 137.7(6), C2–O–Zn 121.1(6), Zn'–O–Zn 97.0(2), O–C2–C3 110.4(8), C2–C3–N 113.4(8).

The Zn–O bond lengths of 1.956 and 1.971 Å in **2b** are comparable to those found in previously reported cadmium and zinc phenoxide complexes (1.864–2.170 Å).^[11–13] The structure of **2b** reveals various important aspects of zinc-catalyzed coupling reactions of epoxides and CO₂ such as ligand (pyridine) dissociation, coordination of the epoxide, and ring opening of the coordinated epoxide by a Lewis base (dissociated pyridine) through C–N bond formation. Ring opening of the epoxide occurs exclusively at the less sterically hindered carbon atom.

The insertion of CO₂ into the metal–oxygen bond of terminal alkoxides or phenoxides is well known,^[14, 15] but CO₂ insertion into bridging phenoxide or alkoxides has not been established. The rapid interaction of CO₂ with **2b** was confirmed by ¹H and ¹³C NMR studies (Figure 2). Upon introduction of ¹³CO₂ into an NMR tube containing **2b** in [D₆]DMSO, a new set of broad resonances appears in the ¹H NMR spectrum, which is likely associated with pyridinium ions in a carbonate species. The ¹³C NMR spectrum also shows a characteristic carbonate resonance at δ = 170 (peak labeled with* in Figure 2D), confirming the formation of a zinc–carbonate species.^[14, 15]

The activities of various zinc complexes containing pyridine or substituted pyridine ligands have been tested for the coupling reactions between CO₂ and epoxides. Table 1 shows that the reactions of alkylene oxides and carbon dioxide in the presence of **1** produce alkylene carbonate in high yield. The catalyst system consisting of ZnBr₂ and pyridine exhibits similar activity to **1a**. However, ZnBr₂ alone is not able to catalyze the reaction, demonstrating the important role of pyridine.

[*] Dr. H. S. Kim
CFC Alternatives Research Center
KIST, P.O. Box 131
Cheongryang, Seoul 130-650 (Korea)
Fax: (+82) 2-958-6869
E-mail: khs@kist.re.kr
Prof. S. O. Kang
Department of Chemistry, Korea University
Seochangdong, Jochiwon, Chungnam (Korea)
Fax: (+82) 41-867-5396
E-mail: sangok@tiger.korea.ac.kr
J. J. Kim, Prof. H. G. Jang
Department of Chemistry, Korea University
Seoul (Korea)
Dr. B. G. Lee, Dr. O. S. Jung
Korea Institute of Science and Technology (Korea)

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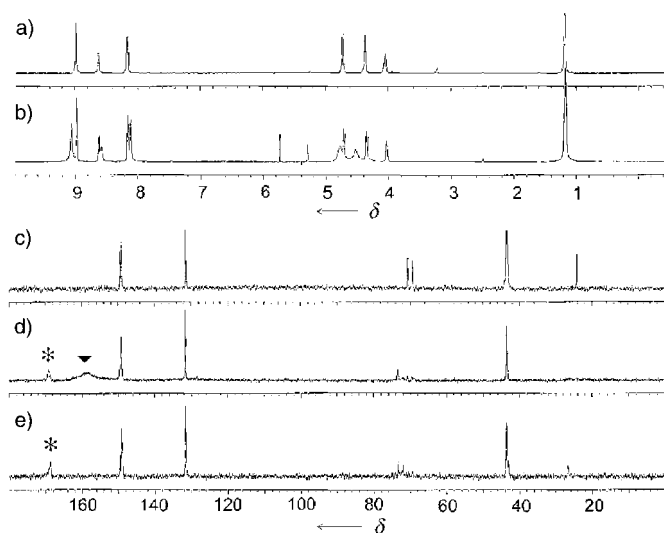


Figure 2. ^1H NMR spectrum of **2b** in a) $[\text{D}_6]\text{DMSO}$ and b) $^{13}\text{CO}_2$ -saturated $[\text{D}_6]\text{DMSO}$. ^{13}C NMR spectra of **2b** in c) $[\text{D}_6]\text{DMSO}$, d) $^{13}\text{CO}_2$ -saturated $[\text{D}_6]\text{DMSO}$ (*: carbonate, $\blacktriangledown$: free dissolved $^{13}\text{CO}_2$), and e) after evacuation of the sample in (d).

Table 1. Activities of various catalysts for the coupling reactions of CO_2 and epoxides.^[a]

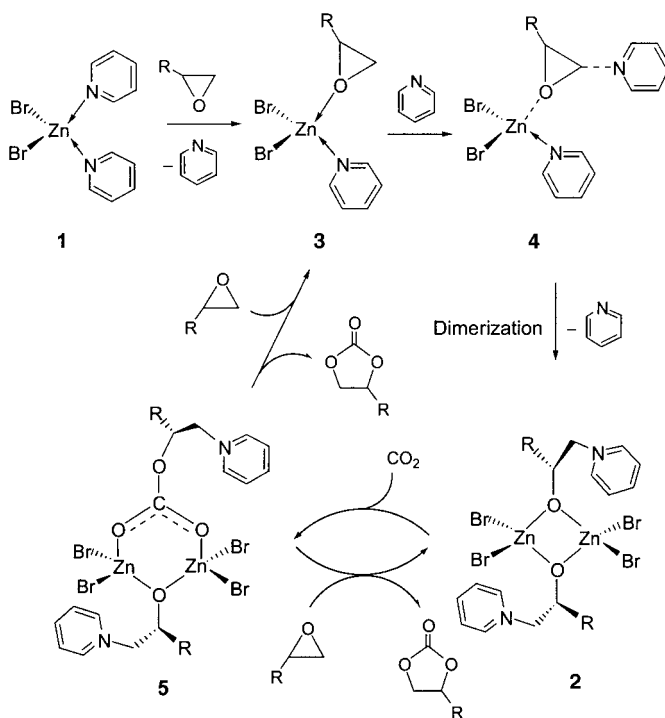
Catalyst	Promoter	Epoxide	Yield [%]	TOF ^[b] [h^{-1}]
ZnBr_2	–	EO	– ^[c]	– ^[c]
–	Py	EO	– ^[c]	– ^[c]
ZnBr_2 ^[d]	Py	EO	76	775
1a	–	EO	83	851
1a	–	PO	8	40
2b	–	PO	47	237
1b	–	PO	80	405
1c	–	PO	62	315
1d	–	PO	≈ 1	≈ 5

[a] Conditions: $T=100^\circ\text{C}$, $p(\text{CO}_2)=3.4\text{ MPa}$, reaction time 1 h; molar ratios: EO/catalyst = 1000, PO/catalyst = 500. [b] TOF = turnover frequency. [c] No reaction. [d] Molar ratio: Py/Zn = 2. EO = ethylene oxide, PO = propylene oxide, Py = pyridine.

Substitution at the 2- or 2,6-positions of the pyridine ligand has a striking effect on the catalytic activity for the coupling reaction between CO_2 and propylene oxide. The catalyst containing pyridine ligands substituted with electron-donating methyl groups (**1b** or **1c**) surprisingly exhibits higher activity than **1a**. In contrast, the catalyst containing an electron-withdrawing chlorine atom at the 2-position of the pyridine ligands (**1d**) shows little catalytic activity. The basicity of the pyridine ligand is believed to play an important role in the ring opening of the coordinated epoxide and in the stabilization of the resulting pyridinium alkoxy ligand.

Interestingly, the activity of **2b** is considerably higher than that of **1a**, indicating the presence of an induction period for the formation of **2b** from **1a**. Surprisingly, complex **2b** retains its integrity even after the coupling reaction of propylene oxide and CO_2 is performed at 100°C under 3.4 MPa of CO_2 for 1 h.^[16] These experimental results strongly suggest that complex **2b** itself is an active species rather than an intermediate. However, the possibility that **2b** exists as a monomeric species under experimental conditions cannot be excluded.

From the activity tests and the mechanistic studies described above, a reasonable mechanism for the coupling reaction of epoxide and CO_2 can be proposed (Scheme 1). Coordination of epoxide with replacement of the pyridine ligands is likely to occur first. Nucleophilic attack of the



Scheme 1. Proposed mechanism for the coupling reaction of CO_2 and epoxide in the presence of **1a** ($\text{R}=\text{H}$, CH_3).

dissociated pyridine on the less sterically hindered carbon atom of the coordinated epoxide and subsequent dimerization of the resulting pyridinium alkoxy ion lead to the formation of **2**. The insertion of CO_2 into the Zn–O bond of **2** would give a carbonate-bridged intermediate **5**. The coordination of an additional epoxide to **5** followed by the elimination of cyclic carbonate can generate **2**. Rapid insertion of carbon dioxide followed by cyclization would generate cyclic carbonate and complex **2** or **3**.

Efforts to tune the catalytic properties of complexes **1** and **2** by modifying the ligand set as well as detailed mechanistic studies are in progress.

Experimental Section

2: General procedure: A solution of **1** (2.6 mmol) in CH_2Cl_2 (30 mL) was treated with the appropriate epoxide (26 mmol) in a 60-mL high-pressure glass reactor and then stored at room temperature for 10 h. The solution was filtered and dried under vacuum to give a relatively air-stable white solid. Crystals of **2b** were directly obtained from the reaction mixture. Crystals started to grow as the reaction proceeded.

2a: Yield: 96%; elemental analysis calcd for $\text{C}_{14}\text{H}_{18}\text{Br}_4\text{N}_2\text{Zn}_2$ (%): C 25.29, H 2.71, N 4.21, Br 48.11, Zn 19.68; found: C 25.00, H 2.65, N 4.19, Br 47.98, Zn 19.51; ^1H NMR (600 MHz, $[\text{D}_6]\text{DMSO}$, 25°C): $\delta=3.90$ (t, $^3J(\text{H,H})=4.8\text{ Hz}$, 2H; CH_2), 4.71 (t, $^3J(\text{H,H})=4.8\text{ Hz}$, 2H; CH_2), 8.20 (t, $^3J(\text{H,H})=6.6\text{ Hz}$, 2H; $\text{C}_5\text{H}_5\text{N}$), 8.66 (t, $^3J(\text{H,H})=6.6\text{ Hz}$, 1H; $\text{C}_5\text{H}_5\text{N}$), 9.05 (d, $^3J(\text{H,H})=6.6\text{ Hz}$, 2H; $\text{C}_5\text{H}_5\text{N}$).

2b: Yield: 98%; elemental analysis calcd for $C_{16}H_{22}Br_4N_2Zn_2$ (%): C 27.73, H 3.18, N 4.04, Br 46.16, Zn 18.89; found: C 27.59, H 3.09, N 3.98, Br 45.50, Zn 18.83; ^1H NMR (600 MHz, $[D_6]DMSO$, 25 °C): δ = 1.16 (d, $^3J(H,H)$ = 1.6 Hz, 3H; CH_3), 4.04 (m, 1H, CH), 4.35 (dd, $^3J(H,H)$ = 12.6 Hz, $^2J(H,H)$ = 3.0 Hz, 1H; CH_2), 4.71 (dd, $^3J(H,H)$ = 12.6 Hz, $^2J(H,H)$ = 8.4 Hz, 1H; CH_2), 8.16 (t, $^3J(H,H)$ = 6.6 Hz, 2H; C_5H_5N), 8.62 (t, $^3J(H,H)$ = 6.6 Hz, 1H; C_5H_5N), 8.97 (d, $^3J(H,H)$ = 6.6 Hz, 2H; C_5H_5N).

Catalysis reaction: All the coupling reactions were conducted in a 100-mL stainless-steel bomb equipped with a magnet bar and an electrical heater. The reactor was charged with the appropriate catalyst and epoxide, and pressurized with CO_2 (≈ 1.4 MPa). The bomb was then heated to a specified temperature with the addition of CO_2 (up to 3.4 MPa) from a reservoir tank to maintain a specified pressure. After the reaction, the bomb was cooled to room temperature and the remaining epoxide was removed. The product mixtures were analyzed by GC, HPLC, and GC-MS. Solid catalyst mixtures were characterized by NMR spectroscopy.

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Rational Construction of Chiral Octanuclear Metallacycles Consisting of Octahedral Co^{III} , Square-Planar Pd^{II} , and Linear Au^I or Ag^I Ions**

Takumi Konno,* Yu Chikamoto, Ken-ichi Okamoto, Tadashi Yamaguchi, Tasuku Ito, and Masakazu Hirotsu

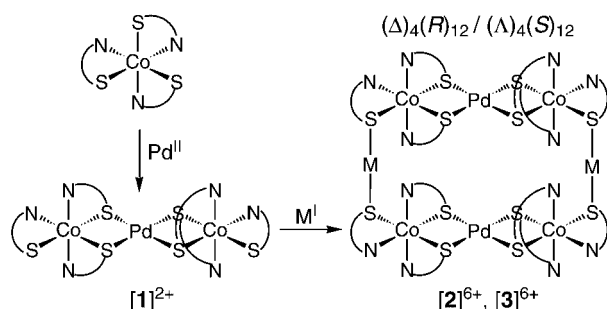
While self-assembly of organic molecules assisted by metal ions has attracted much interest in the field of supermolecular chemistry,^[1] our research interest has been directed to assembly of octahedral metal complexes with simple thiolate ligands, such as 2-aminoethanethiolate (aet) and L-cysteinate (L-cys). It has been shown that the assembly of these types of complexes can be achieved by facile reactions with a variety of metal ions, which utilize the binding ability of a coordinated thiolato sulfur atom toward a second metal ion.^[2–7] For example, a number of trinuclear $[M'\{M(aet)_3\}_2]^{n+}$ ($M' = Fe^{III}$, Co^{III} , Ni^{II}),^[4] pentanuclear $[M'_3\{M(aet)_3\}_2]^{n+}$ ($M' = Ag^I$, Hg^{II}),^[5] and octanuclear $[M'_4O\{M(aet)_3\}_4]^{6+}$ ($M' = Co^{II}$, Zn^{II}) complexes,^[6] in which two or four equivalents of *fac*(S)- $[M(aet)_3]$ ($M = Co^{III}$, Rh^{III} , Ir^{III}) are assembled by forming sulfur-bridged structures with octahedral, linear, or tetrahedral metal ions, have been prepared and their unique stereochemical and spectroscopic properties have been clarified. In these polynuclear structures, all the three thiolato sulfur atoms in each *fac*(S)- $[M(aet)_3]$ unit bridge two metal centers and thus, further assembly of these polynuclear complexes assisted by additional metal ions would not be promising. In this context, it is desirable to prepare sulfur-bridged polynuclear complexes, in which thiolato sulfur atoms are still available for bridging, in order to expand the assembly chemistry based on thiolato metal complexes. One way to do this is to introduce a four-coordinate metal ion, such as $M' = Pd^{II}$, Pt^{II} , at the center of the linear trinuclear structure in $[M'\{M(aet)_3\}_2]^{2+}$. While the reaction of *fac*(S)- $[Co(aet)_3]$ with $[PdCl_4]^{2-}$ in a 1:1 ratio produced a sulfur-bridged $Co_2^{III}Pd^{II}$ pentanuclear complex, $[Pd_2\{Pd(aet)_2\}\{Co(aet)_3\}_2]^{4+}$, in which all of the eight thiolato sulfur atoms from two *fac*(S)- $[Co(aet)_3]$ and one *cis*(S)- $[Pd(aet)_2]$ units are bound to two square-planar Pd^{II} ions,^[7g] we found that the corresponding 2:1 reaction under milder conditions gives the desired trinuclear complex $[Pd\{Co(aet)_3\}_2]^{2+}$, **[1]**²⁺, which readily

[*] Prof. T. Konno, Y. Chikamoto, Dr. M. Hirotsu
Department of Chemistry, Faculty of Engineering
Gunma University
Kiryu, Gunma 376-8515 (Japan)
Fax: (+81) 277-30-1263
E-mail: konno@chem.gunma-u.ac.jp

Prof. K. Okamoto
Department of Chemistry
University of Tsukuba
Tsukuba, Ibaraki 305-8571 (Japan)
Dr. T. Yamaguchi, Prof. T. Ito
Department of Chemistry, Graduate School of Science
Tohoku University
Sendai, Miyagi 980-8578 (Japan)

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reacts with Ag^{I} or Au^{I} to afford cyclic sulfur-bridged octanuclear complexes $[\text{M}'_2\{\text{Pd}[\text{Co}(\text{aet})_3]_2\}]^{6+}$ ($\text{M}' = \text{Au}^{\text{I}}$ ($[\mathbf{2}]^{6+}$), Ag^{I} ($[\mathbf{3}]^{6+}$); Scheme 1). Interestingly, *fac*(S)-[Co(aet)₃] was found to isomerize to adopt a *mer*(S) geometry in $[\mathbf{1}]^{2+}$, which is the key factor to form a cyclic sulfur-bridged



Scheme 1. Synthesis of $[\mathbf{2}]^{6+}$ and $[\mathbf{3}]^{6+}$, by way of $[\mathbf{1}]^{2+}$.

structure in $[\mathbf{2}]^{6+}$ and $[\mathbf{3}]^{6+}$. In this Communication, we report the synthesis and fascinating stereochemical properties of $[\mathbf{1}]^{2+}$, $[\mathbf{2}]^{6+}$, and $[\mathbf{3}]^{6+}$, along with the crystal structures of $[\mathbf{2}]^{6+}$ and $[\mathbf{3}]^{6+}$. To the best of our knowledge, this is the first example of chiral metallacycles composed of three kinds of metal ions with different oxidation states and coordination geometries: octahedral Co^{III} , square-planar Pd^{II} , and linear Ag^{I} or Au^{I} ions.

The reaction of *fac*(S)-[Co(aet)₃]^[4a] with $\text{Na}_2[\text{PdCl}_4]$ in a 2:1 ratio gave an almost black solution, from which a green-black powder ($[\mathbf{1}]\text{Cl}_2 \cdot 5\text{H}_2\text{O}$) was isolated by adding an aqueous solution of NaCl. The nitrate salt of this complex ($[\mathbf{1}](\text{NO}_3)_2 \cdot 1.5\text{H}_2\text{O}$) was obtained by adding an aqueous solution of NaNO_3 to the reaction solution. The elemental and plasma emission analyses of the chloride and nitrate salts are in good agreement with the formulas for the expected $\text{Co}^{\text{III}}\text{Pd}^{\text{II}}\text{Co}^{\text{III}}$ trinuclear $[\text{Pd}\{\text{Co}(\text{aet})_3\}_2]^{2+}$ complex. When a dark green-brown aqueous solution of $[\mathbf{1}]\text{Cl}_2 \cdot 5\text{H}_2\text{O}$ was treated with a colorless aqueous solution containing $[\text{AuCl}\{\text{S}(\text{CH}_2\text{CH}_2\text{OH})_2\}]$,^[8] the intense absorption band at 336 nm characteristic for $[\mathbf{1}]^{2+}$ shifted to a higher energy side (327 nm) and, from the reaction solution, dark green-brown microcrystals ($[\mathbf{2}]\text{Cl}_6 \cdot 16\text{H}_2\text{O}$) were isolated. The plasma emission analysis indicated that $[\mathbf{2}]^{6+}$ contains Co, Pd, and Au atoms in a 2:1:1 ratio and the elemental analysis is also consistent with the 2:1:1 stoichiometry of $[\text{Co}(\text{aet})_3]_2 \cdot \text{PdCl}_2 \cdot \text{AuCl} \cdot 8\text{H}_2\text{O}$.

The structure of $[\mathbf{2}]^{6+}$ was established by a single crystal X-ray analysis of $[\mathbf{2}]\text{Cl}_6 \cdot 16\text{H}_2\text{O}$.^[9] As shown in Figure 1, the entire complex cation $[\mathbf{2}]^{6+}$ consists of four [Co(aet)₃] units and two Pd^{II} and two Au^{I} atoms. Two of the three thiolato sulfur atoms in each octahedral [Co(aet)₃] unit are bound to one Pd^{II} atom to form a linear sulfur-bridged $\text{Co}^{\text{III}}\text{Pd}^{\text{II}}\text{Co}^{\text{III}}$ trinuclear moiety, $[\text{Pd}\{\text{Co}(\text{aet})_3\}_2]^{2+}$ (average bond lengths [Å] are Co–S 2.269(2) and Pd–S 2.323(2)). The two trinuclear moieties are connected with each other by two linear S–Au–S linkages (Au–S = 2.285(2) Å, S–Au–S = 178.85(7)°), completing a cyclic $\text{Co}^{\text{III}}\text{Pd}^{\text{II}}\text{Au}^{\text{I}}$ octanuclear structure in $[\text{Au}_2\{\text{Pd}\{\text{Co}(\text{aet})_3\}_2\}]^{6+}$. Here it should be noted that each [Co(aet)₃] unit in $[\mathbf{2}]^{6+}$ adopts a *mer*(S) geometry, which is distinct from the *fac*(S) geometry of the parental *fac*(S)-

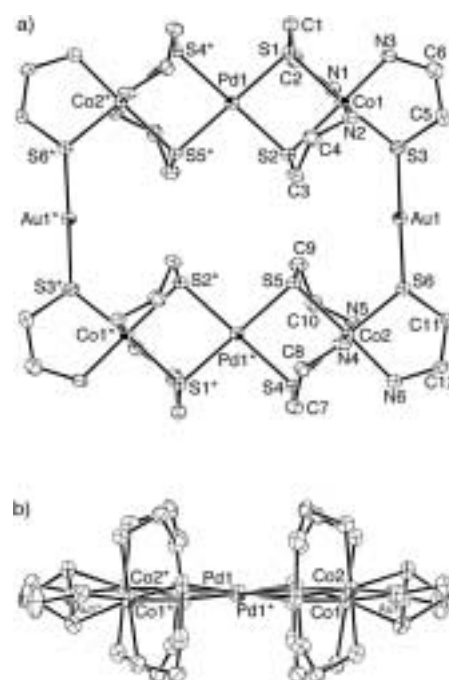


Figure 1. a) Plan and b) elevation of the complex cation $[\mathbf{2}]^{6+}$.

[Co(aet)₃].^[4a, 6b] Two chiral configurations, Δ and Λ , are possible for each *mer*(S)-[Co(aet)₃] unit. However, either the Δ or Λ configurational *mer*(S)-[Co(aet)₃] unit is selectively incorporated in the octanuclear structure in $[\mathbf{2}]^{6+}$ to give only the $\Delta\Delta\Delta\Delta$ or $\Lambda\Lambda\Lambda\Lambda$ isomers. In addition, all 12 asymmetric bridging sulfur atoms are unified to have the *R* configuration for the $(\Delta)_4$ and the *S* configuration for the $(\Lambda)_4$ isomer. Thus, only a pair of enantiomers of approximately D_2 symmetry, $(\Delta)_4(R)_{12}$ and $(\Lambda)_4(S)_{12}$, exist in the crystal; the latter isomer is illustrated in Figure 1. When the solution mixture of $[\mathbf{1}]^{2+}$ with $[\text{AuCl}\{\text{S}(\text{CH}_2\text{CH}_2\text{OH})_2\}]$ was chromatographed on an SP-Sephadex C-25 column (eluent 1.0 M aqueous NaCl), only one band for $[\mathbf{2}]^{6+}$ eluted. Furthermore, the ^{13}C NMR spectrum of $[\mathbf{2}]\text{Cl}_6$ in D_2O exhibits six methylene carbon signals for twelve aet ligands in the complex. These results indicate that the racemate of $(\Delta)_4/(\Lambda)_4$ with a D_2 symmetrical structure was selectively formed for $[\mathbf{2}]^{6+}$, taking account of the X-ray analytical result. This is supported by the fact that $[\mathbf{2}]^{6+}$ was resolved, by column chromatography (SP-Sephadex C-25, eluent 0.5 M aqueous $[\text{Sb}_2((R,R)\text{-tartrate})_2]^{2-}$), into the $(+)\text{CD}_{260}$ and $(-)\text{CD}_{260}$ isomers, which show CD spectra consistent with their enantiomeric relationship.

As illustrated in Figure 2, the absorption spectrum of two moles of $[\mathbf{1}]^{2+}$ coincides well with that of $[\mathbf{2}]^{6+}$. In its ^{13}C NMR spectrum, $[\mathbf{1}]^{2+}$ gives six signals at similar magnetic fields as the signals for $[\mathbf{2}]^{6+}$. From this behavior, $[\mathbf{1}]^{2+}$ is assigned to the sulfur-bridged $\text{Co}^{\text{III}}\text{Pd}^{\text{II}}\text{Co}^{\text{III}}$ trinuclear complex, $[\text{Pd}\{\text{Co}(\text{aet})_3\}_2]^{2+}$, the structure of which is essentially the same as that of the $\text{Co}^{\text{III}}\text{Pd}^{\text{II}}\text{Co}^{\text{III}}$ trinuclear moiety found in $[\mathbf{2}]^{6+}$. That is, in $[\mathbf{1}]^{2+}$ a Pd^{II} ion is coordinated by four thiolato sulfur atoms from two *mer*(S)-[Co(aet)₃] units to form the $(\Delta)_2/(\Lambda)_2$ racemate. Consistent with this assignment, $[\mathbf{1}]^{2+}$ was resolved into the $(+)\text{CD}_{260}$ and $(-)\text{CD}_{260}$ optical isomers by fractional crystallization of its $[\text{Sb}_2((R,R)\text{-tartrate})_2]^{2-}$ diastereo-

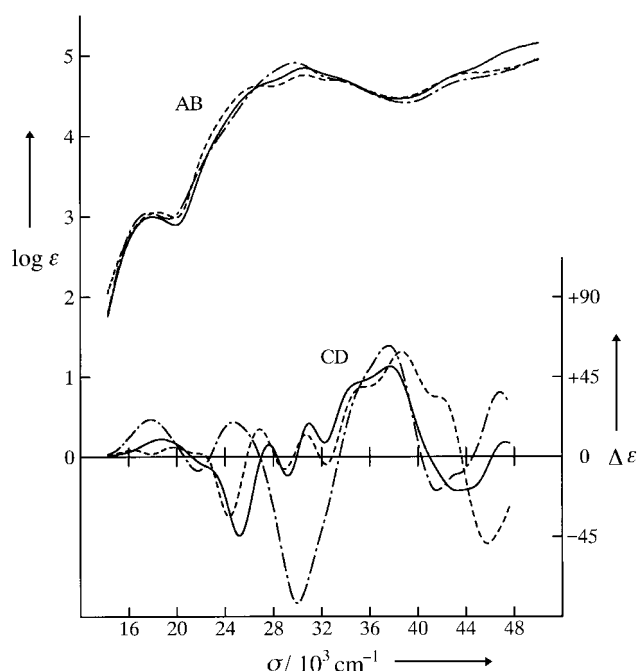


Figure 2. Electronic absorption (AB) and CD spectra of $(+)\text{CD}_{260}\text{-(A)}_2\text{-[1]}^{2+} \times 2$ (•—•), $(+)\text{CD}_{260}\text{-(A)}_4\text{-[2]}^{6+}$ (---), and $(+)\text{CD}_{260}\text{-(A)}_4\text{-[3]}^{6+}$ (—) in H_2O .

meric salts. The overall CD spectral behavior of $(+)\text{CD}_{260}\text{-[1]}^{2+}$ in water (Figure 2) resembles that of the $(A)_2$ isomer of the related sulfur-bridged $\text{Co}^{\text{III}}\text{Pd}^{\text{II}}\text{Co}^{\text{III}}$ trinuclear complex $[\text{Pd}\{\text{Co}(\text{aet})_2(\text{en})\}_2]^{4+}$, in which the central Pd^{II} ion is coordinated by four thiolato sulfur atoms from two terminal $C_2\text{-cis}(S)\text{-[Co(aet)}_2(\text{en})]^+$ units.^[3d] This suggests that the $(+)\text{CD}_{260}$ isomer of $[\text{1}]^{2+}$ has the $(A)_2$ configuration. When $(+)\text{CD}_{260}\text{-(A)}_2\text{-[1]}^{2+}$ was used as the starting complex, instead of the racemate of $[\text{1}]^{2+}$, only the $(+)\text{CD}_{260}$ isomer of $[\text{2}]^{6+}$ was formed. Thus, the $(+)\text{CD}_{260}$ isomer of $[\text{2}]^{6+}$ is assigned to the $(A)_4$ isomer, while the $(-)\text{CD}_{260}$ isomer to the $(A)_4$ one. Contrary to the agreement in the absorption spectra between $[\text{2}]^{6+}$ and two moles of $[\text{1}]^{2+}$, the CD spectrum of $(+)\text{CD}_{260}\text{-(A)}_4\text{-[2]}^{6+}$ differs markedly from that of two moles of $(+)\text{CD}_{260}\text{-(A)}_2\text{-[1]}^{2+}$, especially in the region around $24\text{--}32 \times 10^3 \text{ cm}^{-1}$ (Figure 2). If $[\text{2}]^{6+}$ dissociated in solution, its CD spectrum would be identical to two moles of $[\text{1}]^{2+}$; the difference in the CD spectra suggests that $[\text{2}]^{6+}$ remains intact in solution. The four thiolato sulfur atoms in $[\text{2}]^{6+}$, which became asymmetric by binding with Au^{I} ions, besides their skew cyclic structure, seem to contribute significantly to the CD spectrum.

Treatment of $[\text{1}](\text{NO}_3)_2 \cdot 1.5 \text{H}_2\text{O}$ with AgNO_3 in a ratio of 1:1 in water also produced a dark brown aqueous solution, from which dark green-brown microcrystals ($[\text{3}](\text{NO}_3)_6 \cdot 9 \text{H}_2\text{O}$) were isolated. The plasma emission analysis indicated that $[\text{3}]^{6+}$ contains Co, Pd, and Ag atoms in a 2:1:1 ratio and the elemental analysis is consistent with the formula of $[\text{Ag}_2\{\text{Pd}[\text{Co}(\text{aet})_3]_2\}_2](\text{NO}_3)_6 \cdot 9 \text{H}_2\text{O}$. An X-ray structural analysis of $[\text{3}](\text{NO}_3)_6 \cdot 9 \text{H}_2\text{O}$ ^[9] demonstrated that the complex cation $[\text{3}]^{6+}$ has a sulfur-bridged $\text{Co}_4^{\text{III}}\text{Pd}_2^{\text{II}}\text{Ag}_2^{\text{I}}$ octanuclear structure, in which four $\text{mer}(S)\text{-[Co(aet)}_3]$ units are linked by two Pd^{II} and two Ag^{I} atoms in a cyclic form. The overall structure of $[\text{3}]^{6+}$ is similar to that of $[\text{2}]^{6+}$. In particular, the bond distances and angles around the Co and Pd atoms in

$[\text{3}]^{6+}$ (average lengths [Å] $\text{Co-S} = 2.266(3)$ and $\text{Pd-S} = 2.324(3)$) coincide well with those found in $[\text{2}]^{6+}$. However, the S-Ag-S angles (average 168.28°) in $[\text{3}]^{6+}$ deviate considerably from 180° and, furthermore, the average Ag-S distance of $2.373(3)$ Å in $[\text{3}]^{6+}$ is about 0.09 Å longer than the average Au-S distance in $[\text{2}]^{6+}$. When an aqueous solution of $[\text{3}](\text{NO}_3)_6$ was treated with $[\text{AuCl}\{\text{S}(\text{CH}_2\text{CH}_2\text{OH})_2\}]$ at room temperature, Ag^{I} atoms in $[\text{3}]^{6+}$ were replaced by Au^{I} atoms to produce $[\text{2}]^{6+}$. These results imply that the $\text{Co}_4^{\text{III}}\text{Pd}_2^{\text{II}}\text{Ag}_2^{\text{I}}$ octanuclear metallacycle in $[\text{3}]^{6+}$ is more flexible than the $\text{Co}_4^{\text{III}}\text{Pd}_2^{\text{II}}\text{Ag}_2^{\text{I}}$ metallacycle in $[\text{2}]^{6+}$. The stereochemical behavior of $[\text{3}]^{6+}$ is the same as that of $[\text{2}]^{6+}$, forming only a pair of enantiomers, $(\Delta)_4(R)_{12}$ and $(\Delta)_4(S)_{12}$. While $[\text{3}]^{6+}$ could not be resolved by column chromatography (SP-Sephadex C-25) because of the adsorption in the column, the optically active isomer of $[\text{3}]^{6+}$ was obtained by fractional crystallization of its diastereomeric salts of $[\text{Sb}_2((R,R)\text{-tartrate})_2]^{2-}$. As shown in Figure 2, not only the absorption spectrum but also the CD spectrum of $(+)\text{CD}_{260}\text{-(A)}_4\text{-[3]}^{6+}$ are very similar to that of $(+)\text{CD}_{260}\text{-(A)}_4\text{-[2]}^{6+}$. Moreover, the ^{13}C NMR spectrum $[\text{3}]^{6+}$ exhibits six methylene carbon signals, as does $[\text{2}]^{6+}$. Accordingly, it is reasonable to assume that the sulfur-bridged $\text{Co}_4^{\text{III}}\text{Pd}_2^{\text{II}}\text{Ag}_2^{\text{I}}$ cyclic structure of $[\text{3}]^{6+}$, observed in crystal, is also retained in solution.

In summary, the reaction of $\text{fac}(S)\text{-[Co(aet)}_3]$ with Pd^{II} in water under mild conditions gives a sulfur-bridged $\text{Co}^{\text{III}}\text{Pd}^{\text{II}}\text{-Co}^{\text{III}}$ trinuclear complex $[\text{Pd}\{\text{Co(aet)}_3\}_2]^{2+}$, $[\text{1}]^{2+}$. This result indicates that $\text{fac}(S)\text{-[Co(aet)}_3]$ readily isomerizes to adopt a $\text{mer}(S)$ geometry. Complex $[\text{1}]^{2+}$ was found to function as a bidentate trinuclear-metalloligand toward linear Au^{I} and Ag^{I} ions, forming unprecedented octanuclear metallacycles, $[\text{M}_2\{\text{Pd}^{\text{II}}[\text{Co}^{\text{III}}(\text{aet})_3]_2\}_2]^{6+}$ ($\text{M} = \text{Au}, \text{Ag}$). These cyclic structures are fairly stable in solution and discriminate the absolute configurations of the Co^{III} and bridging sulfur chiral centers. Thus, optically active metallacycles with a total of 16 chiral centers are available. Finally, the present results demonstrate that a variety chiral molecular assemblages consisting of metal ions with different oxidation states and coordination geometries can be constructed by the controlled design of sulfur-bridged polynuclear structures with nonbridging thiolato sulfur atoms.

Experimental Section

$[\text{1}]\text{Cl}_2 \cdot 5 \text{H}_2\text{O}$ and $[\text{1}](\text{NO}_3)_2 \cdot 1.5 \text{H}_2\text{O}$: To a stirred dark-blue suspension of $\text{fac}(S)\text{-[Co(aet)}_3]$ (1.0 g, 3.5 mmol) in water (150 mL) was added $\text{Na}_2[\text{PdCl}_4]$ (0.52 g, 1.8 mmol) and the mixture was stirred at 30°C for 6 h. After removal of unreacted $\text{fac}(S)\text{-[Co(aet)}_3]$ by filtration, a saturated NaCl aqueous solution (25 mL) was added to the filtrate, followed by storage at 4°C for 1 day. The resulting dark-green powder was collected by filtration and recrystallized from water by adding a saturated NaCl aqueous solution. The nitrate salt of $[\text{1}]^{2+}$ was obtained by using a saturated NaNO_3 aqueous solution instead of a saturated NaCl aqueous solution. Yield for $[\text{1}]\text{Cl}_2 \cdot 5 \text{H}_2\text{O}$: 0.94 g (64 %); Yield for $[\text{1}](\text{NO}_3)_2 \cdot 1.5 \text{H}_2\text{O}$: 1.00 g (69 %); elemental analysis for $[\text{Pd}\{\text{Co}(\text{C}_6\text{H}_{18}\text{N}_3\text{S}_3)_2\}_2]\text{Cl}_2 \cdot 5 \text{H}_2\text{O}$: calcd: C 17.16, H 5.28, N 10.01; found: C 17.44, H 5.34, N 10.09; for $[\text{Pd}\{\text{Co}(\text{C}_6\text{H}_{18}\text{N}_3\text{S}_3)_2\}_2](\text{NO}_3)_2 \cdot 1.5 \text{H}_2\text{O}$: calcd: C 17.32, H 4.72, N 13.47; found: C 17.34, H 4.79, N 13.76; ^{13}C NMR (500 MHz, D_2O , sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS)): 31.45, 34.05, 36.17 (CH_2S), 51.86, 56.09, 57.48 (CH_2N); UV/Vis (H_2O): $\sigma_{\text{max}}/10^3$ (log ϵ) = 17.68 (2.75), 22.3 (3.4 sh), 29.59 (4.61), 33.5 (4.4 sh), 44.13 cm^{-1} ($4.4 \text{ M}^{-1} \text{cm}^{-1}$ sh).

[2]Cl₆·16H₂O: To a solution of Na[AuCl₄] (0.10 g, 0.28 mmol) in water (2 mL) was added 2,2'-thiodiethanol (0.16 mL). The resulting colorless solution was added to a solution of [1]Cl₂·5H₂O (0.20 g, 0.24 mmol) in water (40 mL). The mixture was stirred at RT for 30 min, followed by addition of several drops of a saturated NaCl aqueous solution. The reaction solution was allowed to stand at RT for 5 days, which gave dark green-brown microcrystals. Yield: 0.22 g (82%); elemental analysis for [Au₂[Pd[Co(C₆H₁₈N₃S₃)₂]₂]Cl₆·16H₂O: calcd C 12.77, H 4.64, N 7.45; found: C 12.71, H 4.69, N 7.43; ¹³C NMR (500 MHz, D₂O, DSS): δ = 34.86, 35.55, 40.40 (CH₂S), 48.44, 56.00, 57.22 (CH₂N); UV/Vis (H₂O): σ_{max}/10³ (log ε) = 18.36 (3.05), 26.91 (4.63), 30.56 (4.76), 33.6 (4.7 sh), 43.6 cm⁻¹ (4.8 M⁻¹cm⁻¹ sh).

[3](NO₃)₆·9H₂O: To a solution of [1](NO₃)₂·1.5H₂O (0.20 g, 0.24 mmol) in water (40 mL), AgNO₃ (0.04 g, 0.24 mmol) was added. The mixture was stirred at RT for 30 min, followed by addition of several drops of a saturated aqueous NaNO₃. The reaction solution was allowed to stand at RT for 5 days, which gave dark green-brown microcrystals. Yield: 0.20 g (80%); elemental analysis for [Ag₂[Pd[Co(C₆H₁₈N₃S₃)₂]₂](NO₃)₆·9H₂O: calcd: C 13.65, H 4.29, N 11.94; found: C 13.44, H 4.28, N 11.71; ¹³C NMR (500 MHz, D₂O, DSS): δ = 33.03, 35.21, 39.27 (CH₂S), 50.02, 55.98, 57.25 (CH₂N); UV/Vis (H₂O): σ_{max}/10³ (log ε) = 17.82 (3.00), 27.1 (4.6 sh), 30.56 (4.85), 33.8 (4.7 sh), 43.7 cm⁻¹ (4.8 M⁻¹cm⁻¹ sh).

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[9] Crystal structure analysis: General: Bruker CCD area detector, *T* = 203 K, MoK_α radiation (*λ* = 0.71073 Å), semiempirical absorption correction with SADABS. The structures were solved by direct methods (SIR 92) and refined with full-matrix least-squares procedures on *F*² using SHELXL-93. [2]Cl₆·16H₂O: crystal size 0.10 × 0.09 × 0.08 mm, orthorhombic, space group *Pbcn*, *a* = 18.888(2), *b* = 13.397(1), *c* = 28.211(2) Å, *V* = 7138.6(9) Å³, *Z* = 4, ρ_{calcd} = 2.100 g cm⁻³, μ = 61.40 cm⁻¹, ω scan mode, 2θ_{max} = 55.18, 7869 reflections collected, 7869 independent reflections, 5102 observed reflections (*F* > 4σ(*F*)), 352 parameters, semiempirical absorption correction with SADABS, max./min. transmission 0.647/0.547, *R*₁ (*F* > 4σ(*F*)) = 0.038, *wR*₂ (all data) = 0.133, residual electron density 2.22/−1.43 e Å⁻³ (the peaks larger than 1.0 e Å⁻³ were found in the vicinity of heavy atoms). Hydrogen atoms except those of water molecules were placed at calculated positions but were not refined. [3](NO₃)₆·9H₂O: crystal size 0.25 × 0.13 × 0.10 mm, monoclinic, space group *Pn*, *a* = 22.3703(4), *b* = 13.9570(7), *c* = 23.4220(4) Å, β = 107.633(1)°, *V* = 6969.3(3) Å³, *Z* = 4, ρ_{calcd} = 2.013 g cm⁻³, μ = 24.23 cm⁻¹, ω scan mode, 2θ_{max} = 55.28, 22370 reflections collected, 16071 independent reflections, 13955 observed reflections (*F* > 4σ(*F*)), 352 parameters, semiempirical absorption correction with SADABS, max./min. transmission 0.746/0.473, *R*₁ (*F* > 4σ(*F*)) = 0.042, *wR*₂ (all data) = 0.127, residual electron density 1.15/−0.83 e Å⁻³ (the peaks larger than 1.0 e Å⁻³ were found in the vicinity of heavy atoms and nitrate anions). The asymmetric unit contained two crystallographically independent, yet nearly identical, complex cations [3]⁶⁺. Hydrogen atoms were not included in the calculations. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-144580 ([2]Cl₆·16H₂O) and -144581 ([3](NO₃)₆·9H₂O). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Proton-Coupled Electron Transfer from Sulfur: A S-H/S-D Kinetic Isotope Effect of ≥ 31.1**

My-Hang V. Huynh,* Peter S. White, and Thomas J. Meyer*

There is an extensive redox chemistry of high oxidation state ruthenium(IV) oxo,^[1] and osmium(VI) nitrido,^[2] and osmium(IV–VI) hydrazido^[3] complexes based on multiple electron/atom transfers such as O atom,^[4] N⁻ ion,^[3] and H⁻

[*] Dr. M.-H. V. Huynh,^[+] Dr. T. J. Meyer,^[++] Dr. P. S. White
Department of Chemistry, Venable and Kenan Laboratories
University of North Carolina at Chapel Hill
Chapel Hill, NC 27599–3290 (USA)
E-mail: huynh@lanl.gov
tjmeyer@lanl.gov

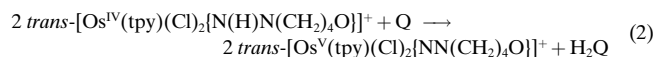
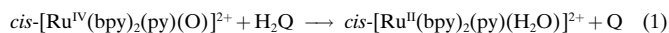
[+] Present address:
Los Alamos National Laboratory
Chemistry Division MS J514
Los Alamos, NM 87545 (USA)
Fax: (+1) 505-667-9905

[++] Present address:
Los Alamos National Laboratory
ALDSSR MS A127
Los Alamos, NM 87545 (USA)
Fax: (+1) 505-667-5450

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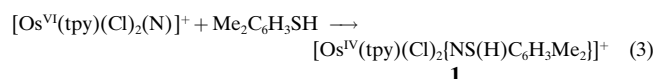
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ion^[5] transfer. Kinetic studies have uncovered reactions which occur with large H/D kinetic isotope effects^[6–8] including $k_{\text{O-H}}/k_{\text{O-D}} = 30 \pm 1$ for the oxidation of hydroquinone (H_2Q) to benzoquinone (Q) by $\text{cis-}[\text{Ru}^{\text{IV}}(\text{bpy})_2(\text{py})(\text{O})]^{2+}$, bpy = 2,2'-bipyridine and py = pyridine, [Eq. (1)],^[6a] and $k_{\text{N-H}}/k_{\text{N-D}} \geq 41.4 \pm 1.3$ for the reduction of Q to H_2Q by $\text{trans-}[\text{Os}^{\text{IV}}(\text{tpy})(\text{Cl})_2[\text{N}(\text{H})\text{N}(\text{CH}_2)_4\text{O}]]^+$, tpy = terpyridine, [Eq. (2)].^[7]



The latter two reactions occur by proton-coupled electron transfer with an electron transferred to or from a largely metal-based $d\pi$ orbital and a proton to or from a coordinated O or N atom. Herein we report what appears to be the first proton-coupled electron transfer from a S-atom. Its existence may have important implications for the electron-transfer reactivity of Fe–S proteins.^[9]

A rapid reaction occurs between the *cis* and *trans* isomers of the osmium(vi) nitrido complex, $[\text{Os}^{\text{VI}}(\text{tpy})(\text{Cl})_2(\text{N})]\text{PF}_6$, and 3,5-dimethylbenzenethiol under argon at room temperature to give the corresponding osmium(iv) sulfimido products, *cis*- and *trans*-**1** ($[\text{1A}]\text{PF}_6 = \text{trans}$ and $[\text{1B}]\text{PF}_6 = \text{cis}$). The net reaction is given in [Eq. 3].



Complex **[1A]**PF₆ has been isolated and characterized by X-ray crystallography (Figure 1). In the structure, the distorted octahedral arrangement of ligands around the Os atom

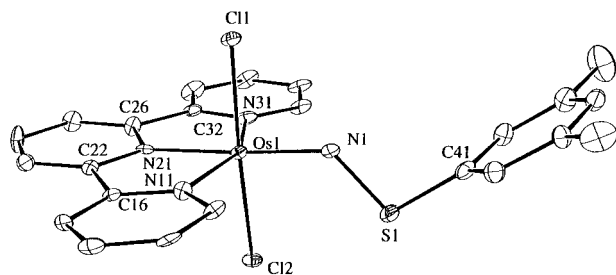
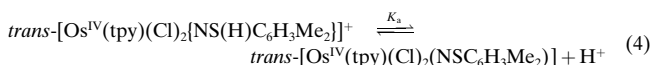


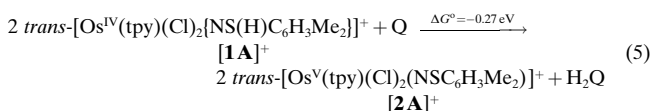
Figure 1. ORTEP plot (thermal ellipsoids set at 30%) of the ion **[1A]**⁺.

in the parent nitrido complex is retained. The Os–N_{tpy} bond lengths range from 2.015(10) Å to 2.129(9) Å with the shortest Os–N_{tpy} bond *trans* to the sulfimido ligand. Angle N1–S1–C41 = 101.6(5)° is consistent with pseudo sp^3 -hybridization and protonation at the S atom.^[10] The bond lengths Os1–N1 = 1.906(10) Å and N1–S1 = 1.706(9) Å and the angle Os1–N1–S1 = 130.4(6)° are consistent with an osmium(iv) complex with an Os1–N1 double bond and a N1–S1 single bond. There are clear structural similarities with known osmium(iv) phosphoraniminato ($\text{trans-}[\text{Os}^{\text{IV}}(\text{tpy})(\text{Cl})_2(\text{NPPH}_3)]^+$)^[2b] and dialkylhydrazido complexes ($[\text{Os}^{\text{IV}}(\text{tpy})(\text{bpy})\{\text{NN}(\text{CH}_2)_4\text{O}\}]^{2+}$,^[11] $\text{cis-}[\text{Os}^{\text{IV}}(\text{tpy})(\text{Cl})(\text{NCCH}_3)\{\text{NN}(\text{CH}_2)_4\text{O}\}]^+$, and $\text{cis-}[\text{Os}^{\text{IV}}(\text{tpy})(\text{NCCH}_3)_2\{\text{NN}(\text{CH}_2)_4\text{O}\}]^{2+}$).^[4]

Cyclic voltammetric measurements in 1:1 (v/v) $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ ($\mu = 1.0 \text{ M HNO}_3$, scan rate = 200 mV s^{-1}) reveal that $E_{1/2}$ for the osmium(v/v) couple decreases by 59 mV pH unit^{−1} from pH = 0 to 1.3 and is pH independent above pH 1.3. From these data, $\text{p}K_{\text{a}} = 1.31 \pm 0.03$ for the acid–base equilibrium in Equation (4), (Supporting Information Figure 1).



As found for the hydrazido $\text{Os}^{\text{V}}/\text{Os}^{\text{IV}}$ couple of [Eq. 2], there is also evidence for proton-coupled electron transfer in the redox chemistry of the sulfur-based couple $[\text{1A}]^+/\text{[2A]}^+$ in [Eq. 5]. When $[\text{1A}]^+$ and Q are mixed, a rapid reaction occurs in which $[\text{1A}]^+$ is oxidized to $[\text{2A}]^+$, and Q is reduced to H_2Q , [Eq. (5)].



The kinetics of this reaction were studied in 1:1 (v/v) $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ and $\text{CH}_3\text{CN}:\text{D}_2\text{O}$ ($[\text{H}^+] = 1.4 \times 10^{-1} \text{ M}$, $\mu = 1.0 \text{ M KNO}_3$, and $T = 25.0 \pm 0.1^\circ \text{C}$) by following characteristic changes in the absorption spectra as $[\text{1A}]^+$ was converted into $[\text{2A}]^+$ under pseudo-first-order conditions in Q. At low $[\text{Q}]$ ($< 2.80 \times 10^{-3} \text{ M}$) but with $[\text{Q}]$ in pseudo-first-order excess, the kinetics are first order in both $[\text{Os}^{\text{IV}}]$ and $[\text{Q}]$ with the observed rate constants $k_{\text{obs}}(\text{H}_2\text{O}) = (1.44 \pm 0.02) \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{\text{obs}}(\text{D}_2\text{O}) = (4.63 \pm 0.09) \times 10^{-1} \text{ M}^{-1} \text{ s}^{-1}$ (Supporting Information Figure 2). A plot of $k_{\text{X}}/k_{\text{D}}$ versus χ_{D} in

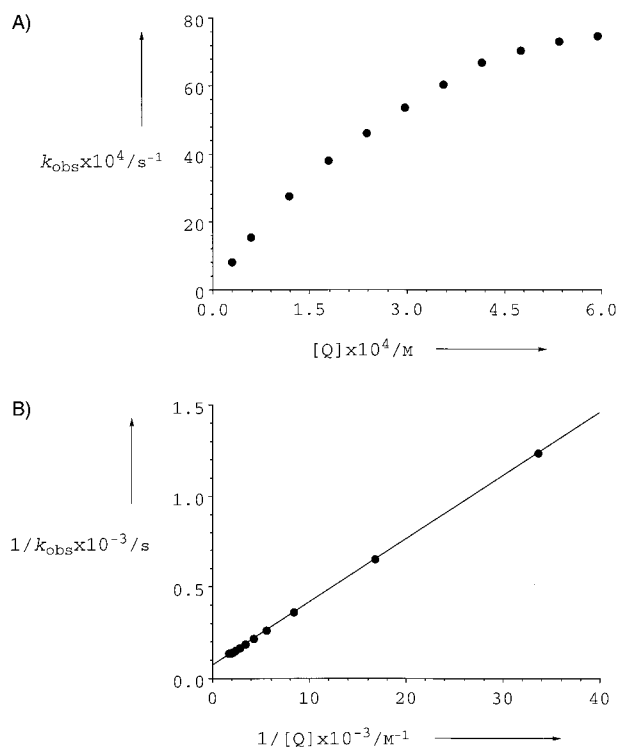
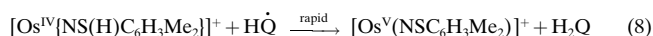
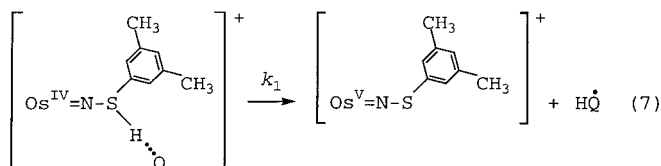
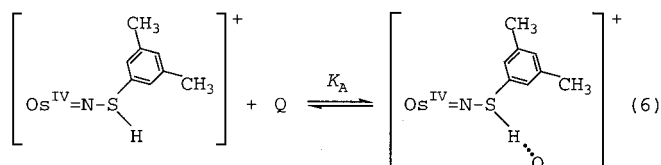


Figure 2. A) Plot of k_{obs} versus $[\text{Q}]$ for the reaction between Q and **[1A]**PF₆ in 1:1 (v/v) $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ ($25.0 \pm 0.1^\circ \text{C}$, $[\text{H}^+] = 1.4 \times 10^{-1} \text{ M}$, and $\mu = 1.0 \text{ M KNO}_3$). B) An inverse–inverse plot ($1/k_{\text{obs}}$ versus $1/[\text{Q}]$) of the plot shown in (A).

CH₃CN:H₂O/D₂O mixtures in which k_x is the rate constant (k_{obs}) at mole fraction D (χ_D) was linear (Supporting Information Figure 3) which reveals that a single proton is involved in the reaction.^[12] From the intercept of the plot at $\chi_D = 0$, the H₂O/D₂O kinetic isotope effect $k_{\text{obs}}(\text{H}_2\text{O})/k_{\text{obs}}(\text{D}_2\text{O})$ is 31.1 ± 0.2 under these conditions.

When studied over a more extended range, the concentration dependence in [Q] is more complex. As illustrated in Figure 2A and by the linear plot of $1/k_{\text{obs}}$ versus $1/[Q]$ (Figure 2B), there is evidence for saturation kinetics with the [Q] dependence consistent with the expression $k_{\text{obs}} = \{(2k_1[Q]/([Q] + K_A))\}$ and the mechanism summarized in Eqs. (6)–(8).^[7]

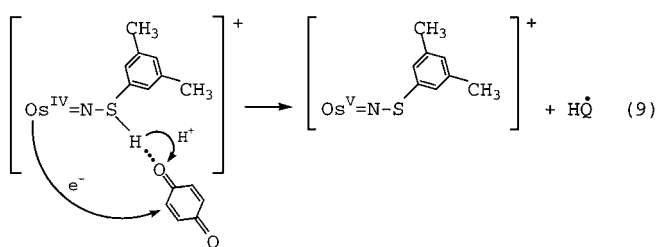


Direct evidence was obtained for the putative intermediate at $[\text{Q}] = 2.17 \times 10^{-4} - 2.60 \times 10^{-3} \text{ M}$ by a shift in λ_{max} (a characteristic band of $[\mathbf{1A}]^+$) from 456 nm to 428 nm which subsequently disappeared to give the spectrum of the final product (Supporting Information Figure 4). This mechanism is similar in detail to mechanisms proposed for the oxidation of H₂Q by *cis*-[Ru^{IV}(bpy)₂(py)(O)]²⁺^[6a] and for the reduction of Q by *trans*-[Os^{IV}(tpy)(Cl)₂(N(H)N(CH₂)₄O)]⁺ in which an intermediate has also been observed.^[7]

As calculated from the intercept, slope, and intercept-to-slope ratios in Figure 2B, $K_A(\text{H}_2\text{O}) = (1.02 \pm 0.02) \times 10^3 \text{ M}^{-1}$ and $k_1(\text{H}_2\text{O}) = (1.42 \pm 0.04) \times 10^{-2} \text{ s}^{-1}$. The kinetics were also measured in 1:1 (v/v) CH₃CN:H₂O/D₂O mixtures. From the [Q] dependence in CH₃CN:D₂O, $K_A(\text{D}_2\text{O})$ and $k_1(\text{D}_2\text{O})$ are $(9.91 \pm 0.15) \times 10^2 \text{ M}^{-1}$ and $(4.71 \pm 0.18) \times 10^{-4} \text{ s}^{-1}$, respectively (Supporting Information Figure 5). From these data, $K_A(\text{H}_2\text{O})/K_A(\text{D}_2\text{O}) = 1.03 \pm 0.03$ and $k_1(\text{H}_2\text{O})/k_1(\text{D}_2\text{O}) = 30.2 \pm 0.3$.

The kinetic analysis reveals a mechanism involving pre-association to form a detectable intermediate, probably stabilized by hydrogen bonding. The magnitude of the kinetic isotope effect points to synchronous electron–proton transfer and a proton-coupled electron-transfer mechanism, [Eq. (9)].

Because of a possible contribution from a parallel electron-transfer pathway between Q and the deprotonated form of $[\mathbf{1A}]^+$, *trans*-[Os^{IV}(tpy)(Cl)₂(NS(C₆H₃)Me₂)]⁺, the kinetic isotope effect of 31.1 is a lower limit. It should be possible to unravel the contribution, if any, of a second pathway by the pH-dependent kinetic study currently under investigation.



Our study appears to provide the first documented example of two phenomena: 1) proton-coupled electron transfer involving a S atom, and 2) proton-coupled electron transfer from an atom not directly bonded to the metal. The reaction is also notable for the large magnitude of the kinetic isotope effect. The observation of S-based, proton-coupled electron transfer could have important implications for the electron-transfer chemistry of iron-sulfur proteins. Related pathways could provide a mechanism for converting complex redox equivalents, involving the transfer of both electrons and protons, into the electron-transfer equivalents available in electron transfer reactions.

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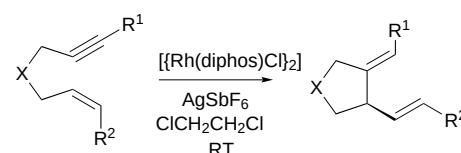
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- [10] In the deprotonated form, *trans*-[Os^{IV}(tpy)(Cl)₂(NSC₆H₃Me₂)], the angle Os1-N1-S1 (129.5(2)°) and bond Os1-N1 (1.890(3) Å) are relatively unchanged while there are significant changes in the angle N1-S1-C41 (104.3(2)°), and the bonds N1-S1 (1.596(4) Å), and S1-C41 (1.784(4) Å cf. with Os1-Cl1 (2.4139(10), Os1-Cl₂ (2.4007(10), Os1-N11 (2.057(3), Os1-N21 (2.012(3), Os1-N31 (2.065(3)) Å in [1A]⁺). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-141160 (1A) and -149866. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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The First Highly Enantioselective Rh-Catalyzed Enyne Cycloisomerization**

Ping Cao and Xumu Zhang*

The development of efficient asymmetric cyclizations is of great interest in organic synthesis due to the existence of many biologically active and naturally occurring cyclic compounds.^[1] However, efficient enantioselective transition metal catalyzed cyclization reactions are rare and remain relatively unexplored.^[2,3] In contrast, the racemic variants of these reactions have been extensively studied. For example, transition metal catalyzed cycloisomerization of 1,6-enynes have been explored in depth.^[4] An asymmetric variant of this transformation would be significant for the synthesis of enantiomerically pure five-membered carbo- and heterocycles. So far, only limited progress has been made in this important enantioselective transformation.^[5] We reasoned that a key to developing such highly enantioselective metal-catalyzed reaction is to find a highly active catalyst for cycloisomerization under mild conditions. Recently, we made a significant advance in developing the first Rh^I-catalyzed enyne cycloisomerization reaction at room temperature. Excellent chemo-, regio-, and diastereoselectivities were achieved (Scheme 1).^[6] Here we report on the first asymmetric Rh-catalyzed enyne cycloisomerizations facilitated by Rh^I



Scheme 1. Rh-catalyzed enyne cycloisomerization.

complexes with Me-DuPhos (DuPhos = 1,2-bis(phospholano)benzene),^[7] BICP (2*R*,2'*R*)-bis(diphenylphosphanyl)-(1*R*,1'*R*)-dicyclopentane (**1**)^[8] and the phosphinite (*R*,*R*,*R*,*R*)-BICPO ligands.^[9]

Using some commercially available chiral phosphane ligands and ligands developed in our laboratory, we screened the asymmetric enyne cycloisomerization reaction with **1a** as a prototypical substrate (Table 1). The highest *ee* was achieved with a Rh/Me-DuPhos catalyst, while no reactivity was observed with a Rh/BINAP system (BINAP = 2,2'-bis(diphenylphosphanyl)-1,1'-dinaphthyl). We found that both the activity and enantioselectivity of this asymmetric

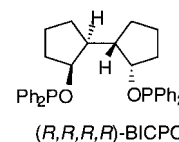


Table 1. Ligand effects on Rh-catalyzed enantioselective enyne cycloisomerization [Eq. (1)].^[a]

Entry	Ligand	Conversion [%] ^[b]	<i>ee</i> [%] ^[c]
1	Me-DuPhos	100	95
2	Et-DuPhos	< 5	63
3	BICP	100	74
4	Me-PennPhos	100	71
5	BINAP	0	–
6	Et-BPE	< 5	5

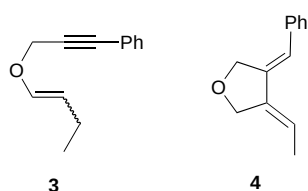
[a] All reactions were carried out with 10 mol % [Rh(ligand)Cl]₂ in ClCH₂CH₂Cl with 0.1 M enyne substrate at room temperature for 2–12 h. The cationic Rh^I catalyst was generated by adding AgSbF₆ to the solution of [Rh(ligand)Cl]₂ in the presence of the substrate. [b] Conversion was determined by GC. [c] The *ee* was determined by GC on a 15 m Supelco β-390 column with a chiral phase.

C–C bond-forming reaction are sensitive to the structure of the chiral ligand. For example, both activity and enantioselectivity were low with the Et-BPE (BPE = 1,2-bis(phospholano)ethane) ligand, and the enantioselectivity dropped significantly on changing from Me-DuPhos to Et-DuPhos. Good enantioselectivities were also obtained with BICP and Me-PennPhos (Me-PennPhos = *P,P'*-1,2-diphenylenebis(endo-2,5-dimethyl-7-phosphabicyclo[2.2.1]heptane)).

On the basis of these results, Me-DuPhos was selected as the preferred ligand for the Rh-catalyzed cycloisomerization of **1a**. We found that the optimal catalyst loading for achieving high reactivity and enantioselectivity is 5–10 mol %. The cationic Rh^I catalyst must be generated in situ by adding one equivalent of AgSbF₆ to the reaction mixture after the substrate has been introduced. Higher catalyst loading

[*] Prof. X. Zhang, P. Cao
Department of Chemistry
The Pennsylvania State University
University Park, PA 16802 (USA)
Fax: (+1) 814-863-8403
E-mail: xumu@chem.psu.edu

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(>20 mol%) leads to the formation of the rearrangement product **3**. After the substrate was consumed, the desired 1,4-diene **2a** isomerized to the more stable conjugated 1,3-diene **4** in the

presence of the Rh^I catalyst. Therefore, the reaction was usually quenched as soon as the substrates were consumed to avoid this isomerization reaction.

Under the optimal reaction conditions, a variety of enyne substrates **1** were tested for cycloisomerization with Rh/Me-DuPhos, Rh/BICP, and Rh/BICPO catalysts (Table 2). For those substrates with an aryl terminal group on the acetylene (**1a–1f**), enantioselectivities achieved with Me-DuPhos were typically higher than those obtained with BICP (Table 2, entries 1–11). However, when R¹ was an alkyl or cyclic alkyl group, no reaction occurred with the Rh/Me-DuPhos catalyst. On the other hand, enantioselectivities with the Rh/BICP catalyst were excellent (Table 2, entries 12–15). Therefore, it appears that the Rh-catalyzed asymmetric enyne isomerization is highly sensitive to subtle variations in substrate substituents. While the detailed reasons for this observation are unknown, a possible explanation is that this catalyst system is responsive to the electronic nature of the ligands and substrates.^[6] For the electron-donating Me-DuPhos system, enyne substrates with aryl groups on the acetylenic terminal are better π acceptors than those with alkyl groups in this position. Higher reactivities and enantioselectivities were observed with less electron-donating substrates (Table 2, entry 4 vs entry 10). For a more electron-donating enyne substrate bearing an alkyl group on the acetylenic terminal,

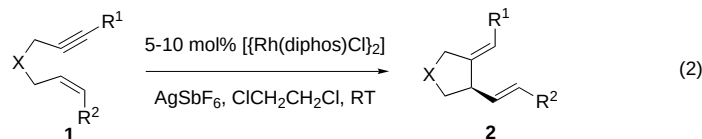
the strong *trans* effect of an electron-donating phosphane (i.e., Me-DuPhos) may prevent strong bonding of the enyne to Rh and thus inhibit the subsequent oxidative cyclization. However, enynes with alkyl groups on the acetylenic terminal may confer the right electronic properties for enyne binding and oxidative cyclization when a less electron-donating phosphane (e.g., BICP) is used. These subtle changes in activities and enantioselectivities indicate that screening of a variety of chiral chelating ligands is needed to identify a highly active and enantioselective enyne cycloisomerization catalyst for a given substrate. A further demonstration of this substrate dependence is illustrated by cycloisomerization of enynes bearing a nitrogen linker (**1i–1k**). A phosphinite-modified Rh catalyst (BICPO/Rh) successfully facilitated the cyclization and gave good enantioselectivities (69–82% *ee*, Table 2, entries 16–18), while Rh/Me-DuPhos and Rh/BICP catalysts were inactive for these transformations.

In conclusion, we have developed the first highly enantioselective Rh-catalyzed enyne cycloisomerization reaction. This asymmetric C–C bond-forming reaction is highly substrate dependent. Our future studies will focus on expanding the substrate scope and exploring the synthetic utility of this asymmetric reaction.

Experimental Section

[[Rh(BICP)Cl]₂]: BICP (0.3 g, 0.6 mmol) was added to [Rh(C₈H₁₂)Cl]₂ (0.15 g, 0.3 mmol) in deoxygenated toluene (5.0 mL) at room temperature. The mixture was stirred for 5 h, and then hexane (4 mL) was added. The solution was cooled to –78 °C for 1 h. Orange crystals formed and were collected by filtration and washed with hexane (6 × 15 mL) to give [[Rh(BICP)Cl]₂] (0.22 g, 85% yield). ¹H NMR (360 MHz, CDCl₃): δ = 8.52 (brs, 8H), 7.42 (m, 8H), 7.14 (m, 8H), 7.08 (m, 8H), 6.88 (m, 8H),

Table 2. Rhodium-catalyzed enantioselective cycloisomerization of 1,6-enynes [Eq. (2)].^[a]



Entry	Substrate	X	R ¹	R ²	Ligand	Cat. loading [mol %]	Time [h]	Yield [%]	<i>ee</i> [%] (config.) ^[b]
1	1a	O	Ph	Me	(<i>R,R</i>)-Me-DuPhos	5	2	62	96 (–)
2					(<i>R,R,R,R</i>)-BICP	5	2	73	74 (–)
3					(<i>R,R,R,R</i>)-BICPO	10	1.5	81	65 (+)
4	1b	O	C ₆ H ₄ (<i>p</i> -Me)	Me	(<i>R,R</i>)-Me-DuPhos	10	4	38	77 (–)
5					(<i>R,R,R,R</i>)-BICP	10	7	58	87 (–)
6	1c	O	C ₆ H ₄ (<i>p</i> -Cl)	Me	(<i>R,R</i>)-Me-DuPhos	5	2	60	95 (–)
7					(<i>R,R,R,R</i>)-BICP	10	0.25	24	83 (–)
8	1e	O	C ₆ H ₄ (<i>m</i> -Cl)	Me	(<i>R,R</i>)-Me-DuPhos	10	0.5	68	93 (–)
9					(<i>R,R,R,R</i>)-BICP	10	7	39	79 (–)
10	1f	O	C ₆ H ₄ (<i>p</i> -CF ₃)	Me	(<i>R,R</i>)-Me-DuPhos	5	1	60	96 (–)
11					(<i>R,R,R,R</i>)-BICP	10	7	47	91 (–)
12	1g	O	cyclopentyl	Me	(<i>R,R</i>)-Me-DuPhos	10	12	–	–
13					(<i>R,R,R,R</i>)-BICP ^[c]	5	2	43	95 (–)
14	1h	O	C ₄ H ₉	Me	(<i>R,R</i>)-Me-DuPhos	10	12	–	–
15					(<i>R,R,R,R</i>)-BICP	5	1	67	98 (–)
16	1i	PhSO ₂ N	Me	Me	(<i>R,R,R,R</i>)-BICPO	3	13	98	82 (–)
17	1j	PhSO ₂ N	Et	Me	(<i>R,R,R,R</i>)-BICPO	3	13	99	80 (–)
18	1k	PhSO ₂ N	Me	Et	(<i>R,R,R,R</i>)-BICPO	3	13	99	69 (–)

[a] All reactions were carried out in ClCH₂CH₂Cl at room temperature with 0.2 mmol substrate at 0.1 M concentration. [[Rh(diphos)Cl]₂]/AgSbF₆ = 1/1 (diphos = 1,2-bis(diphenylphosphanyl) ethane). AgSbF₆ was added to the solution of [[Rh(diphos)Cl]₂] after the substrate had been added. [b] The *ee* value was determined by GC on a Supelco β -390 column with a chiral phase and by HPLC. [c] AgPF₆ was used.

2.56 (m, 8H), 0.76–1.16 (m, 24H); ^{13}C NMR (90 MHz, CDCl_3): δ = 21.92, 24.51, 30.07, 30.47, 32.93, 45.52, 126.02, 127.46, 127.91, 127.99, 128.94, 129.75, 130.67, 133.01, 137.92; ^{31}P NMR (145 MHz, CDCl_3): δ = 47 (d, J = 187 Hz).

General method for enyne cycloisomerization: The enyne (0.4 mmol), $[\text{Rh}(\text{BICP})\text{Cl}]_2$ (5 mol %), and $\text{ClCH}_2\text{CH}_2\text{Cl}$ (2 mL) were introduced into a 25-mL Schlenk tube in a glovebox. The mixture was stirred for 1 min. To this mixture AgSbF_6 (5 mol %) was added, and a precipitate was observed. The mixture was stirred at room temperature, and the course of the reaction was monitored by TLC. The cyclization was normally complete within 2–6 h. The crude mixture was diluted with diethyl ether and filtered to remove silver chloride before purification by flash chromatography.

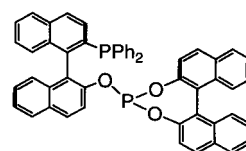
1e: ^1H NMR (360 MHz, CDCl_3): δ = 7.20 (m, 1H), 7.11 (m, 1H), 7.06 (m, 1H), 6.95 (m, 1H), 6.14 (m, 1H), 5.63 (m, 1H), 5.18 (m, 1H), 5.14 (m, 1H), 4.50–4.63 (m, 2H), 4.06 (dd, J = 7.4, 7.4 Hz), 3.49 (dd, J = 8.4, 8.4 Hz), 3.42 (m, 1H); ^{13}C NMR (90 MHz, CDCl_3): δ = 51.20, 70.61, 72.74, 118.41, 121.32, 126.33, 127.12, 128.35, 130.12, 134.12, 136.72, 139.34, 145.92. MS: m/z : 220 [M^+]; HRMS (APCI) calcd for $\text{C}_{14}\text{H}_{13}\text{ClO}$ [M^+ + H]: 221.0733; found: 221.0724.

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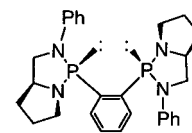
Rhodium-Mediated Asymmetric Hydroformylation with a Novel Bis(diazaphospholidine) Ligand**

Simon Breeden, David J. Cole-Hamilton, Douglas F. Foster, Gary J. Schwarz, and Martin Wills*

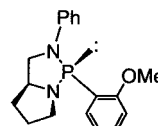
Asymmetric catalytic hydroformylation of alkenes is one of the most efficient and atom-economic of all the known processes for the synthesis of high-value chiral compounds. In one step, three readily available components (alkene, carbon monoxide, hydrogen) are combined in a C–C bond-forming process with no by-products other than unconverted reagents.^[1] Asymmetric hydroformylation of vinyl acetate would convert this inexpensive bulk chemical in one step into a three-carbon synthetic building block which could be employed as an intermediate for the synthesis of many more complex molecules. Despite the scientific and commercial attractiveness of this process, few efficient catalysts are available for this reaction. Of those reported to date, BINAPHOS (**1**) and the closely related BIPHEMPOS (**8**) are among the most efficient (Scheme 1).^[2] As little as 0.20–0.25 mol % of a Rh^I complex of **1** facilitates the asymmetric



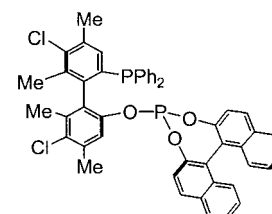
(*R,S*)-BINAPHOS (**1**)



ESPHOS (**6**)



SEMI-ESPHOS (**7**)



(*S,R*)-BIPHEMPOS (**8**)

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[*] Prof. M. Wills, Dr. S. Breeden^[+]

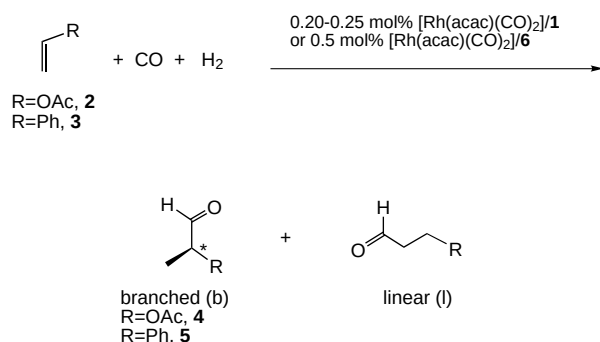
Department of Chemistry
University of Warwick
Coventry, CV4 7AL (UK)
Fax: (+44) 24-7652-4112
E-mail: m.wills@warwick.ac.uk

Prof. D. J. Cole-Hamilton, Dr. D. F. Foster, Dr. G. J. Schwarz
Catalyst Evaluation and Optimisation Service, School of Chemistry
University of St Andrews, Purdie Building
St Andrews, Fife, KY16 9ST (UK)

[+] Present Address:
Department of Chemistry, NUI Galway, Galway (Ireland)

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



Scheme 1. Hydroformylation of vinyl acetate with rhodium(i) complexes of BINAPHOS (**1**) and ESPHOS (**7**). acac = acetylacetonate.

hydroformylation of vinyl acetate (**2**) and styrene (**3**) with high regio- and enantioselectivity (Table 1, entries 1 and 12). The branched-chain products **4** and **5** are formed in up to 94 and 92 % *ee*, respectively.

Recently, we reported the synthesis of, and preliminary studies on, a new class of chiral diazaphospholidine ligands.^[3] The ligands can be prepared in both bidentate (ESPHOS; **6**)^[4] or monodentate (SEMI-ESPHOS; **7**)^[5] form. Both **6** and **7** are readily prepared by the reaction of the appropriate bis(dimethylamino) precursor with a diamine derived from glutamic acid. Although somewhat sensitive to decomposition by hydrolysis or alcoholysis, these heterocycles are stable to the neutral conditions commonly used in hydroformylation reactions. Given the attractiveness of hydroformylation with asymmetric catalysis, we chose to evaluate our ligands in this application.

Hydroformylation of vinyl acetate with 0.5 mol % of a rhodium(i) complex of ESPHOS gave excellent results (Table 1). The best results were achieved at a gas pressure of only 8 bar and 60 °C, which led to essentially quantitative

(98.9 %) conversion after 5 h. The isolated product (90.3 %) was a mixture of branched and linear isomers in a ratio of 94.5:5.5, and the major product had 89 % *ee* (Table 1, entry 5). The temperature was lowered to 30 °C to establish whether the enantiomeric excess could be improved. This resulted in a dramatic decrease in the rate of reaction, so that a higher gas pressure was required to achieve measureable turnover, and in a lower yield (21.6 %), but the enantiomeric excess of the major product increased to 93 % (Table 1, entry 7). These results compare favorably with those achieved with BINAPHOS. Whilst the asymmetric induction achieved with **6** is marginally lower than for **1**, the branched:linear (b:l) ratio is superior (94.5:5.5 vs. 88:12), and the transformation was achieved at much lower gas pressures (8 bar vs. the lowest reported pressure of 20 bar^[2g]) and reaction times (8 h vs. 13 h^[2g]).

Further investigations revealed the crucial importance of the bidentate nature of **6** in the asymmetric hydroformylation process. The use of monodentate ligand **7** under the same conditions as for **6** gave very low yields of essentially racemic product, even when elevated temperatures and pressures were employed (Table 1, entries 8–10). Comparison with triphenylphosphane (Table 1, entry 11) underlines the specific requirement for bidentate ligands. In analogy with other C₂-symmetric ligands, it must be assumed that the origin of the high enantioselectivity results from the stereochemically well-defined environment which is created around the metal upon coordination of the ligand to a rhodium atom (Figure 1).

Having established that **6** is an excellent ligand for hydroformylation of vinyl acetate, we investigated the same reaction of styrene. Surprisingly, whilst rhodium(i) com-

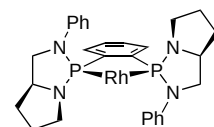


Figure 1. C₂-symmetric environment imposed by ESPHOS upon chelation to rhodium(i).

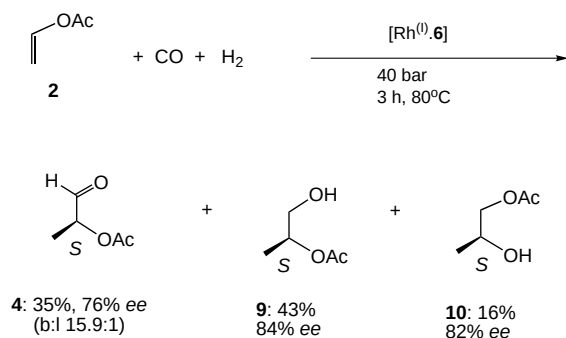
Table 1. Asymmetric hydroformylation of alkenes with catalysis by rhodium complexes.^[a]

Entry	Substrate	Ligand	P:Rh	<i>T</i> [°C]	<i>p</i> [bar]	<i>t</i> [h]	Conversion [%]	Aldehyde yield [%] ^[b]	Aldehyde b:l ^[c]	Aldehyde <i>ee</i> [%]	Alcohol yield [%] ^[d]	Alcohol <i>ee</i> [%]
1	2 ^[e]	1 ^[2c]	4.0	60	100	43	> 99	–	88:12	94(S)	–	–
2	2	6	1.5	80	40	3.0	100	34.9	94:6	76 (S)	58.8	84 (S) ^[h]
3	2	6	1.5	80	40	1.0	98.5	37.0	93:7	84 (S)	54.5	88 (S)
4	2	6	1.5	60	8	2.0	80.0	74.8	93.5:6.5	90 (S)	0.6	–
5	2	6	1.5	60	8	5.0	98.9	90.3	94.5:5.5	89 (S)	3.3	–
6	2	6	1.5	50	8	21	98.6	75.8	94:6	88 (S)	17.1	89 (S)
7	2	6	1.5	30	100	17	23.6	21.6	91.5:8.5	93 (S)	0	–
8	2	7 ^[f]	3.0	80	40	8.0	4.2	1.1	25:75	–	–	–
9	2	7	2.0	80	40	20	10.2	2.5	25:75	–	–	–
10	2	7	2.0	120	100	6.0	21.8	14.7	70:30	< 2 (R)	0.5	–
11	2	PPh ₃	3.0	80	40	4.5	99.7	73.6	81:19	0	4.1	0
12	3	1 ^[2c]	4.0	60	100	36	> 99	–	86:14	92 (S)	–	–
13	3 ^[g]	6	1.5	80	10	1.5	99.6	98.9	78:22	0	–	–
14	3	7	2.0	80	10	2.0	91.3	85.0	60:40	< 1	1.1	–
15	3	PPh ₃	3.0	80	10	1.0	99.1	99.1	55:45	0	–	–

[a] Catalyst prepared in situ from [Rh(acac)(CO)₂] (5 × 10^{−5} mol) and the phosphane in toluene (4 mL) containing styrene (1.0 g, 9.6 × 10^{−3} mmol) or vinyl acetate (1.0 g, 1.04 × 10^{−2} mmol); autoclave stirring speed = 500 rpm. [b] For vinyl acetate reactions, yield refers to 2-acetoxypropanal (1-acetoxypropanal decomposes under the reaction conditions to acetic acid and propanal), and for styrene reactions to 2-phenylpropanal plus 3-phenylpropanal. [c] Refers to aldehyde formation before aldehyde decomposition/hydrogenation. For vinyl acetate reactions, determined from the ratio of branched-chain product (aldehyde + alcohol) to acetic acid, and for styrene reactions from the ratio of branched-chain product (aldehyde + alcohol) to straight-chain product (aldehyde + alcohol). [d] For vinyl acetate reactions, this refers to 2-acetoxy-1-propanol plus 1-acetoxy-2-propanol, and for styrene reactions to 2-phenyl-1-propanol plus 3-phenyl-1-propanol. [e] Acetoxyacetone (0.3–0.6 %) is also a product. [f] Catalyst is unstable. [g] Acetophenone (< 1 %) is also a product. [h] The mixture consists of **8** (43 %, 82 % *ee*) and **9** (16 %, 84 % *ee*).

plexes of **6** and **7** are effective in styrene hydroformylation, the products in both cases were essentially racemic (Table 1, entries 13 and 14). This was unexpected, since most ligands are either good or bad for both substrates. It therefore appears that the presence of a coordinating group in the substrate is essential for high enantioselectivity when diazaphospholindines are used as ligands, somewhat analogous to asymmetric hydrogenation reactions with rhodium/diphosphane catalysts. However, it is possible that racemization may have followed an initially enantioselective reaction in the case of styrene, and we are currently investigating the causes of this unusual pattern of reactivity.

In some studies, we observed the formation of small quantities of alcohol products (from the reduction of the aldehyde **4**) when extended reaction times were employed in hydroformylation. Upon increasing the pressure and the temperature of the reaction, the alcohols **9** and **10** were formed in 43 and 16% yield, respectively, in both cases with 82–84% ee and identical configurations (Scheme 2; Table 1, entry 2). Clearly **9** is formed by the reduction of **4**, and **10** is a rearrangement product of **8**. This behavior contrasts with that



Scheme 2. Hydroformylation of vinyl acetate followed by reduction.

of other rhodium-based systems such as those containing **1** or BIPHEMPOS (**8**), which show no activity for aldehyde hydrogenation. Such hydrogenations must be carried out in a separate step. The quantity of **4** that remained unreduced (35%) had an ee of 76%, which is presumably the result of aldehyde racemization over the extended reaction times. Since both **9** and **10** can be hydrolyzed to the same product, this process provides a direct approach to the asymmetric synthesis of 1,2-diols, which are important reagents and intermediates for fine-chemicals manufacture. We are therefore currently examining the extension of our hydroformylation process to the synthesis of diols and low molecular weight epoxides.

In conclusion, we have described a novel class of chelating phosphorus-donor ligands for asymmetric hydroformylation reactions. Our results demonstrate the need for a bidentate rather than a monodentate structure and an unprecedented requirement for a coordinating group in the substrate to achieve high enantioselectivity. In addition, the same catalyst system can perform the hydrogenation of the aldehyde product and thus provides a route to chiral 1,2-propanediol precursors.

Experimental Section

The preparation of the ligands **6** and **7** has been described.^[3] The catalyst was prepared in situ from $[\text{Rh}(\text{acac})(\text{CO})_2]$ (5×10^{-5} mol) and the phosphane (1.5–4.0 equiv, see Table 1) in toluene (2 mL) in a miniautoclave fitted with a system for injection of substrate and for measuring kinetics at constant pressure. The autoclave was then flushed with CO/H_2 before pressurizing to several bar below the operating pressure. The temperature was raised with stirring to the desired operating temperature, styrene (1.0 g, 9.6×10^{-3} mmol) or vinyl acetate (1.0 g, 1.04×10^{-2} mmol) in toluene (2 mL) was injected, and the pressure was raised to the desired reaction pressure. The pressure was maintained with a mass flow controller. The autoclave stirring speed was 500 rpm. At the end of the reaction, the stirrer was stopped and the reactor cooled rapidly. The liquid products were analyzed by GC with a flame ionization detector (quantitative) and GC-MS (qualitative). The enantioselectivity of the reaction was determined by comparison with authentic product samples.

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arachno- $[\text{Sn}_8]^{6-}$ or closo- $[\text{Li}_2\text{Sn}_8]^{4-}$? Synthesis and Characterization of $\text{A}_4\text{Li}_2\text{Sn}_8$ (A = Rb, K)**

Svilen Bobev and Slavi C. Sevov*

Renewed interest in Zintl phases and Zintl ions in recent years has resulted in some major conceptual developments in these fields. Numerous isolated deltahedral clusters with unusual shapes, such as Tr_{11}^{7-} , Tr_4^{8-} , Tr_5^{7-} , Tr_6^{8-} , Tr_6^{6-} , and

[*] Prof. S. C. Sevov, S. Bobev
Department of Chemistry and Biochemistry
University of Notre Dame
Notre Dame, IN, 46556 (USA)
Fax: (+1) 219-631-6652
E-mail: ssevov@nd.edu

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$\text{Tr}_{12}(\text{Tr})^{11-}$, where Tr (Triel = Group 13) is indium or thallium, were discovered.^[1] Furthermore, it was found that the well-known nine-atom deltahedral Zintl ions Tt_9^{4-} (Tetrel = Group 14) characterized from solutions exist also in the corresponding precursor Zintl phases A_4Tt_9 (A = alkali metal).^[2] This, for the first time, revealed relations between Zintl phases and Zintl ions.^[2] In addition, even the nine-atom silicon clusters Si_9^{4-} , previously unknown from solution studies, were characterized in the Zintl phase $\text{Rb}_{12}\text{Si}_{17}$.^[3] Despite this variety of clusters, isolated *arachno* species, that is, deltahedra with two missing vertices, were not known either in Zintl phases or as Zintl ions. For that matter *closo* species of ten atoms have not been unambiguously characterized yet either.^[4]

Here we report the synthesis and characterization of the first isolated *arachno* cluster of a main group element, namely, *arachno*- Sn_8^{6-} with the shape of a square antiprism. The cluster is found in two compounds, $\text{Rb}_4\text{Li}_2\text{Sn}_8$ and $\text{K}_4\text{Li}_2\text{Sn}_8$, both synthesized by direct fusion of the elements (see Experimental Section). The use of mixed alkali metals was not accidental. This approach is a very powerful tool for changing packing efficiency while keeping constant stoichiometry and charge, and we have used it in syntheses of other novel mixed-cation compounds. For example, mixing Na and Rb or Na and Cs is the only way to stabilize the silicon and germanium clathrate-II structures $\text{A}_8\text{Na}_{16}\text{Tt}_{136}$.^[5] Similarly, the same mixture of cations combined with tin provides the new phase $\text{A}_3\text{Na}_{10}\text{Sn}_{23}$ made of clathrate-II layers separated by a mixture of tin tetrahedra and cations, that is, an intergrowth of clathrate-II and the known phase A_4Sn_4 .^[6] The compounds reported here, $\text{Rb}_4\text{Li}_2\text{Sn}_8$ and $\text{K}_4\text{Li}_2\text{Sn}_8$, were discovered in an attempt to make the Li analogue $\text{A}_3\text{Li}_{10}\text{Sn}_{23}$. They contain the unprecedented *arachno*- Sn_8^{6-} clusters, stabilized by Li^+ as *closo*- $[\text{Li}_2\text{Sn}_8]^{4-}$ and separated by the larger Rb^+ or K^+ ions.

The structures of the two compounds were determined by single-crystal X-ray diffraction (see Experimental Section). Although they crystallize in different space groups and unit cells, their structures are nearly identical. $\text{Rb}_4\text{Li}_2\text{Sn}_8$ crystallizes in $P\bar{1}$ with $Z=4$, while $\text{K}_4\text{Li}_2\text{Sn}_8$ develops a superstructure ($I2/a$) with a fourfold larger cell and $Z=16$. Since the two structures are otherwise virtually the same, only that of $\text{Rb}_4\text{Li}_2\text{Sn}_8$ is described in detail here. It contains two crystallographically different but otherwise similar Sn_8^{6-} clusters. They are almost perfect square antiprisms (D_{4d}) with nearly flat and parallel bases (Figure 1). The deviations from planarity are less than 0.04 Å, and the dihedral angles between the bases are only 0.27(7) and 0.48(7)° for the two clusters. The Sn–Sn distances range from 2.947(3) to 3.037(2) Å (2.989(3)–3.044(2) Å in the square bases and 2.954(2)–3.026(2) Å in the waist) and are comparable with those in other deltahedral tin clusters such as *nido*- Sn_9^{4-} , which has a monocapped square antiprism structure, and its Cr-capped *closo* derivative $[\text{Sn}_9\text{Cr}(\text{CO})_3]^{4-}$.^[7]

The alkali metal cations cap faces, edges, and vertices, as expected, and provide six additional electrons per cluster. The Li–Sn and Rb–Sn distances are in the ranges 2.87(3)–3.20(3) and 3.561(3)–4.378(3) Å, respectively. The clusters are well separated from each other; the shortest intercluster distance is

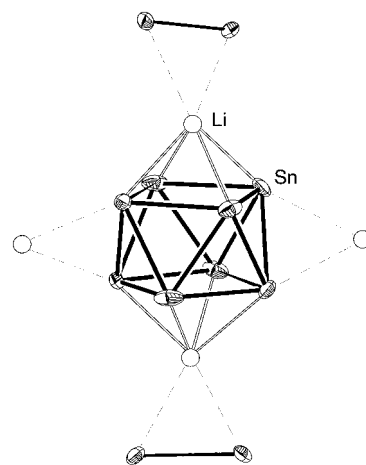


Figure 1. One of the two crystallographically independent *arachno*- Sn_8^{6-} clusters in $\text{Rb}_4\text{Li}_2\text{Sn}_8$ (50% probability thermal ellipsoids) with the shape of a square antiprism (thick full bonds). Also shown are the two lithium atoms (open circles) that cap the square faces (Li–Sn 2.87(3)–3.03(4) Å) and “complete” the cluster to a *closo*- $[\text{Li}_2\text{Sn}_8]^{4-}$. They also interact with Sn–Sn edges of two other Sn_8^{6-} clusters in the same way as the shown cluster interacts with two other lithium atoms through two of its edges (broken lines, Li–Sn 3.03(3)–3.20(3) Å).

4.628(2) Å. The composition, the dark gray to black color, the brittleness, and, more importantly, the diamagnetism of the compound (see Experimental Section) suggest an electronically balanced compound, that is, a Zintl phase. The charge on the cluster is therefore 6– and is indicative of an *arachno* species according to Wade’s rules.^[8]

A second, more intriguing way to view the structure results from recognizing that lithium is: 1) an alkali metal with substantial covalency in its interactions,^[9] and 2) very specifically positioned with respect to the tin clusters in the structure. All lithium atoms cap the square faces of the antiprisms and thus “complete” them to *closo* deltahedra of ten atoms, Li_2Sn_8 (Figure 1). Since the Li–Sn distances are similar to the Sn–Sn distances within the Sn_8 core (see above), the clusters are nearly ideal bicapped square antiprisms. Thus, instead of *arachno*- $[\text{Sn}_8]^{6-}$ the clusters can be viewed as *closo*- $[\text{Li}_2\text{Sn}_8]^{4-}$ species with 22 skeletal electrons that are isoelectronic with the classical $\text{B}_{10}\text{H}_{10}^{2-}$. However, the isoelectronic relation is true only for the delocalized skeletal electrons, but not for the total number of electrons, which includes localized electrons of the B–H bonds in $\text{B}_{10}\text{H}_{10}^{2-}$ or the lone pairs in naked clusters. A naked *closo* cluster of 10 atoms should have $10 \times 2 = 20$ electrons for lone pairs in addition to the 22 skeletal electrons. This, however, is not the case here, since the lithium vertices clearly can not be expected to carry lone pairs, which would result in an enormous negative charge of more than two per lithium atom. The problem is solved very elegantly in this case by the formation of close contacts between a lithium vertex of one cluster and a Sn–Sn edge of another (Figure 1). Thus, instead of a lone pair of electrons, each lithium atom shares two pairs of electrons from the “lone pairs” of the two tin atoms of the nearby cluster in a three-center, four-electron bond. The MO diagram for this model is the same as for a bent H_3^- molecule (an open triangle). There is a low-lying bonding combination of all three atoms, a nonbonding combination of the two end atoms (the tin atoms

in our case), and an antibonding combination. The net result for four electrons is bonding, with two electrons responsible for the two Li–Sn interactions, and a pair of nonbonding electrons delocalized on the two tin atoms. Of course, one can view the bonding as totally localized and consider a resonance structure in which the lithium atom is bonded *exo* to one tin atom (and shares its pairs of electrons), while the second tin atom retains its nonbonding lone pair of electrons. Either way, the important consequence is that the two lithium atoms of one cluster are bonded to two other clusters, and in addition each cluster interacts with two lithium atoms of two other clusters. The clusters are linked to each other in flat layers (Figure 2) that are separated by the Rb^+ or K^+ ions. Thus,

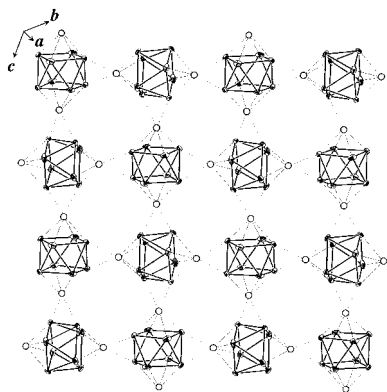


Figure 2. A layer of interconnected four-bonded $[\text{Li}_2\text{Sn}_8]^{4-}$ clusters. Each cluster is connected to two other clusters through its own two lithium atoms (open circles), and two more clusters are bonded to this cluster via their lithium atoms (broken lines). The Rb^+ or K^+ ions (omitted for clarity) are above and below the layer.

each cluster is bonded to four other clusters, whereby each bond carries two electrons, and therefore one electron per bond belongs to the cluster. As a result, the four-bonded *closo*- $[\text{Li}_2\text{Sn}_8]$ cluster requires 22 skeletal electrons (Wade's rules),^[8] four bonds $\times$ one electron per bond = four electrons for intercluster bonding, and six lone pairs on the remaining six vertices $\times$ two electrons per pair = 12 electrons. The total is 38 electrons, and since the number of provided electrons is $2\text{Li} \times 1 + 8\text{Sn} \times 4 = 34$, the cluster needs four additional electrons ($[\text{Li}_2\text{Sn}_8]^{4-}$), and these are provided by the four rubidium or potassium atoms. Attempts to bring the clusters into solution as Zintl ions are currently under way.

Experimental Section

Synthesis: All manipulations were performed in a nitrogen-filled glove box. The compounds were initially detected in the product of reactions of A, Li, and Sn (A = K, Rb) loaded in atomic ratio 3:10:23 in an attempt to produce the Li analogue of $\text{A}_3\text{Na}_{10}\text{Sn}_{23}$.^[6] The reactions were carried out in niobium containers sealed by arc-welding and enclosed in evacuated fused-silica ampoules at 400 °C for 20 days followed by cooling in the turned-off furnace. After the structures and the stoichiometry of the compounds were elucidated from single-crystal X-ray diffraction, the corresponding stoichiometric mixtures were treated under similar reaction conditions. Nevertheless, the products contained impurities of poorly diffracting primitive hexagonal phases, most likely $\text{Rb}_{12}\text{Sn}_{17}$ ($a = 15.22(2)$, $c = 24.76(4)$ Å) and $\text{K}_{12}\text{Sn}_{17}$ ($a = 14.11(3)$, $c = 23.15(6)$ Å). Different temperature profiles did not improve the overall results. Much better yields were achieved when an excess of Sn was used, most likely due to flux effects that provide conditions for more facile reaction and crystal growth.

Structure determination: Single crystals were selected from the initial reaction product and were sealed in capillaries. Data for a crystal of $\text{Rb}_4\text{Li}_2\text{Sn}_8$ (irregular shape, $0.18 \times 0.10 \times 0.08$ mm) were collected on an Enraf-Nonius CAD-4 single-crystal diffractometer with graphite-monochromated $\text{MoK}\alpha$ radiation at room temperature ($\omega/2\theta$ scans, a hemisphere with $2\theta_{\text{max}} = 52^\circ$, 8095 reflections) and were corrected for absorption by using the average of three ψ scans. Crystal data: triclinic, space group $P\bar{1}$, $a = 9.700(1)$, $b = 14.794(3)$, $c = 14.906(1)$ Å, $\alpha = 97.46(1)$, $\beta = 103.461(9)$, $\gamma = 102.49(2)^\circ$; $V = 1994.1(6)$ Å³; $Z = 4$; $\mu = 195.14$ cm⁻¹; $\rho_{\text{calcd}} = 4.348$ g cm⁻³. The refinement of all tin atoms with anisotropic and all lithium atoms with isotropic thermal parameters converged to $R1/wR2$ ($I > 2\sigma_I$) = 0.0637/0.1156 and $R1/wR2$ (all data) = 0.1405/0.1402 for 7780 independent reflections and 234 variables.

Data for a crystal of $\text{K}_4\text{Li}_2\text{Sn}_8$ (irregular shape, $0.16 \times 0.10 \times 0.10$ mm) were collected on a Bruker SMART APEX CCD-based diffractometer with $\text{MoK}\alpha$ radiation at room temperature (ω scans of width 0.3° , a full sphere with $2\theta_{\text{max}} = 66.48^\circ$, 37158/13934 total/independent reflections). The data were corrected for absorption by using the empirical ellipsoidal model SADABS. Crystal data: monoclinic, space group $I2/a$, $a = 18.9638(7)$, $b = 22.0359(8)$, $c = 18.9624(7)$ Å, $\beta = 108.778(1)^\circ$, $V = 7502.3(5)$ Å³; $Z = 16$; $\mu = 113.21$ cm⁻¹; $\rho_{\text{calcd}} = 3.996$ g cm⁻³. The final refinement of all atoms with anisotropic thermal parameters was carried out with interchanging a and c axes (twinning ratio of 0.405) and converged to $R1/wR2$ ($I > 2\sigma_I$) = 0.0427/0.0562 and $R1/wR2$ (all data) = 0.0678/0.0599 for 9145 reflections and 257 variables. Both structures were solved by direct methods and were refined on F^2 with the SHELXTL 5.1 software package.

The reason for the I centering in $\text{K}_4\text{Li}_2\text{Sn}_8$ (and the doubling of the volume) is that the almost equal b and c axes in the $P\bar{1}$ cell of $\text{Rb}_4\text{Li}_2\text{Sn}_8$ of 14.794(3) and 14.906(2) Å, respectively, and the corresponding β and γ angles of 103.461(9) and 102.49(2)°, respectively, become exactly equal in $\text{K}_4\text{Li}_2\text{Sn}_8$. In addition, the a axis is doubled, and this also doubles the volume. Further details on the crystal structure investigations may be obtained from the Fachinformationszentrum Karlsruhe, 76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666, e-mail: crysdata@fiz-karlsruhe.de) on quoting the depository numbers CSD-411278 ($\text{Rb}_4\text{Li}_2\text{Sn}_8$) and CSD-411279 ($\text{K}_4\text{Li}_2\text{Sn}_8$).

Magnetic measurements: The magnetization of 40 mg of selected crystals of $\text{Rb}_4\text{Li}_2\text{Sn}_8$ was measured on a Quantum Design MPMS SQUID magnetometer at 3 T over the temperature range 10–280 K. The data were corrected for the holder and for ion-core diamagnetism. The corrected magnetic susceptibility is negative and temperature independent, and varies between -1.75×10^{-4} and -1.96×10^{-4} emu mol⁻¹. This is consistent with a diamagnetic compound.

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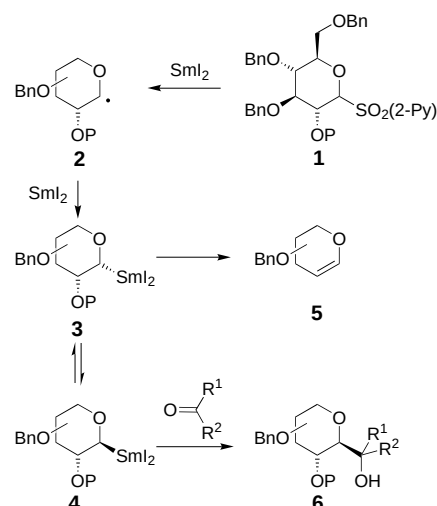
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Reductive Samarium of Anomeric 2-Pyridyl Sulfones with Catalytic Nickel: An Unexpected Improvement in the Synthesis of 1,2-*trans*-Diequatorial C-Glycosyl Compounds

Nicolas Miquel, Gilles Doisneau, and Jean-Marie Beau*

Dedicated to Professor Pierre Sinay
on the occasion of his 62nd birthday

A direct method for the synthesis of C-glycosyl compounds derived from neutral hexoses was recently developed by our group. This procedure, which consists in the reductive samarium of anomeric 2-pyridyl sulfones in the presence of carbonyl compounds (Barbier procedure), yields exclusively 1,2-*trans* C-glycosyl compounds in the hexopyranosyl series, typically the α -C-mannopyranosyl or β -C-glucopyranosyl compounds.^[1–3] In the mechanistic rationale formulated earlier, we proposed that the anomeric radical **2**, which results from the first electron transfer, is further reduced to a kinetic α -oriented organosamarium **3** which, in the *gluco* series, either *syn*-eliminates to glucal **5** or anomerizes to the β -species **4** (Scheme 1). The latter species does not *trans*-eliminate, as it is stable enough at room temperature to react



Scheme 1. Previous mechanistic proposal for the coupling of anomeric 2-pyridyl sulfones with carbonyl compounds mediated by samarium diiodide. 2-Py = 2-pyridyl; P = protecting group.

with the carbonyl compound. In the *manno* series, efficient constructions of carbon-linked dimers, mimics of biologically relevant disaccharides, were developed from this procedure.^[4] With the glucopyranosyl and, more drastically, with the galactopyranosyl series, the C-glycosylation was not efficient enough to be integrated into a synthetic plan.^[1b] The major

drawback was a too high level of the competing β -elimination (elimination/C–C bond formation in an approximately 1/1 ratio), that could only be minimized in the *gluco* series by using a bulky protecting group at O-2 (*tert*-butyldimethylsilyl, P in **1**). We now report an unexpected solution to this problem by incorporating catalytic amounts of nickel(II) iodide (1 mol %) with the reducing agent. Such catalysis by transition metals of some samarium diiodide mediated reactions was introduced by Namy and Kagan.^[5]

Treatment of a solution of sulfone **7a** and cyclohexanone (2 equiv) in THF at 0 °C with a freshly prepared THF solution of SmI₂ containing 1 mol % of nickel(II) iodide, afforded, after a standard workup, the C-glycosyl derivative **8a** in an isolated 94 % yield (Equation 1 and Table 1, entry 1). As determined

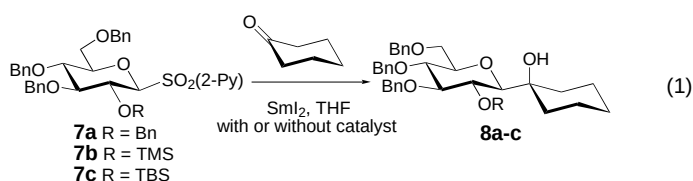


Table 1. SmI₂-induced coupling of glucopyranosyl sulfones **7** with cyclohexanone. 2-Py = 2-pyridyl.

Entry	Pyridyl sulfone	Conditions ^[a]	C-Glycoside (yield) ^[b]	Elimination (yield) ^[b]
1	7a	0 °C, 1 % NiI ₂	8a (94 %)	none
2	7a	0 °C	8a (36 %)	9 (22 %)
3	7a	25 °C	8a (39 %)	9 (51 %)
4	7a	0 °C, 1 % CuCl ₂	8a (30 %)	9 (64 %)
5	7a	0 °C, 1 % FeCl ₃	8a (79 %)	9 (17 %)
6	7a	25 °C, 1 % NiI ₂	8a (60 %)	9 (40 %)
7	7b ^[c]	25 °C	8b (44 %)	9 (37 %)
8	7c ^[c]	25 °C	8c (57 %)	9 (21 %)

[a] For the reaction conditions, see also the Experimental Section.

[b] Yields of isolated products after chromatography on silica gel.

[c] Results taken from ref. [1].

by ¹H NMR analysis, this was the only detectable product of the crude reaction mixture. The absence of the elimination reaction is remarkable and the effect of the nickel catalysis is highlighted by the results obtained in the same reaction under identical conditions without NiI₂, which provided the same coupling product **8a** (36 %) along with the elimination product tri-*O*-benzyl-D-glucal (**9**, 22 %) and the starting sulfone **7a** (27 %) (Table 1, entry 2). Complete conversion of sulfone **7a** only occurred at room temperature (Table 1, entry 3). In a limited study, nickel iodide appears to be the most useful transition metal catalyst to prevent or minimize the competing elimination reaction. Copper(II) chloride has no effect (Table 1, entry 4), whereas the Fe^{III} salt stands as a usable catalyst, although less efficient (Table 1, entry 5). Earlier, we observed that the competing elimination with sulfones in the *gluco* series could be partly suppressed by substituting the trimethylsilyl group at O2 in **7b** by the bulkier *tert*-butyldimethylsilyl group in **7c** (Table 1, entries 7 and 8).^[1] The nickel catalyst not only modifies the rate of the reductive process^[5b] but also the product distribution and, apparently, the overall mechanism.

[*] Prof. Dr. J.-M. Beau, N. Miquel, Dr. G. Doisneau
Université Paris-Sud
Laboratoire de Synthèse de Biomolécules
UMR CNRS 8614, Institut de Chimie Moléculaire
91405 Orsay Cedex (France)
Fax: (+33) 1-6985-3715
E-mail: jmbeau@icmo.u-psud.fr

The new procedure was also successful with the D-galactopyranosyl and L-fucopyranosyl 2-pyridyl sulfones **10** and **16**. These results are presented in Table 2. Addition of the nickel catalyst resulted in an improvement of the C-glycosylation reaction in the *galacto* series from 52 to 80% (Table 2, entries 1 and 2) with a concomitant decrease in the elimination reaction (tri-*O*-benzyl-D-galactal, **12**). Similarly, the L-fucosyl sulfone **16** provided a 96% yield of the C-fucosyl derivative **17** (Table 2, entry 5; 2% of the elimination product di-*O*-benzyl-L-fucal, **18**).

A series, in which sulfones **10**, **16**, and **7** coupled with cyclohexane carbaldehyde, also proceeded smoothly (Table 2), to provide the corresponding β -C-glycosyl products in **15**, **19**, and **20**, respectively (80–82% yield). These results are significant in that a strong increase in the reactivity of SmI₂ makes the Barbier reaction of simple alkyl iodides and aldehydes problematic due to the competing Oppenauer reaction with the aldehyde.^[5b] For products **15** and **19**, only one diastereomer could be detected by ¹H NMR analysis,

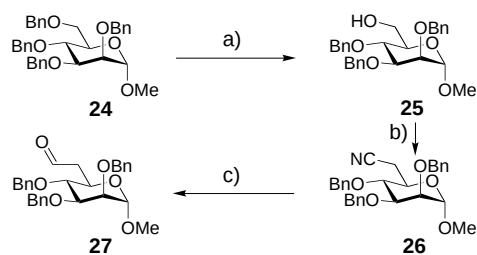
which showed H1 (δ = 3.37 (**15**) and 3.33 (**19**)) with a large value (9.3–9.5 Hz) for the *J*(1,2) coupling constant (hexose atom numbering, see Table 2) and a small value (<1 Hz) for the *J*(1,1') coupling constant, characteristic of an *R* center at C1' in **15** ("D series") and an *S* center at C1' in **19** ("L series").^[6–8] The mannopyranosyl sulfone **21** provided the corresponding C-glycosyl compounds **22** and **23** (Table 2, entries 8 and 9) in yields similar to those obtained without NiI₂.^[1]

The utility of this new procedure has been demonstrated in a fast synthesis of C-glycosyl dimers using aldehyde **27**, obtained by the five-step sequence of reactions shown in Scheme 2.^[9] The 1,6-C-linked disaccharides **28–31** were all provided in good yields (83 to 89%, Table 3, entries 1–4). The addition of the organosamarium reagents to the carbonyl group of aldehyde **27** occurred with a high facial selectivity, to produce the C-glycosyl compounds **29**, **30**, and **31** with a diastereomeric ratio of about 95:5. The major isomers of the diequatorial compounds **28**, **29**, and **30** showed, in their

Table 2. SmI₂-induced C-glycoside formation with catalytic nickel at 0 °C.^[a]

Entry	Pyridyl sulfone	Carbonyl compound	C-Glycoside (yield) ^[b]	Elimination (yield) ^[b]
1	10 , R ¹ = Bn	cyclohexanone	11 (80%)	12 (15%)
2 ^[c]	10 , R ¹ = Bn	cyclohexanone	11 (52%)	12 (40%)
3 ^[c,d]	13 , R ¹ = TMS	cyclohexanone	14 (25%)	12 (35%)
4	10 , R ¹ = Bn	cyclohexanecarbaldehyde	15 (82%; >95:5)	12 (16%)
5	16	cyclohexanone	17 (96%)	18 (2%)
6	16	cyclohexanecarbaldehyde	19 (80%; >95:5)	18 (14%)
7	7	cyclohexanecarbaldehyde	20 (82%; n.d.) ^[e]	9 (16%)
8	21	cyclohexanone	22 (83%)	9 (11%) ^[f]
9	21	cyclohexanecarbaldehyde	23 (87%; n.d.)	9 (8%)

[a] 2-Py = 2-pyridyl. [b] Yields of isolated products after chromatography on silica gel; second values refer to the diastereomeric ratio as determined by ¹H NMR. [c] Reaction performed without NiI₂ at 25 °C. [d] Results taken from reference [1b]. [e] Not determined due to severe overlap in the ¹H NMR spectrum. [f] The protonation product, 1,5-anhydro-2,3,4,6-tetra-*O*-benzyl-D-manno-hexitol, was also formed (2%).



Scheme 2. Synthesis of aldehyde **27**. a) 1) $\text{Ac}_2\text{O}/\text{CF}_3\text{COOH}$ (4/1), 25°C , 1 h, 88%; 2) MeONa , MeOH , 25°C , 12 h, 96%; b) 1.4 equiv PPh_3 , 4 equiv I_2 , imidazole, toluene, 70°C , 1 h, 85%; 2) 1.4 equiv Bu_4NCN , DMF, 0 to 25°C , 3 h, 94%; c) 3 equiv DIBAL-H , CH_2Cl_2 , -78°C , 0.5 h, 80%. DIBAL-H = diisobutylaluminum hydride.

Table 3. SmI_2 -induced C-glycosyl dimer formation with catalytic nickel at 0°C .

Entry	Pyridyl sulfone ^[a]	C-Dimer (yield) ^[b,c]	Diastereomer ratio ^[d]
1		 28 (83%)	n.d. ^[e]
2		 29 (85%)	$\approx 95:5$
3		 30 (89%)	94:6
4		 31 (88%)	95:5

[a] For the reaction conditions, see the Experimental Section. [b] Yields of isolated products (both diastereomers at C7) after chromatography on silica gel. [c] The asymmetric center drawn at C7 is that of the major isomer. [d] Diastereomeric ratio at C7 as determined by HPLC analysis. [e] Not determined due to severe overlap.

^1H NMR spectra, large values (9.0 to 9.5 Hz) for the $J(8,9)$ and $J(9,10)$ coupling constants (tridecopyranoside atom numbering, see Table 3), which indicates an equatorial C7–C8 carbon–carbon bond with a chair conformation for the C8–C13 tetrahydropyran ring. As seen above with **15** and **19**, the small values (<1 –1.3 Hz) for the $J(7,8)$ coupling constants in all three cases are diagnostic of an *R* center at C7 in **28** and **29** (“ β -D series”) and an *S* center at C7 in **30** (“ β -L series”).^[6–8] The *S* center at C7 of the major isomer in **31** has been assigned, on the basis of our previous work in the *manno* series.^[4b, 4c] These observations showed that the facial discrimination establishing the configuration of the exocyclic asymmetric center is exclusively controlled by the asymmetry of the C-glycosyl donor.

In summary, we have extended the reductive samariation protocol to the highly selective synthesis of 1,2-*trans* die-

quatorial C-glycosyl compounds at synthetically useful levels. The results obtained in these new conditions suggest a possible mechanism in which, during the samariation step, the intermediate anomeric radical is reduced primarily under the control of steric factors by a bulky catalytic species containing low-valent nickel (Figure 1).^[10–12] Work is in progress to delineate a precise mechanism for this transformation and to apply this method to the construction of bioactive compounds.

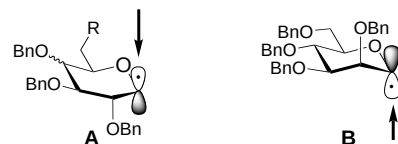


Figure 1. Possible reduction mechanism of the anomeric radical from the *gluco*, *galacto*, or *fuco* series (A) and *manno* series (B) by a catalytic complex containing low-valent nickel under the steric control of the substituent at position 2.

Experimental Section

8a: A solution of NiI_2 in THF (0.01M, 1 mL) was added to a solution of SmI_2 in THF (0.1M, 10 mL) under Ar. This solution (1.03 mL, 94 μmol of SmI_2) was cooled to 0°C and added to a stirred solution of pyridyl sulfone **7a** (25 mg, 37.5 μmol) and cyclohexanone (12 μL , 116 μmol) in THF (0.5 mL) at 0°C under Ar. After stirring at 0°C for 0.5 h, saturated aq. NH_4Cl was added. The reaction mixture was extracted twice with CH_2Cl_2 . The combined organic phases were washed twice with water, dried with Na_2SO_4 , and evaporated to dryness. Flash chromatography (cyclohexane/ EtOAc , 6/1) gave **8a** (22 mg, 94%) as the only product. $[\alpha]_D^{20} = +3$ ($c = 2$, CHCl_3); ^1H NMR (CDCl_3 , 250 MHz): $\delta = 7.37$ – 7.19 (m, 20H; 4 Ph), 5.05, 4.98, 4.86, 4.82, 4.73, 4.63, 4.62, 4.57 (8 $\times$ d, 8H, $J = 11$ Hz; CH_2Ph), 3.81, 3.74 (2 $\times$ t, 2H, $J \approx 9$ Hz; H-2,3), 3.69–3.70 (m, 2H; H-6,6'), 3.64 (dd, 1H, $J(4,5) = 9.7$, $J(3,4) = 8.8$ Hz; H-4), 3.44 (ddd, 1H, $J(4,5) = 9.7$, $J(5,6) = J(5,6') = 3.2$ Hz; H-5), 3.30 (br s, 1H; OH), 3.15 (1d, 1H, $J(1,2) = 9.2$ Hz; H-1), 1.73–1.45 (m, 10H; 5 CH_2); MS (ES): $m/z = 645$ [$M+\text{Na}$]; HR-MS (ES) for $\text{C}_{40}\text{H}_{46}\text{NaO}_6$ [$M+\text{Na}$]: calcd: 645.3192; found: 645.3198.

29: The procedure for the preparation of **8a** was followed with sulfone **10** (25 mg, 37.5 μmol) and aldehyde **27** (27 mg, 37.5 μmol , 1.5 equiv) and provided C-linked disaccharide **29** (32 mg, 85%). ^1H NMR (CDCl_3 , 250 MHz, atom numbering of a tridecopyranoside): $\delta = 7.40$ – 7.22 (m, Ph), 4.99–4.42 (m, 14H; CH_2Ph), 4.67 (d, 1H, $J(1,2) = 1.9$ Hz; H-1), 4.17 (t, 1H, $J(8,9) = J(9,10) = 9.5$ Hz; H-9), 4.02 (d, 1H, $J(10,11) = 3$ Hz; H-11), 3.88 (dd, 1H, $J(3,4) = 9.2$, $J(2,3) = 3.2$ Hz; H-3), 3.81 (dd, 1H, $J(1,2) = 1.9$, $J(2,3) = 3.2$ Hz; H-2), 3.68 (t, 1H, $J(4,5) = 9.5$ Hz, $J(3,4) = 9.2$ Hz; H-4), 3.63 (dd, 1H, $J(9,10) = 9.5$, $J(10,11) = 3$ Hz; H-10), 3.62–3.53 (m, 3H; H-12,13,13'), 3.28 (s, 3H; CH_3), 3.135 (dd, 1H, $J(8,9) = 9.5$, $J(7,8) = 1.3$ Hz; H-8), 2.27 (ddd, 1H, $J = 14$, 11.2, 2.2 Hz; H-6), 1.62 (ddd, 1H, $J = 14$, 10.2, 2.8 Hz; H-6'); MS (ES): $m/z = 1023$ [$M+\text{Na}$]; HR-MS (ES) for $\text{C}_{63}\text{H}_{68}\text{NaO}_{11}$: calcd: 1023.4659; found: 1023.4652.

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Formation of Optically Active Aromatic α -Amino Acids by Catalytic Enantioselective Addition of Imines to Aromatic Compounds**

Steen Saaby, Xiangming Fang, Nicholas Gathergood, and Karl Anker Jørgensen*

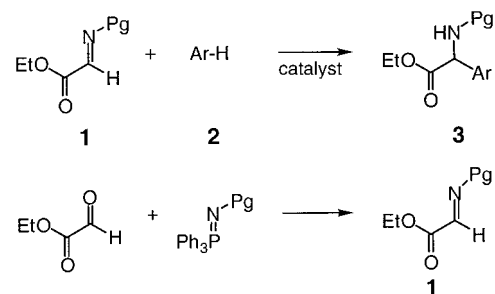
Optically active α -amino acids are vital to life as building blocks of peptides, proteins, and many other natural products. Amino acids are also applied extensively as food additives, pharmaceuticals, and agrochemicals. Furthermore, amino acids are widely used in organic synthesis as targets, and as constituents for reagents and/or catalysts in asymmetric synthesis.

The synthesis of optically active nonproteinogenic α -amino acids is an interesting and important research field which has

received much attention^[1] and the application of asymmetric catalysis towards formation of these compounds from simple starting materials is a fundamental challenge.

Recently, catalysts have been developed for enantioselective addition reactions to imines, including processes such as the *ene*^[2], Mannich-type,^[3] allylation,^[4] and aza-Diels–Alder reactions.^[5] Reports on the catalytic enantioselective addition of imines to aromatics are very few, and, according to our knowledge, only one example of the addition of an imine to indole and pyrrole systems has been reported.^[6] Recently, several catalytic asymmetric Strecker reactions have emerged for the synthesis of optically active, natural, as well as unnatural, aromatic and aliphatic α -amino acids.^[7]

In this paper we present the development of a catalytic enantioselective addition reaction of imines **1** to electron-rich aromatic compounds **2** leading to protected optically active aromatic α -amino acids **3**^[8] (Scheme 1). An advantage of this reaction is that **1** is prepared by an aza-Wittig reaction^[9] and used directly without further purification (Scheme 1).



Scheme 1. Top: Addition of imines **1** to electron-rich aromatic compounds **2** to form protected aromatic α -amino acids **3**. Bottom: Synthesis of the protected imine starting materials **1**. Pg = protecting group.

The combination of CuClO_4 and (*R*)-Tol-BINAP catalyzed the reaction of the α -imine ester **1a** with *N,N*-dimethylaniline (**2a**) in THF at room temperature to give the protected aromatic α -amino acid **3a** in 81 % yield and with 14 % *ee* (see Equation (1) and Table 1, entry 1). Lowering the reaction temperature to -78°C caused a substantial improvement in

Table 1. Optimisation results for the reaction of imine **1a** and *N,N*-dimethylaniline (**2a**) with (*R*)-Tol-BINAP/ CuX as the catalyst.

Entry	CuX	Cat. concentration [mol %]	Solvent	<i>T</i> [°C]	Yield ^[a] [%]	<i>ee</i> [%]
1	CuClO_4	10	THF	RT	81	14
2	CuClO_4	10	THF	-78	81	87
3	CuClO_4	10	CH_2Cl_2	-78	82	85
4	CuClO_4	5	THF	-78	78	93
5	CuPF_6	5	THF	-78	75	96
6	CuPF_6	1	THF	-78	79	81
7 ^[b]	CuPF_6	5	THF	-78	76	93

[a] Yield of isolated products. [b] Large scale reaction with 8.0 mmol of starting material.

[*] Prof. Dr. K. A. Jørgensen, S. Saaby, Dr. X. Fang, Dr. N. Gathergood
Center for Metal Catalyzed Reactions
Department of Chemistry
Aarhus University
8000 Aarhus C (Denmark)
Fax: (+45) 8619-6199
E-mail: kaj@kemi.aau.dk

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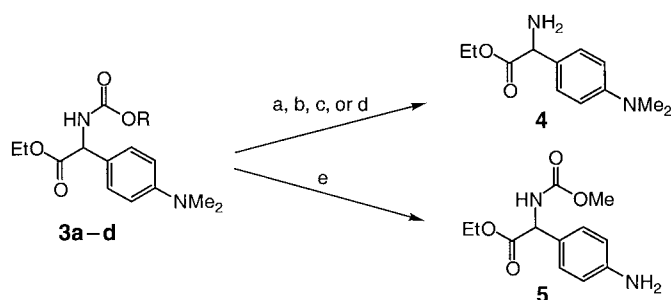
Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

the *ee* value (87%) while maintaining the high yield (81%; entry 2). The optimum catalytic conditions presented in entry 5 gave **3a** in 75% yield with an excellent enantiomeric excess value of 96%. For this catalyst combination, a reduction of the loading to 1 mol% leads also to satisfactory results (entry 6). It should be noted that the reaction is highly regioselective, as only the *para*-substituted product is formed.

To broaden the applicability and scope of the present enantioselective reaction a number of imines with different *N*-protecting groups were tested, and the results are presented in Table 2. Entries 2 and 3 show that the *N*-methoxy- and *N*-benzyloxycarbonyl α -imino esters **1b, c** both react with **2a** giving the aromatic substitution adducts **3b, c** in good yields and excellent enantioselectivities (93% *ee* in both reactions). The reaction of the *N*-Boc protected α -imine ester **1d** with **2a** gave the desired product **3d** in high yield, however, even with optimised solvent, counterion, and temperature conditions, only a moderate enantioselectivity of 64% *ee* was obtained (entries 4–6). The *N*-tosyl α -imino-ester **1e**^[10] also reacts with **2a** giving **3e** in high yield, but with slightly lower enantioselectivity (entry 7) compared to the products **3a–c**, which also have *N*-protecting groups which are readily removed (see below).

Explaining the mode of coordination of the imines **1a–e** to the catalyst is an intriguing problem as the absolute configurations of the products depend on the imine protecting group. Using the (*R*)-Tol-BINAP–Cu^I complex as the catalyst, the *N*-ethoxy-, *N*-methoxy-, and *N*-benzyloxycarbonyl protected imines **1a–c** gave the *R* enantiomers of **3a–c**, while use of the more bulky *N*-Boc and *N*-tosyl protected imines gave the *S* enantiomers of **3d** and **3e**, respectively. At the present stage of investigation we can not account for this observation. Most notable are the results in entry 6 and the dramatic temperature dependency of the reaction (Table 1, entries 1 and 2), which indicate that two competitive coordination modes with opposite chiral induction might be involved.

An important application of the products obtained from the present enantioselective catalytic reaction is, for example, the synthesis of novel nonproteinogenic optically active electron-rich aromatic α -amino acids, and readily removable protecting groups are therefore highly desirable. In Scheme 2, the synthetic procedure is illustrated for the removal of the four different *N*-protecting groups in **3a–d** to give **4** in high yield



Scheme 2. a) When R = Et: TMSI, CH₃CN, 82 °C, 20 min, then EtOH, 82 °C, 3 min, 83%; b) when R = Me: TMSI, CH₃CN, 80 °C, 15 min, then MeOH, 25 °C, 10 min, 90% (isolated as the HI salt); c) when R = Bn: H₂, 20% Pd(OAc)₂, EtOH, RT, 1 h, 98%; d) when R = *t*Bu: 25% TFA in CH₂Cl₂, 25 °C, 30 min, quantitative; e) PhIO, TMSN₃, CH₂Cl₂, –40 °C, 3 h, then work-up in sat. NaHCO₃ (aq.), THF (1:1), RT, 48 h, 92%. TMS = trimethylsilyl, Bn = benzyl, TFA = trifluoroacetic acid.

without detectable racemization; it is also shown that the *N,N*-dimethyl substituent on, for example, **3b** can be deprotected selectively to **5** by a one step procedure.^[11, 12] With the free amino group in **5**, it is now possible to introduce a variety of different new electron-donating and electron-withdrawing substituents on to the aromatic nucleus.

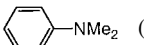
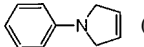
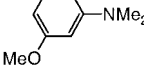
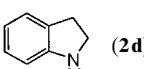
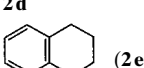
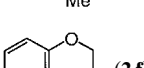
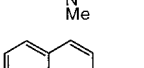
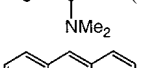
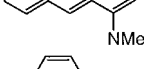
With the conditions for the catalytic addition of imines **1a–e** to **2a** and for the selective deprotection of the products in hand, we examined a variety of electron-rich aromatic compounds with a large structural diversity as potential substrates (Table 3). Mono- and disubstituted, mono- and polyaromatic compounds react in a regioselective reaction, as only one regioisomer of the aromatic electrophilic substitution product is formed. The product **3f** obtained by reaction of imine **1b** with **2b** was isolated in 88% yield and with 86% *ee* (entry 2) and contains a pyrrolidine unit, which is susceptible to further elaboration.^[13] The disubstituted aromatic compound **2c** reacts selectively in the *para* position, relative to the *N,N*-dimethylamino substituent, to give **3g** in good yield and with up to 79% *ee* (entries 3–5). Bicyclic, naphthalene- and anthracene-based substrates also react in a highly enantioselective fashion, giving access to a series of novel optically active aromatic α -amino acid derivatives. The reaction of imine **1b** with *N*-methylindoline (**2d**) gives an almost enantiopure product **3h** (up to 98% *ee*; entries 7 and 8). The aromatic substitution adduct **3h** is interesting as novel optically active 5-indolyl α -amino acids are accessible by

Table 2. Reaction of different *N*-protected imines **1a–e** with *N,N*-dimethylaniline (**2a**) catalyzed by (*R*)-Tol-BINAP/CuPF₆.^[a]

Entry	Imine/Pg	Cat. concentration [mol %]	Solvent	<i>T</i> [°C]	Product	Yield ^[b] [%]	<i>ee</i> [%] (configuration ^[c])
1	1a /CO ₂ Et	5	THF	–78	3a	75	96 (<i>R</i>)
2	1b /CO ₂ Me	5	CH ₂ Cl ₂	–78	3b	75	93 (<i>R</i>)
3	1c /CO ₂ Bn	5	CH ₂ Cl ₂	–78	3c	68	93 (<i>R</i>)
4	1d /Boc	10	DCE	RT	3d	84	64 (<i>S</i>)
5	1d /Boc	1	DCE	RT	3d	72	64 (<i>S</i>)
6	1d /Boc	10	THF	–50	3d	22	14 (<i>R</i>)
7	1e /Ts	5	THF	–78	3e	91	88 (<i>S</i>)

[a] Abbreviations: Pg = protecting group, RT = room temperature, Bn = benzyl, Boc = *tert*-butoxycarbonyl, DCE = 1,2-dichloroethane, Ts = tosyl = toluene-4-sulfonyl, Cbz = benzyloxycarbonyl, DMAP = 4-dimethylaminopyridine. [b] Yield of isolated products. [c] The configuration of **3c** is derived from a crystalline camphanic acid derivative of **3c**, which was analysed by X-ray crystallography; see the Supporting Information for further details. The configurations of the other products were assigned by deprotection (see Scheme 2) followed by standard transformations (CbzCl, Et₃N, DMAP/TsCl, DMAP) and comparison of the samples obtained with **3c**.

Table 3. Results for the reaction of the imines **1b**, **c**, **e** with the substrates **2a–i** using (*R*)-Tol-BINAP/CuPF₆ as the catalyst.^[a]

Entry	Imine	Substrate	Cat. concentration [mol %]	Product	Yield ^[b] [%]	ee [%]
1	1b	 (2a)	5	3b	75	93
2	1b	 (2b)	5	3f	88	86
3	1b	 (2c)	5	3g	68	72
4	1b	2c	10	3g	79	76
5 ^[c]	1b	2c	5	3g	56	79
6	1b	 (2d)	10	3h	63	97
7	1b	2d	20	3h	79	98
8	1b	 (2e)	5	3i	82	87
9	1b	 (2f)	10	3j	44	52
10	1b	 (2g)	5	3k	75	92
11	1c	 (2h)	5	3l	65	89
12 ^[c]	1e	 (2i)	10	3m	83	79

[a] All reactions were performed at -78°C , except the reaction in entry 12 which was performed at 0°C . All reactions were performed with CH_2Cl_2 as the solvent, except for the reactions in entries 5 and 12 which were performed in THF. The observed most active position for aromatic electrophilic substitution in all these substrates is the 4-position, relative to the nitrogen substituent. The major product from substrate **2i** is also addition in the 4-position. [b] Yield of isolated products.

oxidation of the indoline moiety using mild procedures.^[14] *N,N*-Dimethyl-1-aminonaphthalene (**2g**) and *N,N*-dimethyl-1-aminoanthracene (**2h**) also react in an enantioselective reaction giving the corresponding aromatic α -amino acids in good yields and with high *ee* values (92% and 89%, respectively; entries 10 and 11).

The catalytic reaction shows high substrate tolerance, as a series of diverse products are formed in generally good yields and with excellent *ee* values (up to 98%). The optically active aromatic α -amino acids are formed with easily removable protecting groups on the amino functionality and show potential and applicability in various fields of chemistry.

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Hydrogen/Deuterium Exchange Catalyzed by an Unusually Stable Mercury–Toluene Complex**

Alexander S. Borovik, Simon G. Bott, and Andrew R. Barron*

The chemistry of mercury(II) salts, in particular the trifluoroacetate, with aromatic hydrocarbons is well developed with regard to electrophilic attack on aromatic compounds, that is, aromatic mercuration. Olah et al. have shown that arenemercurinium ion complexes are involved, in a fast exchange, as intermediate complexes in aromatic mercuration.^[1] Extensive studies by Kochi and co-workers showed that the activation of the arene is related to a charge-transfer transition in the π complex.^[2, 3] As part of this work they also isolated a η^6 complex of C_6Me_6 and $[Hg(O_2CCF_3)_2]$.^[4] Crabtree and co-workers have demonstrated that photolysis of metallic mercury in the presence of aromatic compounds results in a variety of C–C bond-forming reactions and have proposed a π complex as a key intermediate.^[5] Thus, the activation of aromatic compounds by mercury salts is well established. Our interest in this area was initially stimulated by the possibility of exploring the activation of small molecules by Lewis acids,^[6, 7] in particular the use of Group 13 halides to enhance the Lewis acidity of other metal halides, for example, mercury.

The reaction of $HgCl_2$ with two molar equivalents of $GaCl_3$ in toluene yields an orange photosensitive solution from which yellow crystals may be obtained in high yield. The solubility in toluene suggests a simple Lewis acid base complex rather than a cation/anion pair. Although the EI mass spectrum and elemental analysis are consistent with the presence of toluene, solution ^1H and ^{13}C NMR spectroscopy do not allow an unambiguous characterization (see below). However, an X-ray crystallographic structure determination showed that the molecular formula was $[Hg(\eta^2-C_6H_5Me)_2-(GaCl_4)_2]$ (**1**), and that the toluene ligands are π bound to the mercury center (Figure 1).^[8, 9] Complex **1** represented only the second unsupported π -aryl complex of mercury to be structurally characterized, although a number of intramolecular π complexes have been structurally characterized.^[10, 11] Arene complexes of mercury(II) with SbF_6^- and AsF_6^- ions have been proposed based upon NMR data, but no structural evidence was obtained,^[12] however, based on the structure of **1** these assignments were undoubtedly correct.

Each toluene in **1** is bound in an asymmetric η^2 manner with the $Hg(1)$ –C(4) bond *para* to the toluene methyl group (2.349(9) Å) being significantly shorter than the $Hg(1)$ –C(3)

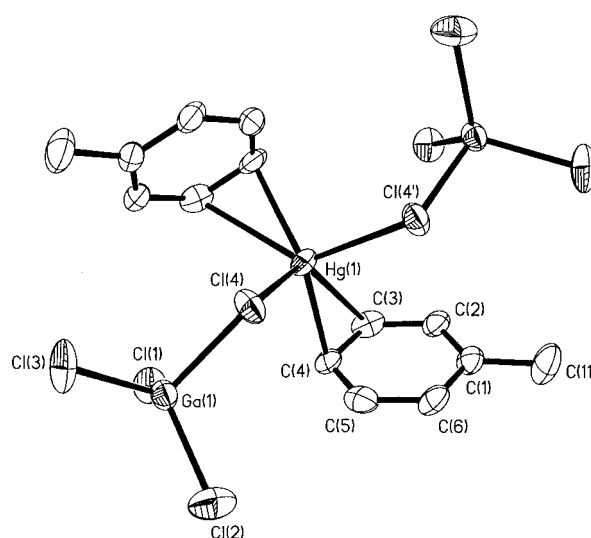


Figure 1. Molecular structure of **1**. Thermal ellipsoids are set at the 30% probability level and the hydrogen atoms are omitted for clarity.

bond in the *meta* position (2.71(1) Å). Noteworthy is that these values are either side of the values reported by Lau and Kochi for $[Hg_2(\mu-O_2CCF_3)_4(\eta^2-C_6Me_6)_2]$ (2.56 and 2.58 Å^[2]) and significantly shorter than observed for the intramolecular coordination discussed above (ca. 3.2 Å^[10, 11]). The relative Hg–C bond lengths would suggest that the methyl is *para* directing as would be expected from traditional Friedel–Craft chemistry. It is also worth noting that a typical Hg–C σ bond is 2.1–2.2 Å in length which is only slightly shorter than the Hg–C_{para} interaction.^[13] Although the Kochi complex was the first (and only) crystallographically characterized mercury–arene π complex prior to our work, several examples have been spectroscopically characterized.^[14, 15] Of note is the isostructural relationship of **1** to the reported $[Ag(C_6H_4Me_2)(ClO_4)]$ complex,^[16] in which the arene rings in the silver complex are also bound in an asymmetric η^2 manner. It was proposed that the perchlorate group played a significant role in stabilizing the Ag^I -aromatic complex and the $[GaCl_4]^-$ ion clearly performs a similar function with the mercury.

Solid-state ^{199}Hg and ^{13}C MAS NMR (MAS = magic angle spinning) spectra for **1** are consistent with the X-ray structure.^[17] In the ^{199}Hg NMR spectrum the chemical shift of the mercury center ($\delta = -1970$) is downfield to that reported for $HgCl_2$ ($\delta = -1497$ ^[18]) and closer to that of Hg^{II} aquated salts ($\delta = -2253$ to -2361 ^[19]) than simple Lewis base complexes, for example, $[HgCl_2\{P(nBu)_3\}_2]$ ($\delta = -404$ ^[20]). The UV/Vis spectrum of **1** shows an intense absorption at 305 nm ($\epsilon = 7000 \text{ mol}^{-1} \text{ cm}^{-1}$). The band at 305 nm is likely that of the π – π^* transition for the coordinated toluene,^[21] however, it is also consistent with the toluene–Hg charge-transfer transitions observed for arene complexes of $[Hg_2(\mu-O_2CCF_3)_4]$.^[2] However, two observations differentiate the present system from $[Hg_2(\mu-O_2CCF_3)_4(\eta^2\text{-arene})_2]$ complexes. First, the absorption for **1** is at significantly lower energy than those observed for $[Hg_2(\mu-O_2CCF_3)_4(\eta^2\text{-arene})_2]$ (288–315 nm^[2]) or the toluene complexes of $HgCl_2$ (274 nm^[15]). Second, whereas the absorption for $[Hg_2(\mu-O_2CCF_3)_4$

[*] Prof. A. R. Barron, A. S. Borovik

Department of Chemistry and Center for Nanoscale Science and Technology

Rice University, Houston, TX 77005 (USA)

Fax: (+1) 713-348-5619

E-mail: arb@rice.edu

Prof. S. G. Bott

Department of Chemistry, University of Houston

Houston, TX 77204 (USA)

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(η^2 -arene)₂] is transient the absorption for **1** is sustained indefinitely. Both of these observations suggest a stronger interaction between mercury and the aromatic group than previously observed. Exposure of **1** to ambient light results in rapid decomposition, however, sensitivity to O₂ and CO is negligible.

As was noted above, solution NMR spectra could not be obtained for [Hg(η^2 -C₆H₅Me)₂(GaCl₄)₂]; dissolution in C₆D₆ results in the rapid (< 5 min, 25 °C), quantitative formation of C₆D₅Me and C₆D₅H, as confirmed by ¹H and ¹³C NMR spectroscopy as well as GC/MS. When C₆H₅Me and C₆D₆ are mixed in the presence of < 0.1 mol % of **1** complete scrambling of the aromatic hydrogen/deuterium atoms occurs, indicating that the H/D exchange reaction is catalytic. Furthermore, H/D exchange is catalyzed for C₆D₆ and xylenes in the presence of < 0.1 mol % of [Hg(η^2 -C₆H₅Me)₂(GaCl₄)₂], suggesting that the identity of the initial aromatic is not vital in defining the catalytic exchange.

Hydrogen/deuterium exchange reactions for aromatic compounds are known to occur in the presence of strong acids or transition metals.^[22] For example, addition of H₂SO₄ to a mixture of D₂O and C₆H₆ allows for the synthesis of C₆D₆.^[23] Other suitable acid catalysts include HO₂CCF₃ and HBr. In all cases an acid with a suitable pK_a (< 0.2) is required. In the present system we propose that the acidity of the aromatic protons/deuteriums of the coordinated aromatic molecule (toluene or benzene) is increased from their uncomplexed values (pK_a (benzene) = 43^[24]) through activation by the mercury/gallium complex. Initial ab initio calculations suggest that the coordination of benzene to a "Hg(GaCl₄)₂" moiety results in a threefold increase in negative charge at the carbon associated with the shortest Hg–C bond, that is, Hg(1)–C(4) in [Hg(η^2 -C₆H₅Me)₂(GaCl₄)₂].^[25]

Experimental Section

Toluene (25 mL) was added to the solid mixture of anhydrous HgCl₂ (1.00 g, 3.68 mmol) and GaCl₃ (1.298 g, 7.37 mmol). The resulting orange solution was heated at approximately 100 °C while being vigorously stirred to allow all the HgCl₂ to dissolve. After about 15 min heating was stopped and the reaction flask was wrapped in aluminum foil to prevent the decomposition reaction caused by bright light. Large yellow crystals grew within 1 h at room temperature, yield: 90 %; m.p. 78 °C.

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Stabilization of a Reactive Cationic Species by Supramolecular Encapsulation**

Marco Ziegler, Julia L. Brumaghim, and Kenneth N. Raymond*

In the study of molecular capsules, the stabilization of reactive species and reactions of encapsulated guests are areas of great interest as possible methods for drug delivery and for use as molecular reaction vessels.^[1–4] Organic capsule molecules such as hemicarcerands are covalently linked cages with large internal cavities and wide pores for the incorporation of guest species. These molecular capsules have been found to stabilize cyclobutadiene,^[5] *o*-benzyne,^[6] 1,2,4,6-cycloheptatriene,^[7] and dibenzoyl peroxide^[8] as encapsulated guests, and sulfenic and selenic acids through covalent linkage to the capsule molecules.^[9]

More recently, self-assembly has been used to synthesize structures held together by weaker interactions including metal coordination and hydrogen bonds.^[3, 10–12] These cage complexes are also capable of selectively encapsulating guest molecules, including the encapsulation of C-shaped molecules reported by Kusukawa and Fujita,^[13] and the cation inclusions reported by Raymond et al.^[14] Here we report that the [Ga₄L₆]^{12–} supramolecular tetrahedron^[15] (Figure 1) is capable of encapsulating and stabilizing the reactive cationic species [Me₂C(OH)PEt₃]⁺, the first trapping of such a species.

The ¹H NMR spectrum recorded upon addition of PEt₃ to a solution of Na₁₂[Ga₄L₆] in D₂O shows upfield-shifted resonance signals in the δ = –1 to –2 range (Figure 2). Such upfield-shifted resonance signals are characteristic of encapsulated guest molecules.^[10, 14] The doublet of triplets observed at δ = –1.12 (*J*_{H,P} = 17, *J*_{H,H} = 8 Hz) is similar to signals seen for the methyl groups of triethylphosphine. A corresponding signal for the methylene groups of a PEt₃ moiety can be seen at δ = –1.56. The presence of two doublets at δ = –1.42 (*J*_{H,P} = 15 Hz) and δ = –1.53 (*J*_{H,P} = 15 Hz), however, indicated that this molecule

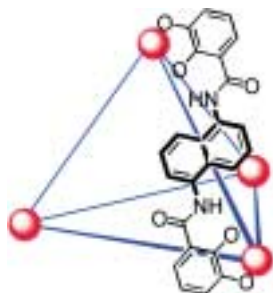
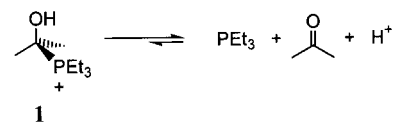


Figure 1. Schematic drawing of the [Ga₄L₆]^{12–} tetrahedron showing the structure of the ligand (L); blue lines represent ligand molecules, and red circles represent gallium atoms.

was not simply triethylphosphine. We assigned these encapsulated guest signals to the phosphine–acetone adduct, [Me₂C(OH)PEt₃]⁺ (**1**), formed from the reaction of PEt₃ with small amounts of acetone present from the synthesis of the tetrahedral cluster. Thus, the doublets observed at δ = –1.42 and δ = –1.53 were assigned to the two methyl groups from the Me₂COH moiety. Diastereotopic splitting of the methyl protons in the ¹H NMR spectrum is an effect which has been previously observed for encapsulated guest molecules.^[15] Integration of the observed signals support these assignments.

Encapsulation of this phosphorus-containing cation can be observed by ³¹P NMR as well as ¹H NMR spectroscopy. In the ³¹P{¹H} NMR spectrum of this mixture, several resonance signals are observed: at δ = 31.2 (s), 37.1 (s), 48.5 (1:1:1 t, *J*_{P,D} = 44 Hz), 63.8 (s), and 68.3 (s). The signal at δ = 37.1 is attributed to that of the encapsulated guest, while the remaining signals are due to unidentified decomposition products of PEt₃ in water.^[16] The same ¹H and ³¹P NMR signals of the tetrahedral [Ga₄L₆]^{12–} cluster with its [Me₂C(OH)PEt₃]⁺ guest were observed when this experiment was repeated in CD₃OD.

The cationic species **1** is formed by the reaction of triethylphosphine and acetone in the presence of protons. Although this species has been synthesized and isolated under anhydrous conditions,^[17–19] it decomposes in aqueous solutions (due to the negligible concentration of acetone) according to the equilibrium shown in Scheme 1. Thus, observation of **1** in



Scheme 1. Decomposition equilibrium of [Me₂C(OH)PEt₃]⁺ (**1**) in the presence of water.

D₂O indicates that encapsulation inside the tetrahedral cluster stabilizes this reactive species. However, because of the low concentration of acetone in the D₂O solution, this encapsulated species decomposes as it slowly comes out of the cluster cavity. Because of its instability in low acetone concentrations, it is likely that **1** is in fact formed inside the tetrahedral cavity. ¹H NMR evidence indicates that the “empty” [Ga₄L₆]^{12–} cluster actually contains acetone molecules as guests inside the tetrahedral cavity. We infer that protonated phosphine, HPEt₃⁺, diffused into the cluster cavity and reacted with an encapsulated acetone molecule to form **1**.

To confirm our identification of the encapsulated guest molecule, the phosphonium salt [Me₂C(OH)PEt₃][Br] was independently synthesized.^[20] When this phosphonium salt was added to solutions of Na₁₂[Ga₄L₆] in CD₃OD,^[21] ¹H NMR signals which corresponded to those observed previously upon addition of PEt₃ were seen in the ¹H NMR spectra.

[*] Prof. Dr. K. N. Raymond, Dr. M. Ziegler, Dr. J. L. Brumaghim
Department of Chemistry
University of California
Berkeley, CA 94720 (USA)
Fax: (+1) 510-486-5283
E-mail: raymond@socrates.berkeley.edu

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Observation of identical ^1H and ^{31}P NMR resonance signals with addition of either PET_3 or $[\text{Me}_2\text{C}(\text{OH})\text{PET}_3][\text{Br}]$ to a solution of the tetrahedral cluster is further evidence that the encapsulated species is in fact **1**.

ESI mass spectrometry has previously proven useful for characterizing multiply charged coordination compounds of this type in solution.^[22–24] In this case also, mass spectrometry provided conclusive proof that **1** was encapsulated as a guest. In the electrospray mass spectrum of $\text{Na}_{11}[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]$ in methanol (Figure 3a), signals for $\text{Na}_8[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{3-}$ and

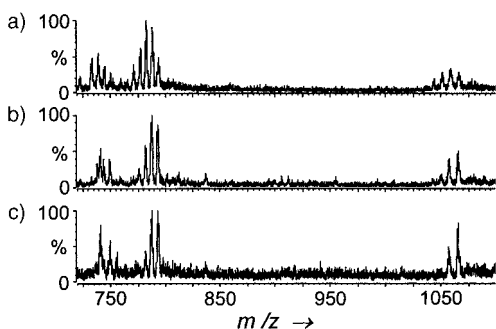


Figure 3. a) Electrospray mass spectrum of $\text{Na}_{11}[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]$; b) mass spectrum after addition of three equivalents of NaOH; c) mass spectrum after addition of six equivalents of NaOH.

$\text{Na}_7[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{4-}$ were observed at m/z 1066 and m/z 794, respectively. Weaker signals at m/z 751 correspond to the empty $\text{Na}_8[\text{Ga}_4\text{L}_6]^{4-}$ cluster. The exchange of Na^+ for H^+ ions accounts for the distribution of signals seen for all species observed in this spectrum. Addition of three and six equivalents of NaOH decreased the amount of protonated species (Figure 3b and 3c). High-resolution FT mass spectra for the $\text{Na}_8[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{3-}$ and $\text{Na}_7[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{4-}$ species have confirmed the chemical composition of the cluster (Figure 4).^[25]

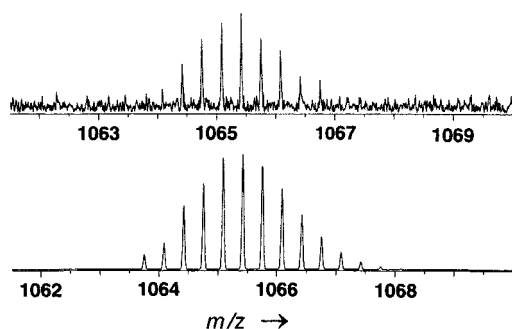


Figure 4. High-resolution mass spectrum of $\text{Na}_{11}[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{3-}$ (top) and calculated spectrum (bottom).

Incorporation of the cationic $[\text{Me}_2\text{C}(\text{OH})\text{PET}_3]^+$ species instead of the neutral PET_3 into the $\text{Na}_{12}[\text{Ga}_4\text{L}_6]$ cluster gives further proof that cationic guest molecules are preferentially encapsulated in this tetrahedral cluster as was previously observed.^[14] Most remarkable, however, is the stabilization of this phosphine–acetone adduct when encapsulated as a guest. Formation of this molecule inside the tetrahedral cluster would also account for the observation of this adduct in wet

methanolic and even aqueous solutions. This stabilization indicates that the guest cation **1** only slowly diffuses out of the host cavity in solution, remaining stable in D_2O for several hours, and in CD_3OD for at least one day.

In summary, the stabilization of the ion $[\text{Me}_2\text{C}(\text{OH})\text{PET}_3]^+$ by encapsulation in the tetrahedral cluster $\text{Na}_{12}[\text{Ga}_4\text{L}_6]$ is the first example of guest stabilization in a self-assembled supramolecular cluster. Similar to some reactions in micelles, stabilization of encapsulated guests in these nanoscale clusters could make it possible to perform reactions otherwise disfavored by dilute solution conditions.

Experimental Section

^1H and $^{31}\text{P}\{^1\text{H}\}$ NMR spectra were obtained on a DRX-500 spectrometer at 500 MHz and 122 MHz, respectively. Chemical shifts for ^1H and ^{31}P NMR spectra are reported in ppm (δ) relative to SiMe_4 and H_3PO_4 , respectively. Triethylphosphine was purchased from Aldrich and used as received. $\text{Na}_{12}[\text{Ga}_4\text{L}_6]$ ^[15] and $[\text{Me}_2\text{C}(\text{OH})\text{PET}_3][\text{Br}]$ ^[19] were synthesized by using published procedures.

Electrospray mass spectrometry was performed on a Quattro (Micromass) triple quadrupole mass spectrometer. High-resolution mass spectral data were acquired by using a Bruker Apex II 7 Tesla actively shielded FTICR mass spectrometer equipped with an Analytica electrospray source (off axis probe). Sample solutions were 300 pmol μL^{-1} in methanol with Na^+ as counterions. Calibration was performed with NaI standard solutions.

NMR studies of $\text{Na}_{12}[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]$: NMR spectra were obtained for a sample of $[\text{Na}_{12}[\text{Ga}_4\text{L}_6]]$ (33 mg, 11 μmol) in D_2O with added PET_3 (4.8 μL , 32.5 μmol). ^1H NMR (500 MHz, D_2O): δ = 8.20 (d, 12H; Ar_NH), 7.84 (d, 12H; Ar_NH), 7.41 (dd, 12H; Ar_CH), 7.12 (t, 12H; Ar_NH), 6.82 (dd, 12H; Ar_CH), 6.69 (t, 12H; Ar_CH), −1.12 (dt, 9H; PCH_2CH_3), −1.42 (d, 3H; CMe_2), overlapping signals at −1.53 (d, CMe_2) and −1.56 (m, PCH_2CH_3) with total integration of 9H.

Mass spectra of $\text{Na}_{11}[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]$: Mass spectrometry samples were obtained by removing an aliquot (160 μL) from the NMR samples and diluting the solution to 0.5 mL with methanol. MS (ESI): m/z : 1065.6 ($\text{Na}_8[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{3-}$), 1058.4 ($\text{HNa}_7[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{3-}$), 1050.7 ($\text{H}_2\text{Na}_6[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{3-}$), 1044.1 ($\text{H}_3\text{Na}_5[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{3-}$), 793.6 ($\text{Na}_7[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{4-}$), 788.1 ($\text{HNa}_6[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{4-}$), 782.9 ($\text{H}_2\text{Na}_5[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{4-}$), 777.3 ($\text{H}_3\text{Na}_4[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{4-}$), 771.5 ($\text{H}_4\text{Na}_3[\text{Ga}_4\text{L}_6 \supset \mathbf{1}]^{4-}$), 750.7 ($\text{Na}_8[\text{Ga}_4\text{L}_6]^{4-}$), 745.3 ($\text{HNa}_7[\text{Ga}_4\text{L}_6]^{4-}$), 739.7 ($\text{H}_2\text{Na}_6[\text{Ga}_4\text{L}_6]^{4-}$), 733.2 ($\text{H}_3\text{Na}_5[\text{Ga}_4\text{L}_6]^{4-}$).

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Nanosized $\text{Pd}_{145}(\text{CO})_x(\text{PET}_3)_{30}$ Containing a Capped Three-Shell 145-Atom Metal-Core Geometry of Pseudo Icosahedral Symmetry**

Nguyet T. Tran, Douglas R. Powell, and Lawrence F. Dahl*

Dedicated to Professor Dr. Heinrich Vahrenkamp on the occasion of his 60th birthday

Presented herein are the geometrical features of a stoichiometric capped three-shell palladium carbonyl phosphane cluster **1**. Its pseudo I_h metal core (see Figure 1) consists of a central Pd atom surrounded by 12 Pd atoms (shell 1), which in turn are encapsulated by 42 Pd atoms (shell 2), which in turn are encapsulated by 60 Pd atoms (shell 3). Thirty additional Pd atoms cap the 30 square polygons of the third-shell polyhedron with a triethylphosphane ligand attached to each capping Pd atom. It is currently presumed that 60 CO groups (crystal-disordered) edge-bridge the 30 capping Pd atoms with one-half of the square basal Pd atoms (in accordance with an IR carbonyl spectrum). The diameter of the entire Pd_{145} core of **1** between two centrosymmetrically opposite capping Pd atoms is 1.65 nm; the spherical-like geometry of

the centered three-shell Pd_{115} kernel (that is, without the 30 capping Pd atoms) is evidenced by the similarity of the corresponding distances for the centrosymmetrically opposite pentagonal faces (1.22 nm), square faces (1.27 nm), and triangular faces (1.27 nm) of the third shell.

This remarkable Pd_{145} nanocluster, whose metal-core geometry was unambiguously characterized from complete crystallographic analyses of X-ray data for two crystals (from different preparations, crystals A and B),^[1, 2] was isolated from the reduction of a monomeric square-planar palladium precursor, $[\text{Pd}(\text{PET}_3)_2\text{Cl}_2]$.^[3, 4] The geometrical conformity of the 55 interior metal atoms within the third shell of **1** to the well-known two-shell icosahedral Mackay hard-sphere model^[5] had not been previously observed in single-crystal X-ray crystallographic determinations.^[6, 7] The third-shell polyhedron in this unprecedented close-packed multi-shell metal carbonyl cluster possesses 60 equivalent vertices along with 12 pentagonal, 20 equilateral triangular, and 30 square faces;^[8] this semi-regular (Archimedean) polyhedron, named rhombicosidodecahedron (with Schläfli symbol 3.4.5.4),^[9] is a heretofore crystallographically unknown stereoisomer of the universally familiar C_{60} buckyball, an icosahedrally truncated semi-regular polyhedron of I_h symmetry with 60 equivalent vertices and 12 pentagonal and 20 hexagonal faces.

Because the formation of **1** is extremely sensitive to reaction boundary conditions, extensive systematic preparative efforts have been made during the last 24 months to provide a reproducible but (as yet) low-yield synthesis. The Goddess Fortuna recently revisited our laboratory in that crystals of **1** were again isolated and its crystal structure redetermined. The fact that **1** has 55 interior Pd atoms makes it an ideal model for experimental operational tests of previous interpretations of size-dependent physical properties (attributed to quantum-size effects) reported^[10] for several giant noncrystalline, (N,O)-ligated, full-shell palladium clusters (ideally formulated from other measurements as five-shell Pd_{561} species by the Moiseev and Schmid groups^[11, 12] and as seven-/eight-shell $\text{Pd}_{1415}/\text{Pd}_{2057}$ species by Schmid et al.^[13]) and for larger Pd (colloid) particles.

That the 115-atom 3-shell core of **1** (i.e., minus the 30 capping atoms) was actually proposed by Farges et al.^[14] as a possible full-shell structural model for noble-gas clusters produced in free-jet expansion into a vacuum is somewhat astonishing (i.e., mass spectra of charged noble-gas clusters provide no subshell magic-number evidence for the 115-atom cluster being particularly abundant because of special stability^[14, 15]). Their model represents a geometrical modification of the classic Mackay hard-sphere multilayer icosahedral (MIC) models based upon a 13-atom centered icosahedron being considered as 20 identical (slightly distorted) *fcc* tetrahedra that share a common vertex and are connected to one another through adjacent shared faces.

Formation of the Mackay 55-atom 2-shell MIC model arises from each of the 20 tetrahedra adding a 6-atom v_2 triangle to its 3-atom v_1 triangular surface (111) face. The resulting second complete MIC shell ($n=2$) consists of 42 atoms, that may originate from the addition of 30 atoms to the midpoints of the 30 edges of a 12-atom icosahedron in the second shell. In general, the number of atoms in a complete n^{th} shell is given

[*] Prof. L. F. Dahl, Dr. N. T. Tran, Dr. D. R. Powell
Department of Chemistry
University of Wisconsin-Madison
1101 University Avenue, Madison, WI 53706 (USA)
Fax: (+1) 608-262-6143
E-mail: dahl@chem.wisc.edu

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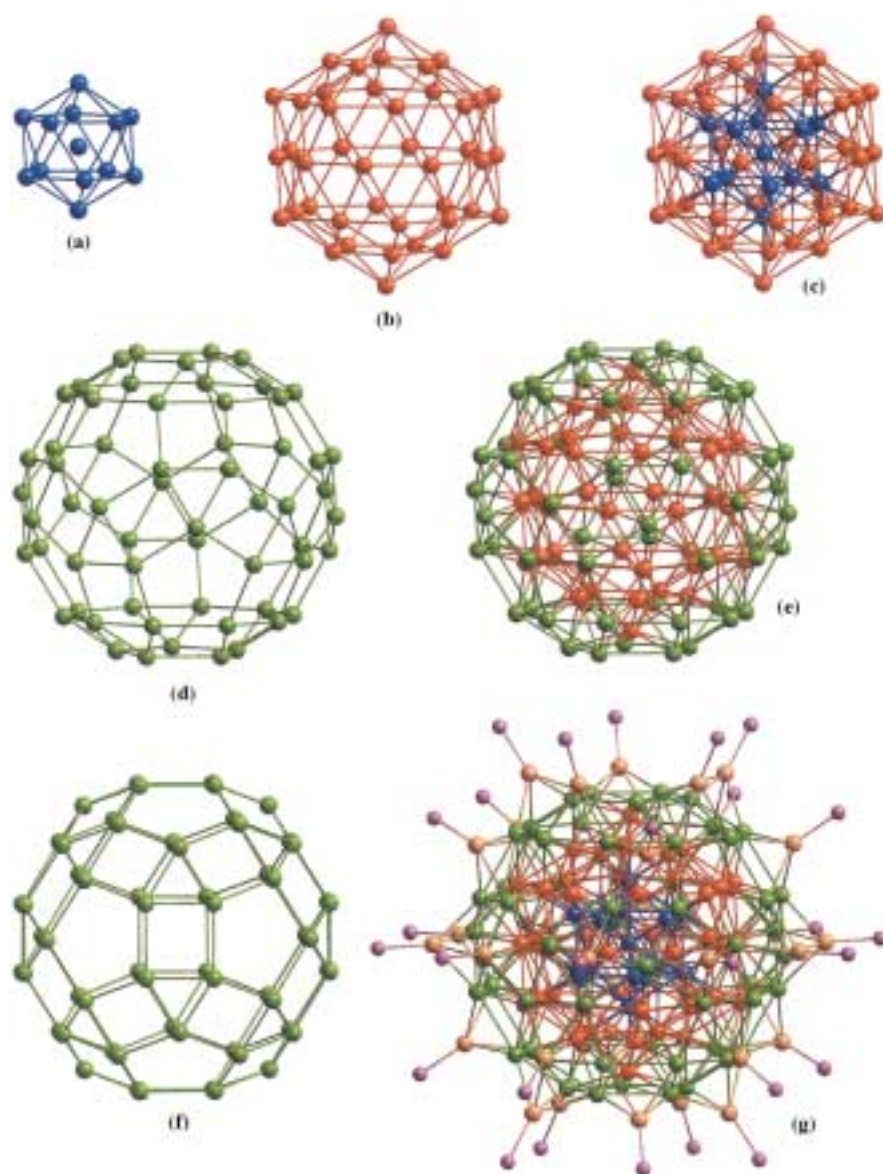


Figure 1. Formal concentric construction of the capped three-shell nanosized Pd_{145} core of pseudo I_h symmetry in $\text{Pd}_{145}(\text{CO})_x(\text{PET}_3)_{30}$: a) 12-atom ν_1 icosahedron surrounding the central atom (ν_n denotes $(n+1)$ equally spaced atoms along each edge). This centrosymmetrically centered icosahedron, one of the five regular Platonic solids (with equivalent vertices and identical faces composed of only one polygon), comprises: (1) six five-fold axes through opposite pairs of 12 vertices; (2) 10 three-fold axes through midpoints of opposite pairs of 20 triangular faces; and (3) 15 two-fold axes through midpoints of opposite pairs of 30 edges. b) 42-atom ν_2 icosahedron (shell 2) generated by the addition of an atom to the midpoint of each of the 30 edges of the 12-atom ν_1 icosahedron. c) Shell 2 encapsulating the Pd-centered shell 1 to give a 55-atom Mackay icosahedron. d) 60-atom polyhedron (shell 3), mathematically named a rhombicosidodecahedron (3.4.5.4). The numbers N (in parentheses) designate the Schläfli symbol which represents in cyclic order the polygons (N -gons) meeting at each equivalent vertex. e) Shell 3 encapsulating shell 2. f) Another orientation of the outermost third-shell polyhedron that more clearly shows the 60 equivalent vertices along with the 62 faces (namely, the 12 pentagons, 20 equilateral triangles, and 30 squares). This Archimedean polyhedron, a semi-regular one in that all 60 vertices are identical but there are three different polygons, has pseudo I_h symmetry. g) Entire 3-shell Pd_{145} core including 30 exopolyhedral Pd atoms (with attached triethylphosphane P atoms (purple)) that form square pyramids by capping of the 30 square polygons of the third shell.

by $(10n^2+2)$; the total number of atoms (N) in a full n -shell MIC model corresponds to the well-known magic-number sequence $N=13$ ($n=1$), 55 ($n=2$), 147 ($n=3$), 309 ($n=4$), 561 ($n=5$), etc.^[5a, 15a] Noteworthy is that this same sequence of magic numbers is obtained for full-shell *ccp* (i.e., *fcc*) clusters. In fact, this full-shell sequence is responsible for the idealized

formulations of the renowned $\text{Au}_{55}(\text{PPh}_3)_{12}\text{Cl}_6$ ($n=2$) from Schmid et al.^[16] and the above-mentioned ligand-stabilized Pd_{561} ($n=5$),^[11, 12] Pd_{1415} ($n=7$),^[13] and Pd_{2057} ($n=8$)^[13] clusters.

In the formal cluster-growth sequences of the third MIC layer (i.e., shell 3), Farges et al.^[14] recognized that the addition of atoms to the 55-atom Mackay icosahedron can occur in two ways and thereby can give rise to two types of surface arrangements: one cluster-growth pattern involves the addition of atoms to the edge-sites and vertices of the 6-atom ν_2 triangular surface (111) face of a tetrahedron such that *fcc* (*abca*) layer-packing is maintained; complete surface coverage of each of the 20 tetrahedra by a 10-atom ν_3 triangular layer involves the addition of 92 atoms ($n=3$) to give the 147-atom 3-shell Mackay icosahedron. The other cluster-growth pattern arises from the addition of atoms to the three internal *hcp* sites of the 6-atom ν_2 triangular surface (111) face of a tetrahedron in the second shell (Figure 2). The complete third-shell layer

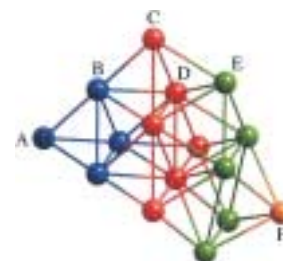


Figure 2. Pd_{16} fragment of the Pd_{145} core displaying the capped three-shell atomic arrangement with atom-labeling; see text and refs [4],[24] for further details.

then forms the above-described 60-vertex rhombicosidodecahedron (i.e., 3 atoms per face $\times$ 20 faces) and thereby produces the 115-atom 3-shell cluster (without the capping atoms) observed in **1**. Farges et al.^[14] denoted the first pattern as a *regular* MIC arrangement and the latter pattern as a *twin* arrangement. Subsequently, the *regular* atom-site surface arrangement for shell 3 was also designated as edge-capping/vertex (ECV3)^[15b,c] or simply the Mackay overlay,^[17] while the *twin* arrangement was designated as face-capping/vertex (FCV3)^[15b,c, 18] or the anti-Mackay layer.^[17]

Of prime interest is that in the formal construction of the third icosahedral layer, Farges et al.^[14a] stated that experimental electron diffraction patterns of the noncrystalline structures of argon clusters indicate a transformation from a *twin* to a *regular* surface MIC arrangement. They concluded from a comparison of the experimental and calculated diffraction functions for $N=116$ (incomplete third layer), $N=147$ (three complete layers), and $N=178$ (incomplete fourth layer) that each of these clusters has a *regular* MIC structure. The matching of experimentally observed shell and subshell magic numbers^[14, 15] with structural models obtained by pairwise additive potential calculations^[14, 15, 17] for noble-gas clusters favors the existence of icosahedral geometries for particular N -atom sizes with cluster-growth occurring by anti-Mackay overlayers for a relatively empty third shell but by *regular* Mackay overlayers for a nearly full third shell.^[19–21]

Riley, Parks, and co-workers^[22] have pioneered the development of a chemical probe method to determine by mass spectrometry how the number and nature of particular surface sites of photoionized nickel clusters vary with cluster size and temperature. Based upon saturation-coverage binding patterns of different adsorbates (e.g., N_2 , H_2 , CO , NH_3 , H_2O) under equilibrium conditions, they suggested from pronounced chemical-reactivity magic numbers that “naked” as well as ligated gas-phase nickel clusters in the size-range from 10 to 28 atoms and from 49 to approximately 200 atoms form predominantly icosahedral-type geometries.^[22]

Recent, more sophisticated theoretical calculations^[23] of equilibrium geometries of Ni_N clusters ($N \leq 55$)^[23a–c] and Pd_N clusters ($N \leq 23$)^[23a] instead produced highly diversified lowest-energy geometries which are notably dissimilar to the previously proposed icosahedral structures except for those containing a stable icosahedral core (i.e., corresponding to $N=13, 19, 23, 51–55$). These calculations (which are based upon density functional theory (DFT) concepts) also predicted the existence of geometrically different isomers with analogous binding energies for a considerable number of size-selected clusters along with the occurrence of multiple isomeric transformations. Geometric changes were linked to the underlying electronic structures of transition metal clusters.^[23c] DePristo and co-workers^[23a,b] proposed that the major driving force for the creation of the most stable structures for Ni_N and Pd_N clusters involves a maximization of the minimum coordination for any surface atom (giving rise to more uniform surface coordination numbers). Proposed consequences^[23a,b] are nearly spherical cluster shapes, a large range of nearest-neighbor interatomic distances, high coordination numbers, and large electron densities about the core atom(s) of the clusters. In contrast, equilibrium geometries of noble-gas clusters, which are stabilized by weak dispersion forces, were proposed to involve maximization of the number of nearest-neighbor interatomic contacts (that correspond to similar distances for close-packed geometries).^[14, 15, 17]

Ligation effects arising from the capping of the 30 square polygons in the third-shell polyhedron of **1** by 30 $Pd(PET_3)$ fragments along with doubly bridging CO units linking the capping Pd atoms to two of the four square-basal Pd atoms no doubt play a dominant role in the energetically preferred

formation of the 60-atom semi-regular icosahedral polyhedron of shell 3.^[14] The layer-by-layer growth pattern of the ligated capped 3-shell Pd_{145} core of pseudo- I_h symmetry presumably involves an energetic compromise between maximization of atom-connectivities (resulting in the lowest surface Pd coordination number being seven^[24]) and the minimization of icosahedral strain energy.

Table 1 presents distances averaged under I_h symmetry based upon the atom-labeling palladium fragment given in Figure 2. This table not only reveals that the corresponding average (means) for the 11 different types of Pd–Pd distances for crystals A and B agree within 0.01 Å but also that the dispersion of individual Pd–Pd separations for each of the means for crystal A is unusually small. Only those for crystal A are utilized in this discussion.

Table 1. The mean metal–metal and metal–phosphorus connectivities under pseudo- I_h icosahedral symmetry in $Pd_{145}(CO)_x(PEt_3)_{30}$ for crystals A and B^[a] and corresponding individual ranges for crystal A.

Connectivity ^[b]	$N^{[c]}$	Mean [Å] ^[a]	Range [Å]
Pd(A)–Pd(B)	12	2.63 (2.63)	2.629(3)–2.633(3)
Pd(B)–Pd(B)	30	2.77 (2.77)	2.757(4)–2.778(5)
Pd(B)–Pd(C)	12	2.74 (2.73)	2.734(4)–2.737(4)
Pd(B)–Pd(D)	60	2.70 (2.70)	2.696(4)–2.709(4)
Pd(C)–Pd(D)	60	2.82 (2.82)	2.811(6)–2.836(6)
Pd(D)–Pd(D)	60	2.82 (2.82)	2.810(5)–2.825(6)
Pd(C)–Pd(E)	60	2.73 (2.73)	2.714(7)–2.749(7)
Pd(D)–Pd(E)	120	2.74 (2.73)	2.724(6)–2.756(6)
Pd(E)–Pd(E)			
(triangle/square shared edge)	60	2.82 (2.82)	2.795(8)–2.844(9)
Pd(E)–Pd(E)			
(pentagon/square shared edge)	60	3.09 (3.09)	3.070(8)–3.112(9)
Pd(E)–Pd(F)	120	2.84 (2.84)	2.809(9)–2.874(9)
Pd(F)–P	30	2.32 (2.30)	2.181(13)–2.363(15)

[a] The means for crystal B are given in parentheses. [b] Atom-labeling is given in Figure 2. [c] N denotes the number of symmetry-equivalent connectivities under I_h symmetry.

For each of the identically deformed 20 tetrahedra within the inner 13-atom centered icosahedral shell of **1**, the experimentally determined uniform contraction of the three radial edges relative to the three tangential edges by 5.0% (i.e., $[(2.77–2.63 \text{ Å})/2.77 \text{ Å}] \times 100$) is in complete agreement with the theoretically predicted value of approximately 5%.^[5a, 12] This radial compression is also evidenced on going from shell 1 to shell 2; the relative growth increment along the radial (intershell) direction increases by 4.2% (i.e., $[(2.74–2.63 \text{ Å})/2.63 \text{ Å}] \times 100$), while that along the tangential (intra-shell) direction increases by only 1.8% (i.e., $[(2.82–2.77 \text{ Å})/2.77 \text{ Å}] \times 100$). For shell 3 both the intershell Pd(C)–Pd(E) and Pd(D)–Pd(E) means are virtually identical with that of the radial Pd(B)–Pd(C) mean between the first and second shell (2.74 Å). That the tangential (intra-shell) Pd(E)–Pd(E) mean of 2.82 Å for the shared edge between each triangle and square polygon is 0.27 Å shorter than that of 3.09 Å for the shared edge between each pentagon and square polygon reflects considerably larger bonding interactions presumably arising from sterically imposed strain effects. Noteworthy is that the Pd–Pd distance found in *ccp* Pd metal is 2.75 Å.^[25]

Current/planned collaborations include detailed physical characterization of **1** involving mass spectrometric, electro-

chemical, TEM, EXAFS, PES, and variable-temperature magnetic susceptibility measurements. A preliminary investigation has already indicated the existence of PMe_3 and PPh_3 analogues.

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- [1] Crystal structure analysis: Intensity data were collected at 133(2) K for two black prism-shaped crystals on a Bruker SMART CCD area detector mounted on a Bruker P4 goniometer with $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). The crystal structures were solved by direct methods and refined by full-matrix anisotropic least-squares methods (based on F^2). $\text{Pd}_{145}(\text{CO})_x(\text{PET}_3)_{30}$ (with $x = 60$): $M = 20653.1$, cubic, $\text{Pa}\bar{3}$, $Z = 4$; $F(000) = 37960$. Crystal A: $a = b = c = 35.1022(4) \text{ \AA}$, $\alpha = \beta = \gamma = 90^\circ$, $V = 43251.7(9) \text{ \AA}^3$, $\rho_{\text{calc}} = 3.172 \text{ Mg m}^{-3}$. 34 693 reflections were obtained by $0.4^\circ \varphi$ oscillation frames (90 s/frame) over $10.46^\circ \leq 2\theta \leq 44.98^\circ$. An empirical absorption correction (SADABS) was applied ($\mu(\text{MoK}\alpha) = 6.050 \text{ mm}^{-1}$; max./min. transmission, 0.352/0.191). Refinement (479 parameters; 1072 restraints) on 9300 independent merged reflections ($R_{\text{int}} = 0.080$) converged at $wR_2(F^2) = 0.336$ for all data; $R_1(F) = 0.093$ for 4308 observed data ($I > 2\sigma(I)$); GOF (on F^2) = 1.049; max./min. residual electron density, $1.84/-1.36 \text{ e \AA}^{-3}$. Crystal B: $a = b = c = 35.019(3) \text{ \AA}$, $\alpha = \beta = \gamma = 90^\circ$, $V = 42945(6) \text{ \AA}^3$, $\rho_{\text{calc}} = 3.194 \text{ Mg m}^{-3}$. 96 721 reflections were obtained by $0.2^\circ \varphi$ oscillation frames (120 s/frame) over $2.60^\circ \leq 2\theta \leq 45.00^\circ$. An empirical absorption correction (SADABS) was applied ($\mu(\text{MoK}\alpha) = 6.03 \text{ mm}^{-1}$; max./min. transmission, 0.289/0.263). Refinement (479 parameters; 1072 restraints) on 8733 independent merged reflections ($R_{\text{int}} = 0.143$) converged at $wR_2(F^2) = 0.409$ for all data; $R_1(F) = 0.098$ for 2875 observed data ($I > 2\sigma(I)$); GOF (on F^2) = 1.134; max./min. residual electron density, $1.49/-1.22 \text{ e \AA}^{-3}$. Geometric restraints were placed upon the positional parameters of all light atoms. The ethyl carbon atoms on the phosphorus atoms were modeled as "crystal-ordered" in spite of the methyl carbon peaks being particularly weak on Fourier difference maps. The crystal-disordered CO groups were modeled as two composite doubly bridging CO groups in two orientations based upon structural determinations of other palladium carbonyl phosphane clusters^[2] exhibiting two doubly bridging CO groups per square-pyramidal capping Pd atom. Equal occupancies were assumed for *trans* CO groups on opposite sides and $x/(1-x)$ refined occupancies for each set of *cis* pairs that are 90° apart by rotations about the Pd-P axes; the refined occupancies of the sets of *cis*-related CO groups ranged from 0.50(4)/0.50(4) to 0.70(4)/0.30(4). Displacement parameters of the carbon and oxygen atoms were assigned as 1.2 and 1.5 times, respectively, the isotropic equivalent values of the associated non-hydrogen atoms. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-149270 and -149271. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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- [3] $[\text{Pd}(\text{PET}_3)_2\text{Cl}_2]$ (0.25 g; 0.60 mmol) and $[\text{Au}(\text{PPh}_3)\text{Cl}]$ (0.050 g; 0.10 mmol) were dissolved in DMF (10 mL) under N_2 . This solution was transferred under CO into DMF (20 mL) containing NaOH pellets and kept under CO atmosphere. After two days the reaction, that had turned slowly to dark brown with a black precipitate, was terminated by filtration into $[\text{PPh}_3]\text{Br}$ (1.0 g). Addition of H_2O to the ice-cooled solution produced a dark brown solid that was extracted successively with MeOH, MeCN, and THF. Cluster **1** was isolated from the MeOH extract in low yields. An IR spectrum (THF) exhibited one strong band at 1870.4 cm^{-1} characteristic of a bridging carbonyl. Compound **1** forms thermally stable crystals that are soluble in polar solvents. Two geometrically intriguing $\text{Au}_2\text{Pd}_{21}$ and $\text{Au}_2\text{Pd}_{41}$ carbonyl phosphane clusters were isolated as by-products; details of their structural determinations and spectroscopic characterizations will be presented elsewhere.

- [4] Because the central atom in both the $\text{Au}_2\text{Pd}_{41}$ and $\text{Au}_2\text{Pd}_{21}$ clusters is Au, the possibility that the central atom encapsulated by the 12 Pd atoms in shell 1 of **1** is also Au instead of Pd was carefully examined. However, this possibility can be ruled out conclusively from the X-ray crystallographic refinements of both crystals. The crystallographic analysis involves a comparison of the equivalent isotropic atomic displacement values obtained from anisotropic least-squares refinements of the independent atoms of the molecular cluster under crystallographic $\bar{3}$ site symmetry; the crystallographically asymmetric part of the Pd_{145} core (Figure 2) is comprised of the central Pd(A) atom located at the $\bar{3}$ (S_6) site, two independent Pd(B) atoms in shell 1, two independent Pd(C) and five independent Pd(D) atoms in shell 2, 10 independent Pd(E) atoms in shell 3, and five independent capping Pd(F) atoms. When the central atom is designated as Pd, the equivalent isotropic value of $0.057(2) \text{ \AA}$ determined for this atom from least-squares refinement of the CCD X-ray data for crystal A is in excellent agreement with those of $0.059(1)$ and $0.060(1) \text{ \AA}$ for the two independent Pd(B) atoms in shell 1; similarly, for crystal B the corresponding central Pd(A) value of $0.066(3) \text{ \AA}$ agrees closely with those of $0.063(2)$ and $0.064(2) \text{ \AA}$ for the two independent Pd(B) atoms in shell 1. However, when only the central atom was arbitrarily assigned as Au, its resulting equivalent isotropic value increased in a separate least-squares refinement to an unrealistic higher value of $0.128(2) \text{ \AA}$ versus those of $0.060(1)$ and $0.059(1) \text{ \AA}$ for the two independent Pd(B) atoms in shell 1; similarly, for crystal B the equivalent isotropic value of the central atom increased to a totally unreasonable value of $0.163(4) \text{ \AA}$ versus $0.063(2)$ and $0.063(2) \text{ \AA}$ for the two independent Pd(B) atoms.
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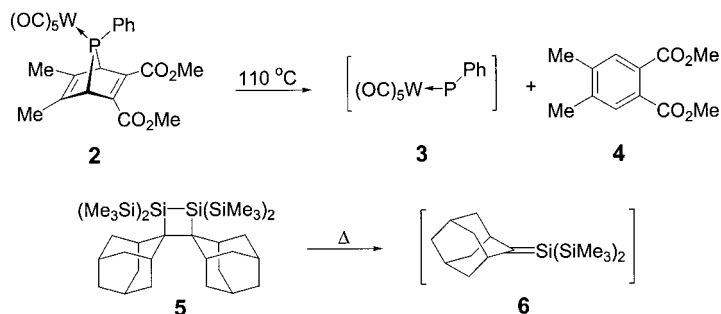

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A Novel CSiP Ring from the Reaction of a Complexed Phosphinidene and a Silene**

Mark J. M. Vlaar, Andreas W. Ehlers, Frans J. J. de Kanter, Marius Schakel, Anthony L. Spek, Martin Lutz, Nadejda Sigal, Yitzhak Apeloig, and Koop Lammertsma*

Cyclopropanes and their three-membered heterocyclic analogues have long been recognized as important chemical structures. Even three-membered rings containing the second-row elements phosphorus and silicon are now well established. Moreover, many phosphiranes and siliranes are already known containing a second heteroatom such as O,^[1] N,^[2] or S.^[3] However, no phosphasilirane has yet been isolated. So far, only ³¹P and ²⁹Si NMR data have been reported by Drieß and Pritzkow for a CPSi ring structure (**1**), which they could not isolate from the reaction mixture.^[4] Here we report on a novel synthesis leading to a transition metal complexed phosphasilirane, and present its spectroscopic characterization and single-crystal structure determination as well as the results of G3(MP2) ab initio calculations on the strain energy of the parent CPSiH₃ compound.

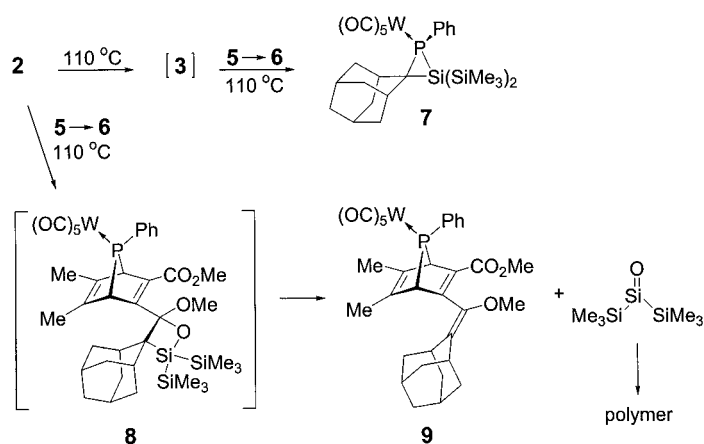
In situ generated terminal complexed phosphinidenes such as **3**^[5] are convenient singlet carbene-like building blocks^[6] for the synthesis of phosphirane complexes by addition to olefins.



Hence, the addition of this phosphinidene to a silene seemed to us an obvious path to a phosphasilirane complex. However, only a few stable silenes have been synthesized and the

number of methods that give transient silenes under the reaction conditions used to generate **3** is even more limited.^[7] A welcome exception is silene **6**,^[8] which is generated in situ by retrocycloaddition from its dimer **5**. Silene **6** has been applied successfully as a building block in several high-yield trapping reactions.^[8, 9] The thermal equilibrium between the silene and its dimer is most effective at around 100 °C, the temperature at which complexed phosphinidene **3** is generated. Therefore **6** appeared to us as a plausible candidate to explore the reactivity of silenes toward complexed phosphinidenes.

To maximize the concentration of the two reactive intermediates (phosphinidene complex **3** and silene **6**), a mixture of silene dimer **5** and a twofold excess (based on silene) of phosphinidene precursor **2** was heated in toluene at 110 °C. After 90 min the reaction was stopped, and the reaction mixture was purified by column chromatography at –15 °C to give after crystallization bright yellow crystals of phosphasilirane complex **7** and light yellow crystals of **9** in remarkably high yields of 25 and 30 %, respectively.



Phosphasilirane **7** is a remarkably stable compound with characteristic spectroscopic properties. Compared to the data for the reported phosphasilirane **1**, its ³¹P NMR resonance at $\delta = -116.9$ is deshielded by 80.5 ppm, its ²⁹Si NMR resonance at $\delta = -97.9$ is shielded by 43.4 ppm, and its ⁽¹⁺²⁾J(P,Si) coupling constant of 22.9 Hz is smaller by 26.8 Hz. These differences between **7** and **1** may be attributed to the presence of the W(CO)₅ and Si(CH₃)₃ substituents. The ¹J(P,W) coupling constant of 213.6 Hz for **7** is about 40 Hz smaller than is generally observed for W(CO)₅-complexed 1-phenylphosphiranes. This is consistent with its rather long P–W bond of 2.5509(9) Å (Figure 1).^[10] Likewise, the P1–C1 bond of **7** of 1.880(3) Å is slightly elongated and its endocyclic angle at P1 of 54.60(11)° is accordingly larger by 5°.^[11] The Si1–C1 bond length of 1.919(3) Å and the endocyclic bond angle at Si1 of 52.99(10)° are slightly larger than those typically observed in siliranes.^[12]

Silene **5** does not only react with the in situ generated phosphinidene complex **3** but also with its precursor **2** to give **9**. The ³¹P NMR resonance of **9** at $\delta = 184.4$ is characteristic for a 7-phosphanorbornadiene. Due to the absence of ²⁹Si NMR chemical shifts and the complexity of both the ¹H and

[*] Prof. Dr. K. Lammertsma, M. J. M. Vlaar, Dr. A. W. Ehlers, Dr. F. J. J. de Kanter, Dr. M. Schakel
Department of Organic and Inorganic Chemistry
Faculty of Sciences, Vrije Universiteit
De Boelelaan 1083, 1081 HV, Amsterdam (The Netherlands)
Fax: (+31)20-444-7488
E-mail: lammert@chem.vu.nl

Dr. A. L. Spek, Dr. M. Lutz
Bijvoet Center for Biomolecular Research
Crystal and Structural Chemistry, Utrecht University
Padualaan 8, 3584 CH, Utrecht (The Netherlands)

N. Sigal, Prof. Dr. Y. Apeloig
Department of Chemistry
Technion-Israel Institute of Technology, Haifa 32000 (Israel)

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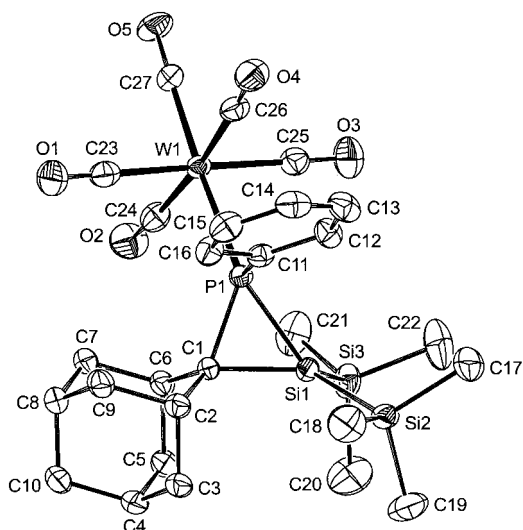


Figure 1. Structure of **7** in the crystal (displacement ellipsoids drawn at the 50% probability level; hydrogen atoms are omitted for clarity). Selected bond lengths [Å], angles, and torsion angles [°]: P1–W1 2.5510(9), P1–Si1 2.2436(13), P1–C1 1.880(3), Si1–C1 1.918(3), Si1–Si2 2.3699(14), Si1–Si3 2.3603(15), C1–C2 1.541(5), C1–C6 1.536(5); Si1–P1–C1 54.59(11), P1–Si1–C1 53.00(10), Si1–C1–P1 72.41(12), W1–P1–C11 110.17(11), C2–C1–C6 109.3(3), Si2–Si1–Si3 114.12(6); W1–P1–Si1–C1 –111.73(14), C11–P1–C1–Si1 –94.76(15).

^{13}C NMR spectra, we resorted to a single-crystal X-ray structure determination (Figure 2)^[10] to establish the structure of **9**. Its formation is readily rationalized by a [2+2] cycloaddition of the silene to an ester carbonyl group of **2**, giving the rather crowded compound **8**, which undergoes a [2+2] retroaddition to afford **9** and a silanone. The latter is not observed as it polymerizes instantaneously. While we did not observe intermediate **8** there is ample precedence for the formation of 1,2-silaoxetanes from silenes and non-enolizable ketones as well as for its dissociation into alkenes and silanones.^[13] It is relevant to note in this context that the [2+2] cycloaddition of an ester and a silene is also a first; silene

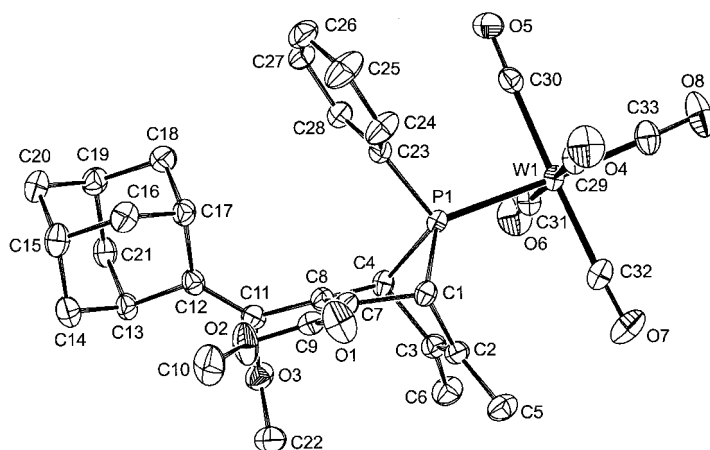


Figure 2. Structure of **9** in the crystal (displacement ellipsoids drawn at the 50% probability level; hydrogen atoms are omitted for clarity). Selected bond lengths [Å], and angles [°]: P1–W1 2.4846(6), P1–C1 1.866(2), P1–C4 1.876(2), P1–C23 1.823(2), C1–C2 1.546(3), C3–C4 1.541(3), C2–C3 1.336(3), C1–C7 1.523(3), C4–C8 1.529(3), C7–C8 1.356(3), C8–C11 1.479(3), C11–C12 1.334(3); C1–P1–C4 79.29(10), P1–C1–C2 97.00(15), P1–C4–C3 96.41(14), C1–P1–W1 123.15(8), C4–P1–W1 123.76(7), P1–C1–C7 101.72(15), P1–C4–C8 102.40(15).

additions to α,β -unsaturated esters have been reported to occur in a [4+2] fashion.^[14] The formation of **9** is all the more remarkable, since 7-phosphanorbornadiene complexes usually decompose quite rapidly at 110 °C (e.g. **2** $\rightarrow$ **3**) and the same would be expected for **9**. Because the crystal structures of **9** and the $\text{Cr}(\text{CO})_5$ complex of **2**^[15] show very similar bond lengths and angles for the 7-phosphanorbornadiene unit, we speculate that the higher thermal stability of **9** toward cheletropic elimination of $\text{PhPW}(\text{CO})_5$ is related to a reduced exothermicity for formation of the aromatic fragment.

We resorted to ab initio MO theory to obtain the strain energy for the parent phosphasilirane **10**, the MP2 geometry of which is given in Figure 3. Comparison with the X-ray crystal structure of **7** shows that the bond lengths within the CPSi ring of **7** are 0.02 Å (Si–P), 0.03 Å (C–P), and 0.06 Å (C–Si) longer than in the parent **10**, reflecting the effect of steric congestion around the ring. From the ΔH_f values calculated at G3(MP2)^[16] for **10** (30.5 kcal mol^{–1}) and the other molecules of the homodesmotic reaction shown in Equation (1), a strain

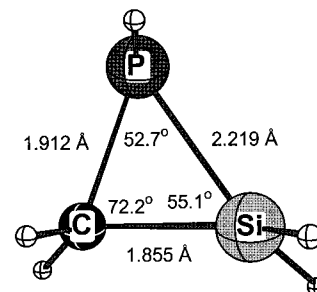
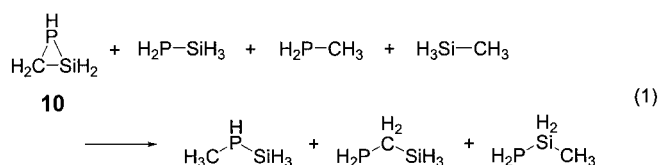


Figure 3. MP2-optimized structure of phosphasilirane PSiH_3 .



energy of 26.5 kcal mol^{–1} is deduced. This strain energy is similar in magnitude to that of cyclopropane and larger than that of phosphirane (22.2 kcal mol^{–1}),^[17] but substantially smaller than the value of 42.3 kcal mol^{–1} for silirane (all G3(MP2)).^[18] This reduction in strain energy of 16.9 kcal mol^{–1} from the large value for silirane has been related to the preference of Si for angles near 90°.^[18a] The elongated bond lengths of phosphasilirane (CPSiH_3) as compared to silirane (C_2SiH_6) enlarge the endocyclic angle at Si from 49.0 to 52.7.

Experimental Section

The experiment was performed under an atmosphere of dry nitrogen. NMR spectra were recorded on Bruker WM 250 (^{31}P) and MSL 400 (^1H , ^{13}C , ^{29}Si) spectrometers by using SiMe_4 (^1H , ^{13}C , ^{29}Si) and 85% H_3PO_4 (^{31}P) as external standards. High-resolution mass spectra (HRMS) were recorded on a Jeol HX-1010 and IR spectra on a Mattson 630 Galaxy spectrophotometer.

7 and **9**: Silene dimer **5** (0.17 g, 0.28 mmol) and complex **2** (0.73 g, 1.1 mmol) were heated in refluxing toluene (10 mL) for 90 min. Workup by solvent evaporation, chromatography (–15 °C, activated silica (pentane/ CH_2Cl_2 (3/1); CH_2Cl_2)), and crystallization (hexane/ CH_2Cl_2) yielded yellow crystals of **7** (0.10 g, 25%, bright) and **9** (0.12 g, 30%, pale).

7: m.p. 159–161 °C; ^{29}Si NMR (C_6D_6): $\delta = -9.0$ (d, $^2J(\text{Si},\text{P}) = 4.7$ Hz; $\text{Si}(\text{CH}_3)_3$), -10.3 (d, $^2J(\text{Si},\text{P}) = 4.1$ Hz; $\text{Si}(\text{CH}_3)_3$), -97.9 (d, $^{1+2}J(\text{Si},\text{P}) = 22.9$ Hz; PSi); ^{31}P NMR (C_6D_6): $\delta = -116.9$ ($^1J(\text{P},\text{W}) = 213.6$ Hz); ^{13}C NMR (C_6D_6): $\delta = 199.6$ (d, $^2J(\text{C},\text{P}) = 25.5$ Hz; *trans*-CO), 198.5 (d, $^2J(\text{C},\text{P}) = 6.8$ Hz, $^1J(\text{C},\text{W}) = 126.4$ Hz; *cis*-CO), 138.8 (d, $^1J(\text{C},\text{P}) = 15.2$ Hz; *ipso*-Ph), 129.4 (d, $^4J(\text{C},\text{P}) = 1.9$ Hz; *p*-Ph), 128.8 (d, $^2J(\text{C},\text{P}) = 8.6$ Hz; *o*-Ph), 128.5 (*m*-Ph), 43.7 (d, $^1J(\text{C},\text{P}) = 34.6$ Hz; PCSi), 41.9 (d, $^1J(\text{C},\text{P}) = 6.4$ Hz; adamantyl(Ad)-CH₂), 41.3 (d, $^1J(\text{C},\text{P}) = 13.8$ Hz; Ad-CH₂), 38.8 (s; Ad-CH₂), 38.4 (s; Ad-CH₂), 36.8 (d, $^1J(\text{C},\text{P}) = 4.1$ Hz; Ad-CH), 36.3 (d, $^1J(\text{C},\text{P}) = 2.4$ Hz; Ad-CH₂), 33.7 (d, $^1J(\text{C},\text{P}) = 6.2$ Hz; Ad-CH), 28.6 (s; Ad-CH), 28.1 (s; Ad-CH), 2.3 (d, $^3J(\text{C},\text{P}) = 1.6$ Hz; CH₃), 1.7 (s; CH₃); ^1H NMR (C_6D_6): $\delta = 7.6$ – 7.7 (m, 2H; Ph), 7.0 – 7.1 (m, 3H; Ph), 1.8 – 2.9 (m, 14H; Ad), 0.48 (s, 9H, CH₃), 0.00 (s, 9H, CH₃); HRMS: calcd. for $\text{C}_{27}\text{H}_{37}\text{Si}_3\text{PO}_3\text{W}$: m/z 740.11963; found: 740.1212; IR (CH_2Cl_2): $\tilde{\nu} = 1933$ (s), 2066 (w) ($\nu(\text{CO})$) cm^{-1} .

9: m.p. 167–168 °C; ^{31}P NMR (CDCl_3): $\delta = 184.4$ ($^1J(\text{P},\text{W}) = 235.1$ Hz); ^{13}C NMR (CDCl_3): $\delta = 199.4$ (d, $^2J(\text{C},\text{P}) = 25.0$ Hz; *trans*-CO), 196.7 (d, $^2J(\text{C},\text{P}) = 6.7$ Hz, $^1J(\text{C},\text{W}) = 125.4$ Hz; *cis*-CO), 165.5 (d, $^3J(\text{C},\text{P}) = 3.0$ Hz; C=O), 153.7 (d, $^3J(\text{C},\text{P}) = 4.9$ Hz; O=C=C), 142.5 (d, $^2J(\text{C},\text{P}) = 12.5$ Hz; C=C–C=O), 139.3 (d, $^1J(\text{C},\text{P}) = 17.6$ Hz; *ipso*-Ph), 138.7 (d, $^2J(\text{C},\text{P}) = 11.6$ Hz; C=CCH₃), 138.5 (s; C=C–CH₃), 136.8 (d, $^2J(\text{C},\text{P}) = 2.7$ Hz; C=C–C=O), 136.3 (s; O=C=C), 128.8 – 129.6 (m; Ph), 64.3 (d, $^1J(\text{C},\text{P}) = 19.0$ Hz; PCH), 58.7 (s; C=C–OCH₃), 57.0 (d, $^1J(\text{C},\text{P}) = 23.1$ Hz; PCH), 51.9 (s, O=C–O–CH₃), 39.3 (s, Ad-CH₂), 39.2 (s, Ad-CH₂), 38.6 (s, Ad-CH₂), 37.3 (s, Ad-CH₂), 32.7 (s, Ad-CH), 30.3 (s, Ad-CH), 28.6 (s, Ad-CH), 28.5 (s, Ad-CH), 16.4 (d, $^3J(\text{C},\text{P}) = 2.2$ Hz; CH₃), 16.2 (d, $^3J(\text{C},\text{P}) = 2.1$ Hz; CH₃); ^1H NMR (CDCl_3): $\delta = 7.2$ – 7.4 (m, 5H; Ph), 4.2 (d, 1H, $^4J(\text{H},\text{H}) \approx 3.0$ Hz; PCH), 3.7 (pseudo-t, 1H, $^4J(\text{H},\text{H}) \approx ^2J(\text{H},\text{P}) \approx 3.0$ Hz; PCH), 3.7 (s, 3H; H₃CO–C=O), 3.0 (m, 1H; CH–Ad), 2.8 (s, 3H; H₃C–O–C=O), 2.1 (s, 3H; C=C–CH₃), 2.0 (s, 3H; C=C–CH₃), 1.3 – 1.9 (m, 13H; Ad); HRMS: calcd for $\text{C}_{27}\text{H}_{37}\text{Si}_3\text{PO}_3\text{W}$ (%): 772.14227; found: 772.1379; IR (CH_2Cl_2): $\tilde{\nu} = 1939$ (s), 2070 (w) ($\nu(\text{CO})$) cm^{-1} .

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Virtual Screening for Bioactive Molecules by Evolutionary De Novo Design**

Gisbert Schneider,* Odile Clément-Chomienne, Laurence Hilfiger, Petra Schneider, Stefan Kirsch, Hans-Joachim Böhm, and Werner Neidhart

One of the goals of computational chemistry is to be able to design novel molecular structures with biological activity comparable to a defined known drug (the “template”) and with a significantly different architecture. There is a need to develop techniques that can be applied if a high-resolution receptor model is unavailable, complementing more conventional structure-based molecular modeling and design. Such algorithms would be particularly helpful for combinatorial library shaping and fast-follower strategies in drug development. Two major problems must be solved to successfully address this task. First, the tool must be able to systematically navigate and efficiently search in a vast chemical space containing billions of virtual compounds, as it is impossible to exhaustively screen all possible molecules for a desired biological activity. Second, the conceptual view of a molecule must be defined in such a way that allows rapid comparison of the template with novel compounds, yielding a measure of functional rather than structural similarity.

For the sake of “backbone (scaffold)-switching” the various pharmacophore concepts provide a straightforward approach.^[1] Adaptive stochastic searching, notably evolutionary algorithms, represents a practicable solution to optimization in a high-dimensional search space. This is especially true when no or only little information is available about the ruggedness of the associated “fitness landscape”.^[2] Here we present an efficient computational molecular design strategy that implements pharmacophore-guided evolutionary search-

ing in chemical space. Experimental proof of the concepts is demonstrated by the successful de novo design of a new structural class of potent K⁺-channel inhibitors. The algorithm is referred to as TOPAS (TOPOlogy-Assigning System) in this report.^[3] TOPAS provides a solution to template-based de novo design, in which novel molecules are assembled taking a given bioactive compound as the reference point (template structure).

A challenging task in de novo design is the generation of molecular structures with “druglike” properties. To reduce the risk of generating molecular architectures with inadequate features, or which are difficult to synthesize—a problem encountered by many de novo design procedures^[4]—TOPAS was equipped with a stock of building blocks obtained from retrosynthetic fragmentation of compounds listed in the Derwent World Drug Index (WDI).^[5] The idea is that assembly of such building blocks by a limited set of chemical reactions might lead to chemically feasible novel structures, from both the medicinal chemistry and the synthesis planning perspective. All structures contained in the WDI, which had an entry related to “mechanism” or “activity”, were subjected to retrosynthetic fragmentation. This approach is similar to the RECAP procedure developed by Hann and co-workers.^[6] Eleven reaction schemes were applied to perform fragmentation (for details, see the original publication).^[6] We used the Daylight tool kit for implementation of reaction schemes.^[7] A total of 24 563 unique building blocks was obtained from our analysis.^[3] The same eleven reactions were used by TOPAS to assemble novel molecules.

TOPAS is based on a special evolutionary algorithm, a (1, λ) evolution strategy.^[2, 8] This type of evolutionary algorithm has been successfully applied to peptide design experiments in previous applications.^[9] The common feature of evolutionary algorithms is a cyclic variation-selection process. “Parents” breed “offspring”, and the fittest of each “generation” becomes the parent of the subsequent optimization cycle. Starting from an arbitrary point in search space (a randomly selected chemical structure), TOPAS generates a set of λ variant structures which satisfy a bell-shaped distribution centered in the chemical space coordinates of the parent structure. This means that most of the variants will be very similar to their parent, and the number of offspring at a certain distance point will be less as the distance-to-parent increases.^[3] Distance in chemical space is defined by a topological pharmacophore measure (see below).

Evolution strategies are often applied to real-valued function optimization problems.^[2] Algorithms generally operate directly on the parameters to be optimized, in contrast to genetic algorithms, which usually operate on a separately coded transformation of the objective variables (the so-called “chromosome”).^[10] In addition, evolution strategies include a second-level optimization of “strategy parameters”, that is, tunable variables that in part determine how each parent will generate offspring. This enables an adaptive stochastic search to be performed and guarantees a finite probability of breeding very dissimilar structures (“snoopers” in chemical space).^[9] The width of the variant distribution is determined by the variance or standard deviation, σ , of the bell-shaped curve reflecting the distance-to-parent probability. The σ

[*] Priv.-Doz. Dr. G. Schneider, O. Clément-Chomienne, L. Hilfiger, P. Schneider, S. Kirsch, H.-J. Böhm, W. Neidhart
Hoffmann-La Roche Ltd.
Pharmaceuticals Division
4070 Basel (Switzerland)
Fax: (+41) 61-68-8904
E-mail: gisbert.schneider@Roche.com

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value may be regarded as a measure of library diversity: Small values lead to narrow distributions of variants, while large values often result in novel structures that are very dissimilar to the parent molecule.

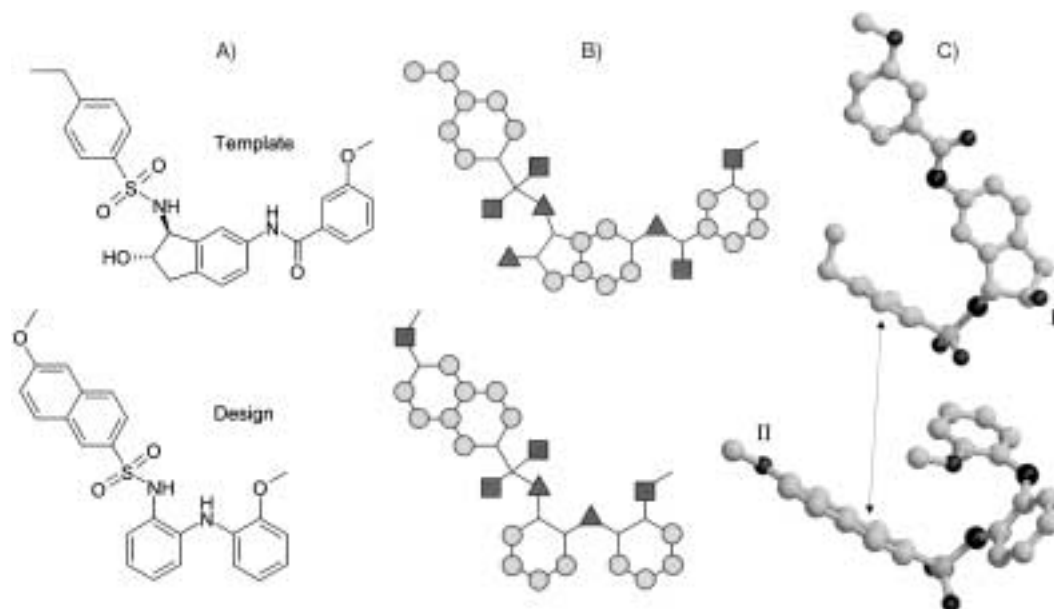
At the start of a virtual screening experiment, σ should be set to a large value to explore chemical space for potential optima ("universal" library). At later stages σ will have to have small values to facilitate local hill-climbing (focused or biased libraries). In contrast to related techniques, there exists no predefined "cooling schedule" or a fixed decaying function for σ . Its value may freely change during optimization to be able to adapt to the actual requirements of the search.^[2] Large σ values will automatically evolve if large steps are favorable, and small values will result when only a small move in chemical space leads to success on the fitness landscape. Further details on this mechanism, alternative implementation schemes, and a comparison of evolutionary algorithms can be found elsewhere.^[2, 10]

In TOPAS, fitness is defined as the pairwise similarity between the template and the variant structures. This is done using a measure of topological pharmacophore similarity.^[3] Molecules are encoded by two-point pharmacophores using generalized atom types (hydrogen-bond donors/acceptors, positive/negative charge, lipophilic). This is similar to correlation vector representation of molecular structures and properties.^[11] According to this abstraction, a molecule is a combination of pairs of generalized atom types topologically connected by bonds. Similarity between two molecules was defined as the Euclidian distance between correlation vectors. The main advantage of this approach is that molecules are similar if they exhibit a similar distribution of generalized atom types in their two-dimensional structure, irrespective of the chemical way their atoms are connected.^[12] A downside is the fact that important three-dimensional and full topological

structural information is neglected. Whenever such information is crucial for bioactivity, the design algorithm may fail to produce useful results. Recently, novel potent Ca^{2+} -channel blocking agents were retrieved from a large database (CATS program) using the same definition of pharmacophore similarity.^[13] This finding motivated us to apply the correlation vector scheme for de novo design by TOPAS. In this work, additional penalty terms were added to the fitness function to avoid undesired structures. The fitness value was decreased if a) the total number of atoms exceeded 50, b) the sum of oxygen and nitrogen atoms was greater than 12, or c) if there were more than seven potential hydrogen-bond donors present in a given molecule. These crude filters are thought to facilitate the design of bioavailable molecules.^[14]

For a challenging test of TOPAS we selected a known potent K^{+} -channel blocking agent as the template molecule: *trans*-(2*S*,3*S*)-*N*-[3-(4-ethylbenzenesulfonylamino)-2-hydroxy-indan-5-yl]-3-methoxy-benzamide (Scheme 1 a, top).^[15] In our electrophysiological studies this compound had an IC_{50} of $0.11 \pm 0.02 \mu\text{M}$ for the inhibition of the human $\text{Kv}1.5$ potassium channel (hKv1.5). Inhibitors of voltage-dependent potassium channels induce a decrease in potassium ion movement across cell membrane. In cardiac cells this decrease leads to the prolongation of the action potential. Increasing myocardial refractoriness by prolonging the action potential can be useful for the treatment of cardiac arrhythmia.^[16] Blocking potassium channels and depolarizing the resting membrane potential has been shown to regulate a variety of biological processes, like T-cell activation under immune-reactive conditions.^[17]

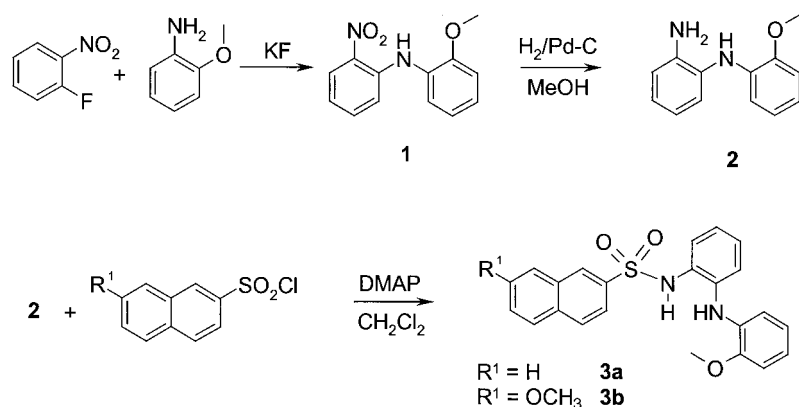
We performed several design runs with TOPAS, yielding slightly different "best" molecular structures after 100 generations (not shown). Starting from a randomly assembled molecular structure, 100 variants were virtually synthesized



Scheme 1. Models of the template structure (top) and the designed structure (bottom). A and B are two-dimensional representations. In B, generalized atom types are shown (squares, hydrogen-bond acceptors; triangles, hydrogen-bond donors; circles, lipophilic atoms). Three-dimensional models are shown in C.^[20] These structures were superimposed by a pharmacophore matching routine. For clarity, they are given in a side-by-side view. Two functional groups are labeled by I and II (heteroatoms are shown in dark gray).

per generation and the best of each generation was the parent of the next cycle. The fittest molecule obtained was a naphthylsulfonamide (Scheme 1a, bottom). From a purely structural perspective, there is no obvious similarity between the template and the designed structure besides a common sulfonamide group. However, assignment of generalized atom types indicates a resemblance of the two molecules, and a mutual pharmacophore pattern is uncovered (Scheme 1b). This example demonstrates that simplistic abstractions from chemical structure can sometimes help reveal “hidden” molecular similarities. To get a more precise picture of relevant features, three-dimensional structural models were superimposed by using a pharmacophore matching routine. Both molecules, the template and the design, seem to favor a U-shaped structure, resulting in a good fit of the planar aromatic ring systems and the adjacent sulfonamide moieties (Scheme 1c). From this we concluded that the hydroxy group **I** does not significantly contribute to the relevant pharmacophore motif. In addition, omitting methoxy group **II** of the designed structure might be beneficial for bioactivity, a very lipophilic system seems to be required in this position. Indeed, after completion of our work experimental evidence was published to support this view.^[18]

Two molecules were synthesized according to Scheme 2. Compound **3a** was synthesized to investigate the effect of the methoxy group **II** on the biological activity; compound **3b** is the original design recommended by TOPAS. Accordingly, 1-fluoro-2-nitrobenzene was allowed to react with *o*-anisidine



Scheme 2. Synthetic scheme for the molecules **3a** and **3b** designed by TOPAS. DMAP = 4-(dimethylamino)pyridine.

in an aromatic nucleophilic substitution to give (2-methoxyphenyl)-(2-nitrophenyl)amine (**1**) in near-quantitative yields. Subsequent reduction of the nitro derivative **1** was performed by catalytic hydrogenation with palladium as catalyst to yield the diamine **2** in 87.9% yield. Condensation of **2** with 7-methoxynaphthalene-2-sulfonyl chloride or naphthalene-2-sulfonyl chloride in methylene chloride with 4-(dimethylamino)pyridine (DMAP) as a base gave rise to the sulfonamides **3a** and **3b** as colorless solids in 42 and 35% yields, respectively, after chromatography.

Electrophysiological measurement undoubtedly proved K⁺-channel blocking activity by **3a** and **3b** (IC₅₀ = 0.47 and 7.34 μM, respectively; Figure 1). This finding demonstrates

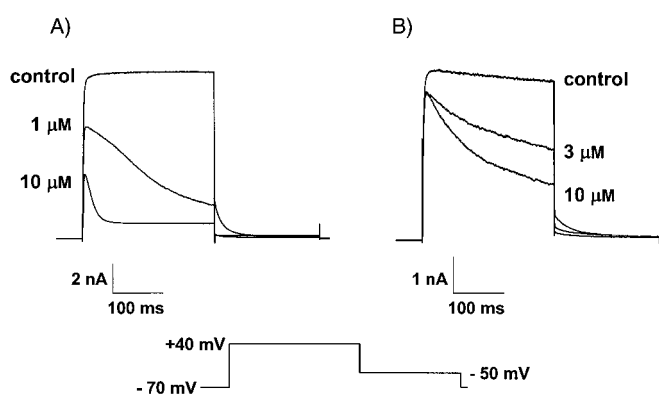


Figure 1. Inhibition of voltage-gated K⁺ flux via the hKv1.5 channel in transfected mammalian CHO cells. Representative families of whole-cell currents are shown, evoked by 250 ms test pulses to +40 mV followed by a 200 ms step to −50 mV. A) Effect of structure **3a** at 1 μM and 10 μM. B) Effect of structure **3b** at 3 μM and 10 μM.

that the TOPAS design technique can be used for generation of novel molecular structures exhibiting biological activity that is comparable to a structurally different template molecule. It must, however, be stressed that the original design **3b** was approximately two orders of magnitude less active (IC₅₀ = 7.34 ± 0.22 μM) than the template (IC₅₀ = 0.11 ± 0.02 μM). Nevertheless, it represents a valuable novel chemical class, which is currently being further explored. Straight-forward structural modifications—as suggested by our molecular modeling studies—already led to the improved compound **3a** exhibiting K⁺-channel blocking activity comparable to the original template (IC₅₀ = 0.47 ± 0.09 μM).

Our TOPAS strategy offers a number of advantages: 1) an adaptive stochastic search is performed in virtual chemical space; 2) the molecules generated are not restricted to a predefined combinatorial class (like e.g., peptides or Ugi reaction products); 3) novel structures are assembled from drug-derived building blocks using a set of “simple” chemical reactions; 4) a large diversity of molecular fragments can be explored; and 5) the design algorithm may be applied when a high-resolution receptor structure is unavailable. A particularly appealing future application is ligand design to influence G-protein-coupled receptor activity.

A significant advantage of using building blocks derived from known drugs is that reassembly of fragments potentially produces “druglike” structures. However, this technique may limit exploration of hitherto unknown “druglike” regions in chemical space. In similar fashion, exploration of chemical space may be further limited in the use of a small set of reaction schemes. An additional concern is that it is rarely known a priori which parts of a template molecule are relevant or irrelevant for a particular biological function. This can easily result in inactive molecular designs. One must not forget that it is comparably easy to find novel structures exhibiting some desired bioactivity using virtual screening tools, whereas the de novo design of real drugs is only slowly

emerging.^[19] We are convinced that fragment-based evolutionary design approaches like TOPAS that are able to optimize for several fitness functions in parallel—including predictions of metabolic and pharmacokinetic parameters—will lead to further significant progress in this area of computational chemistry and virtual screening.

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Chirally Modified *n*-Butyllithium: Tuning the Composition, Structure, and Enantioselectivity with Modular Fencholates**

Bernd Goldfuss,* Melanie Steigelmann, and Frank Rominger

This work is dedicated to Prof. Dr. Günter Helmchen on the occasion of his 60th birthday.

Chirally modified organolithium reagents are eminent as reagents in enantioselective syntheses^[1] and as initiators in asymmetric–anionic polymerization.^[2] The control of reactivity and selectivity by suitable moderators is crucial for successful application.^[3] Studies in solution^[4] and in the solid state^[1a,g,h, 5] yield precious information on the nature of chiral organolithium systems^[6] and open possibilities for a rational design of new reagents.^[7] Although *n*-butyllithium (*n*BuLi) is among the most widely employed organolithium reagents and several X-ray diffraction studies have been performed on achiral *n*BuLi complexes,^[8] there is little structural information on chirally modified *n*BuLi species.^[5b,c, 9]

Recently, we reported the synthesis and the X-ray crystal structure of the enantiopure *n*BuLi lithium fencholate complex **1**.^[9] Complex **1** forms spontaneously as a colorless precipitate on mixing anisylfenchole (**2**) and a solution of *n*BuLi in hexane and contains one equivalent of *n*BuLi as well as three lithium fencholate moieties **2**-Li (Figure 1).^[9] The

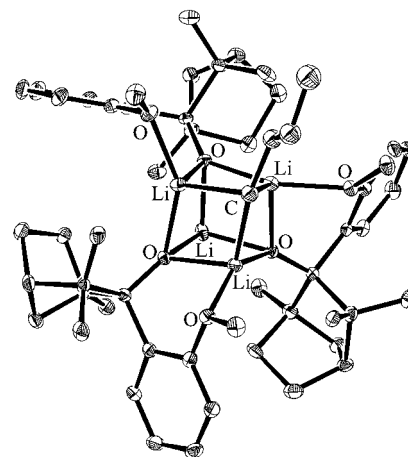


Figure 1. Molecular structure of **1**. The hydrogen atoms are omitted.

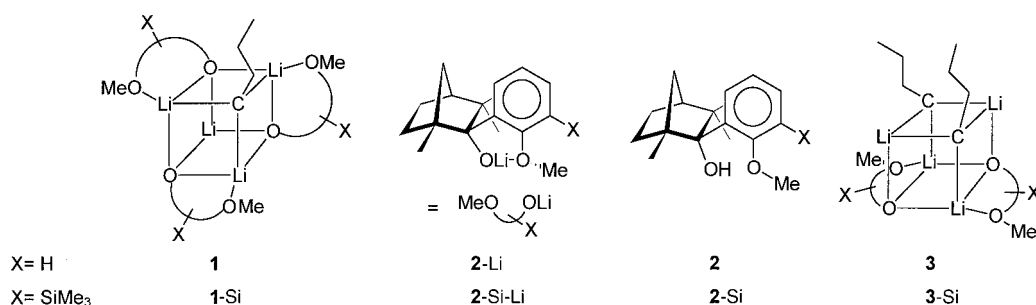
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[*] Dr. B. Goldfuss, Dipl.-Chem. M. Steigelmann, Dr. F. Rominger^[+]
Organisch-Chemisches Institut der Universität Heidelberg
Im Neuenheimer Feld 270, 69120 Heidelberg (Germany)
Fax: (+49) 6221-54-4205
www.uni-heidelberg.de/institute/fak12/OC/goldfuss
E-mail: Bernd.Goldfuss@urz.uni-heidelberg.de

[+] X-ray crystal structure analysis

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introduction of **2-Si**, which contains an SiMe₃ substituent *ortho* to the methoxy group, as a precatalyst increases (relative to **2**) both the reactivity and the enantioselectivity of diethylzinc additions to benzaldehyde.^[10] Herein we show that **2-Si** is also capable of trapping *n*BuLi ($\rightarrow$ **3-Si**) and thus chirally modifying it. The SiMe₃ substituent in **2-Si** influences



the composition and the structure of the enantiopure *n*BuLi complex and also increases the enantioselectivities of *n*BuLi addition reactions to benzaldehyde.

Addition of *n*BuLi (in hexane) to **2-Si** results in the formation of a colorless precipitate which was identified, after recrystallization and single-crystal X-ray analysis, as the *n*BuLi fencholate complex **3-Si** (Figure 2).^[11] Two *n*BuLi molecules and two lithium fencholate units form a distorted

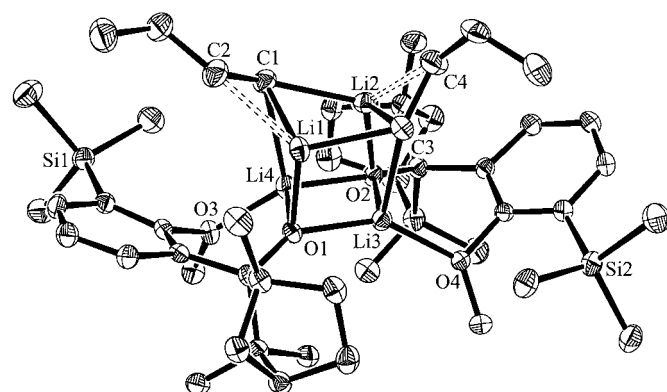


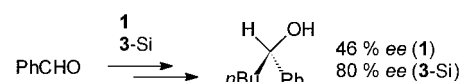
Figure 2. Molecular structure of **3-Si**. The hydrogen atoms and one *n*-hexane molecule are omitted. Selected bond lengths [Å] and angles [°]: C1–Li1 2.157(6), C1–Li2 2.172(6), C1–Li4 2.446(6), C3–Li1 2.192(6), C3–Li2 2.173(6), C3–Li3 2.450(6), O1–Li1 1.891(5), O1–Li3 1.926(5), O1–Li4 1.881(5), O2–Li2 1.919(5), O2–Li3 1.884(5), O2–Li4 1.896(5), C2–Li1 2.381(6), C4–Li2 2.359(6), O3–Li4 1.947(5), O4–Li3 1.974(5), Li1–H_{C2} 2.25, Li1–H_{C3} 2.32, Li2–H_{C4} 2.18, Li2–H_{C3} 2.31; Li1–C1–C2 78.5(2), Li2–C3–C4 77.0(2).

Li₄C₂O₂ cube in **3-Si** (Figure 2).^[12] While in **1** lithium ions next to the *n*-butylide units are coordinated by methoxy groups,^[9] no analogous Li–O bonds are apparent for Li1 and Li2 in **3-Si**. Instead, these lithium ions form short bonds to the β-CH₂ units of the *n*-butylide groups (Li1–C2: 2.381(6) Å, Li2–C4: 2.359(6) Å). Intraaggregate secondary Li–CH₂ interactions are also found in (cC₆H₁₁Li)₆,^[13] {(cCHCMe₂CMe₂)CH₂–Li}₆,^[14] (*n*BuLi·LiOtBu)₆,^[8e] (*n*BuLi)₆,^[15] (*t*BuLi)₄,^[15] (*i*Pr–Li)₆,^[16] (*t*BuCH₂CH₂Li·THF)₄,^[17] as well as with cyclopropyl-ring edges.^[18] These secondary Li–C interactions in **3-Si**

clearly direct *trans* (at C1) and *gauche* (at C3) *n*-butylide units towards the Li–C edges of the Li₄C₂O₂ cube (Figure 2).

Like **2**, the trimethylsilyl derivative **2-Si** is also able to “trap” *n*BuLi, whereby an aggregate is formed, but not with a *n*BuLi:lithium fencholate ratio of 1:3 as found in **1** but with a ratio of 2:2. Both **1** and **3-Si** can butylate benzaldehyde, with however, different enantioselectivities (Scheme 1).^[19] While **1** yields (*R*)-1-phenyl-1-pentanol in 46% *ee*, **3-Si** affords, under analogous conditions, 80% *ee*.^[20]

To explore the origins of the increased *n*BuLi content (relative to **1**) in **3-Si** caused by SiMe₃ substitution, the energies of formation for the structurally characterized compounds **1** and **3-Si** as well



Scheme 1. In enantioselective additions to benzaldehyde complexes **1** and **3-Si** yield preferentially (*R*)-1-phenyl-1-pentanol.

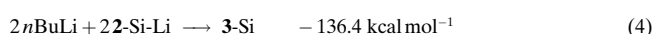
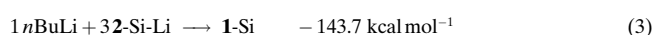
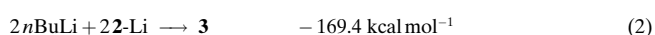
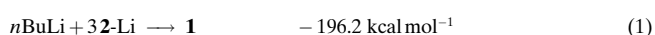
as for the possible, but not observed, species **1-Si** and **3** were computed (ONIOM^[21](B3LYP^[22]/6-31 + G*:UFF^[23]), Table 1, Equations (1)–(4)).^[24] For ligand **2-Li** (X = H) the most exothermic energy of formation is computed for complex **1** with a *n*-butylide:fencholate ratio of 1:3 [Eq. (1), –196.2 kcal mol^{–1}]. The formation of complex **3** from *n*BuLi

Table 1. Total energies *E* (ONIOM(B3LYP/6-31 + G*:UFF)) for the evaluation of the relative energies of formation of *n*BuLi lithium fencholates according to Equations (1)–(4).^[a]

Compound	<i>E</i> [a.u.]	Compound	<i>E</i> [a.u.]
<i>n</i> BuLi	–47.40173	1	–297.29345
2-Li	–83.19300	1-Si	–297.21014
2-Si-Li	–83.19314	3	–261.45943
		3-Si	–261.40716

[a] All fully optimized structures (C₁ symmetry, with the exception of *n*BuLi(C_s)) were characterized as minima (NIMAG = 0) by frequency calculations. Zero-point energy corrections are not included.^[21]

and **2-Li** in a ratio of 2:2 is, in comparison to **1**, less favored by 27 kcal mol^{–1} [Eq. (2), –169.4 kcal mol^{–1}]. This explains the preferential formation of **1**.^[9] The SiMe₃ substituent in **2-Si** supports the 2:2 composition more: the formation of **3-Si** with an *n*-butylide:fencholate ratio of 2:2 [Eq. (4), –136.4 kcal mol^{–1}] is only 7 kcal mol^{–1} less favorable than the formation of **1-Si** with a 1:3 composition [Eq. (3), –143.7 kcal mol^{–1}] and **3-Si** is formed experimentally.^[25]



Equations 1 and 3 show a significant destabilization (53 kcal mol⁻¹) of **1**-Si relative to **1**, while more similar energies of formation difference 33 kcal mol⁻¹) are evaluated in Equations 2 and 4 for **3** and in **3**-Si.

Why **1**-Si is less stable than **1** is clearly shown in the computed structure of **1**-Si (Figure 3). The arrangement of three bulky trimethylsilylaryl moieties gives rise to repulsive interactions between the SiMe₃ groups and the *endo*-situated methyl groups of the bicyclo[2.2.1]heptane fragments (Figure 3). Similar repulsive interactions between *endo* methyl groups were identified as origin for the favored (*R*)-biaryl conformation in 2,2'-(bis-2-hydroxy-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl)-1,1'-biphenyl.^[26]

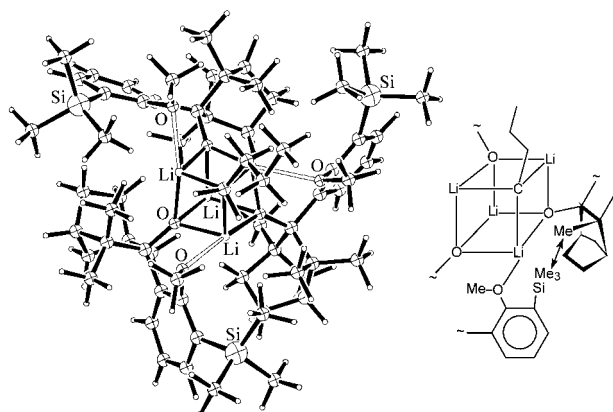


Figure 3. ONIOM(B3LYP/6-31+G*:UFF) optimized structure of **1**-Si. The repulsive interactions between SiMe₃ and the *endo* methyl groups of the bicyclo[2.2.1]heptane fragments are shown schematically (right).

The stereochemical influence of SiMe₃ groups directs an increase in the *n*BuLi:lithium fencholate ratio from 1:3 in **1** to 2:2 in **3**-Si. Furthermore, agostic interactions are formed between the lithium ions and the β-CH₂ units in **3**-Si. These contacts offer a possible method to modify the reactivities (e.g. an increased propensity for β-hydride eliminations)^[27] and selectivities of the mixed anionic aggregate **3**-Si, relative to **1**. Indeed higher enantioselectivities are observed in the reactions of **3**-Si with benzaldehyde than in that of **1** with benzaldehyde. Thus, the targeted variation of the *ortho* substituents X (e.g. H versus SiMe₃) of the anisylfencholate units provides many possibilities for tuning the compositions, structures, and selectivities of chirally modified *n*-butyllithium reagents.

Experimental Section

For the syntheses of **1** and **2**-Si see ref [9] and [10a].

Synthesis and crystallization of **3**-Si: *n*BuLi (1.0 mmol, 1.6 M in hexane, 0.63 mL) was added at 0 °C to **2**-Si (166 mg, 0.5 mmol) and the mixture was stirred at 25 °C for 30 min. Repeated cooling to -78 °C yielded a colorless, crystalline precipitate, which after recrystallization afforded single crystals of **3**-Si.^[11]

Enantioselective butylation of **1** and **3**-Si: The complexes **1** or **3**-Si were prepared from **2** (3 mmol, 781 mg) or **2**-Si (2 mmol, 665 mg), respectively, with *n*BuLi (1.6 M, 4 mmol, 2.50 mL) in hexane. The reaction mixture was cooled to -78 °C and after 20 min benzaldehyde (for **1**: 1 mmol, 106 mg; for **3**-Si: 2 mmol, 212 mg) was added. After 2 h at -78 °C the mixture was warmed to 25 °C, stirred for 2 h and then hydrolyzed. After purification by

chromatography (for **1**: silica gel, toluene:acetone = 29:1; for **3**-Si: petroleum ether:ethyl acetate = 9:1) the product (*R*)-1-phenyl-1-pentanol was obtained in a yield of 34% for **1** and 38% for **3**-Si, with 46 and 80% *ee*, respectively (GC, HPLC: DAICEL OB-H, isopropanol:hexanes = 0.8:99.2).

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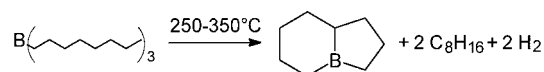
- [11] X-ray structure analysis of **3-Si**: SHELXTL V5.10, $C_{34}H_{94}Li_4O_4Si_2$, $M = 891.23$, $T = 200(2)$ K, $\lambda = 0.71073$ Å, monoclinic, space group: $P2_1$, $Z = 2$, $a = 10.3514(1)$, $b = 21.8005(3)$, $c = 12.3909(1)$ Å, $\beta = 98.703(1)^\circ$, $V = 2764.01(5)$ Å³, density (calcd): 1.071 g cm⁻³, reflections collected: 20901, independent reflections: 9020 ($R_{int} = 0.0343$), observed reflections: 7114 ($I > 2\sigma(I)$), absorption coefficient $\mu = 0.104$ mm⁻¹, absorption correction: semi-empirical from equivalents, max/min transmission: 0.96 and 0.71, refinement method: full-matrix least-squares on F^2 , data/restraints/parameter: 9020/26/609, Flack parameter 0.10(11), final R indices ($I > 2\sigma(I)$), $R1 = 0.048$, $wR2 = 0.102$, largest differential peak and hole: 0.21 und -0.21 e Å⁻³. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-145225. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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C–H Activation by Direct Borane – Hydrocarbon Dehydrogenation: Kinetic and Thermodynamic Aspects**

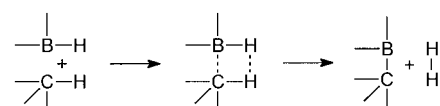
Bernd Goldfuss,* Paul Knochel,* Lars O. Bromm, Kolja Knapp

Dedicated to Prof. Manfred Regitz on the occasion of his 65th birthday

Selective C–H activations and functionalizations of hydrocarbons are challenging chemical processes with enormous synthetic potential.^[1] Transition metal catalyzed^[2,3] and metal-free^[4] C–H activations are being studied intensively.^[5] Allylic C–H activations with organoborane reagents have been established as useful methods in organic synthesis.^[6] Köster and Rotermund discovered in 1960 that the pyrolysis of triorganoboranes (e.g. tri-*n*-octylborane) yielded bicycloborane, alkene, and molecular hydrogen (Scheme 1).^[7] After further studies Köster et al. proposed a four-center mechanism for the observed cleavage of C–H and the formation of C–B bonds (Scheme 2).^[8]



Scheme 1. Pyrolysis of tri-*n*-octylborane.



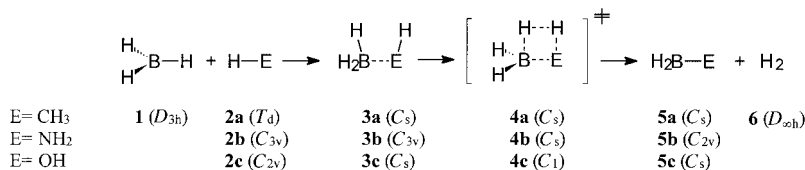
Scheme 2. The four-center mechanism of borane–hydrocarbon dehydrogenation reactions proposed by Köster.

Recently, similar intramolecular C–H activations of *tert*-butyl and phenyl groups in organoboranes in solution were reported^[6c] without transition metal catalysts.^[3] To analyze the factors influencing the reactivity and selectivity of these *direct*, uncatalyzed borane–hydrocarbon dehydrogenations, we have studied the kinetic and thermodynamic aspects by theoretical methods.

[*] Dr. B. Goldfuss,
Organisch-Chemisches Institut der Universität Heidelberg
Im Neuenheimer Feld 270, 69120 Heidelberg (Germany)
www.uni-heidelberg.de/institute/fak12/OC/goldfuss
Fax: (+49) 6221-54-4205
E-mail: Bernd.Goldfuss@urz.uni-heidelberg.de
Prof. Dr. P. Knochel, Dipl.-Chem. L. O. Bromm,
Dipl.-Chem. K. Knapp
Ludwig-Maximilians-Universität
Butenandtstrasse 5–13, 81377 München (Germany)
Fax: (+49) 89-2180-7680
E-mail: knoch@cup.uni-muenchen.de

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While reactions of borane **1** with ammonia (**2b**) and water (**2c**) are well known, there are no reports on the analogous direct borane–methane (**2a**) dehydrogenation reaction (Scheme 3).^[9] With borane both ammonia and water



Scheme 3. Reactions of borane with methane, ammonia, and water (Tables 1 and 2).

form stable adduct complexes^[10] (see the adduct formation energies E_{ad} of **3b** and **3c** calculated at the B3LYP/6-311++G**^[11, 12] level, Tables 1 and 2).^[13] The subsequent elimination of hydrogen (**6**) via the transition structures **4b** and **4c** (activation energy, E_{a} Table 2)^[14] yield the exothermic and exergonic products **5b** and **5c** (total reaction energy E_{r} , Table 2, Scheme 3). Methane (**2a**) and borane form only a weakly bound van der Waals complex **3a** (see E_{ad} in Table 2). However, relatively small activation barriers relative to the educts $E_{\text{a}}(\text{Ed})$, are computed for the hydrogen elimination between methane and borane via the transition structure **4a** (Figure 1) ($\Delta E^\ddagger = 23.5 \text{ kcal mol}^{-1}$, $\Delta H^\ddagger = 21.8 \text{ kcal mol}^{-1}$,

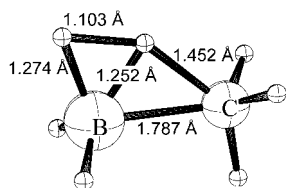


Figure 1. The transition structure **4a** of the methane–borane dehydrogenation optimized at the B3LYP/6-311++G** level.

$\Delta G^\ddagger = 30.5 \text{ kcal mol}^{-1}$, Table 2).^[15] The attack of the electrophilic boron atom occurs at the C–H bond and induces the elimination of H₂ from the transition **4a** (Figure 1).^[16] The analogy to the four-center mechanism proposed by Köster (Scheme 2) is apparent. While ammonia and water yield products (**5b** and **5c**) in strongly exothermic and exergonic reactions the formation of methylborane **5a** is slightly endothermic and endergonic (see E_{r} in Table 2).^[17]

Activation of C–H bonds through direct, intramolecular dehydrogenation reactions has been observed for *tert*-butyl groups and also for phenyl moieties in organoborane compounds.^[6e]

From benzene and borane a weakly bound van der Waals complex is formed (E_{ad} in Table 3).^[18] The transition structure for the benzene–borane dehydrogenation (Figure 2)^[19] is similar to that of the methane–borane dehydrogenation (Figure 1), however smaller activation barriers ($E_{\text{a}}(\text{Ed})$ values for X = H in Table 3) and an exothermic energy of reaction (E_{r}) are predicted.^[20] The influence of electronic effects on the dehydrogenations of arenes is clearly demonstrated in mono-substituted benzene derivatives, in which the borane attacks the *para* position (Scheme 4, Table 3). The lowest activation energy barriers (E_{a}) and the most exothermic energies of reaction (E_{r}) are found for electron-donating substituents (e.g. X = NMe₂, OMe). In contrast, electron-withdrawing substituents in the *para* position cause increased activation barriers and less exothermic reactions. There is a good correlation between Hammett σ_{p} values of the *para* substituents X and electronic activation energies (Figure 3).^[21] In transition structures with electron-donating substituents, the H–H bonds of the leaving dihydrogen units are shorter than in the transition structures with electron-withdrawing-substituents (e.g. NMe₂: 1.093 Å versus NO₂: 1.147 Å, Table 4).

Table 1. Electronic,^[a] enthalpic,^[b] and Gibbs' free energies^[b] [a.u.] for the starting materials, first-formed adducts, transition structures, and products of the reactions between BH₃ and H–E (Scheme 3).

E	1	2	3	4 ^[c]	5	6
CH ₃ (a)	–26.59500 –26.59117 –26.61254	–40.48940 –40.48559 –40.50672	–67.08341 –67.07597 –67.11258	–67.04689 (1139) –67.04205 –67.07064	–65.91272 –65.90789 –65.93604	–1.16951 –1.16620 –1.18100
NH ₂ (b)		–56.54846 –56.54464 –56.56649	–83.18046 –83.17569 –83.20290	–83.12560 (1442) –83.12101 –83.14887	–82.02631 –82.02214 –82.04806	
OH (c)		–76.43726 –76.43348 –76.45490	–103.04509 –103.03992 –103.06898	–103.01126 (1421) –103.00696 –103.03432	–101.91276 –101.90877 –101.93494	

[a] B3LYP/6-311++G** optimized structures, unscaled zero-point energy corrections are included. [b] $T = 298.15 \text{ K}$, $p = 1 \text{ atm}$. [c] Transition structures with imaginary frequency in parenthesis [cm^{-1}].

Table 2. Relative electronic,^[a] enthalpic,^[b] and Gibbs' free energies^[b] [kcal mol^{-1}] of adduct formations of **3** (E_{ad}), energy barriers of dehydrogenations relative to **3** $E_{\text{a}}(\text{vdW})$, and relative to the starting materials **1** and **2** $E_{\text{a}}(\text{Ed})$, and the total reaction energies relative to the starting materials E_{r} (Table 1, Scheme 3).

E	E_{ad}	$E_{\text{a}}(\text{vdW})$	$E_{\text{a}}(\text{Ed})$	E_{r}
CH ₃ (a)	0.6 (–0.1), ^[c] 0.5, 4.2	22.9, 21.3, 26.3	23.5, 21.8, 30.5	1.4, 1.7, 1.4
CH ₃ (a) ^[d]	–0.1 (–1.2), ^[c] –0.4, 4.3	22.2, 20.8, 24.8	22.1, 20.4, 29.1	0.0, 0.3, 0.1
NH ₂ (b)	–23.2, –25.0, –15.0	34.4, 34.3, 33.9	11.2, 9.3, 18.9	–32.9, –33.0, –31.4
OH (c)	–8.0, –9.6, –1.0	21.2, 20.7, 21.8	13.2, 11.1, 20.8	–31.4, –31.6, –30.4

[a] B3LYP/6-311++G** optimized structures, unscaled zero-point energies are included. [b] $T = 298.15 \text{ K}$, $p = 1 \text{ atm}$. [c] No zero-point energy correction. [d] MP2(fc)/6-311++G** optimized structures, unscaled zero-point energies are included.

Table 3. Hammett parameter σ_p of the substituents X and the relative electronic,^[a] enthalpic,^[b] and Gibbs' free energies,^[b] [kcal mol⁻¹] of adduct formation (E_{ad}), activation barriers (E_a), and total reaction energies (E_r) for the reaction shown in Scheme 4.

X	$\sigma_p(X)^{[c]}$	$E_{ad}^{[d]}$	$E_a(vdW)^{[e]}$	$E_a(Ed)^{[f]}$	$E_r^{[g]}$
NMe ₂	-0.63	-1.8 (-3.8), -2.5, 6.7	17.4, 17.0, 18.0	15.6, 14.5, 24.7	-9.8, -9.5, -6.6
OMe	-0.28	-0.8 (-2.7), -0.9, 7.1	17.5, 16.5, 19.2	16.7, 15.5, 26.3	-7.8, -7.2, -5.1
<i>t</i> Bu	-0.15	-0.3 (-2.1), -0.4, 7.3	17.4, 16.3, 19.4	17.1, 15.9, 26.7	-6.3, -5.7, -3.7
Me	-0.14	-0.4 (-2.1), 0.1, 5.4	17.6, 16.4, 20.3	17.1, 16.5, 25.7	-6.4, -5.8, -3.8
H ^[h]	0.0	-0.2 (-1.7), -0.2, 5.6	17.8, 16.6, 20.0	17.6, 16.4, 25.6	-5.6, -4.9, -4.0
F	+0.15	-0.1 (-1.8), -0.2, 6.7	17.9, 16.7, 20.2	17.8, 16.5, 26.9	-6.3, -5.6, -3.6
Cl	+0.24	-0.1 (-1.5), 0.0, 6.7	17.9, 16.7, 20.3	17.8, 16.6, 27.0	-5.9, -5.2, -3.2
Br	+0.26	0.0 (-1.5), 0.0, 6.8	17.8, 16.7, 20.2	17.9, 16.7, 27.0	-5.8, -5.1, -3.1
COMe	+0.47	0.1 (-1.2), 0.3, 6.4	18.0, 16.7, 21.2	18.1, 17.0, 27.7	-4.6, -3.9, -2.0
CF ₃	+0.53	0.3 (-1.0), 1.0, 5.4	18.2, 16.9, 21.3	18.5, 17.9, 26.7	-4.5, -3.8, -1.8
CN	+0.70	0.4 (-0.9), 0.5, 6.6	18.2, 16.9, 21.2	18.6, 17.4, 27.8	-4.4, -3.8, -1.8
NO ₂	+0.81	0.5 (-0.7), 0.7, 6.5	18.4, 17.1, 21.5	18.9, 17.8, 28.1	-3.9, -3.2, -1.4

[a] B3LYP/6-311 ++ G** optimized structures, unscaled zero-point energies are included. [b] $T = 298.15$ K, $p = 1$ atm. [c] Hammett values.^[21] [d] Electronic energies of adduct formation, in parentheses are the values without zero-point energy correction. [e] Activation barrier relative to van der Waals complexes. [f] Activation barrier relative to isolated starting materials molecules. [g] Energy of reaction relative to isolated starting materials molecules. [h] See Figure 2.

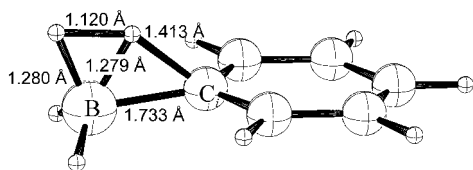
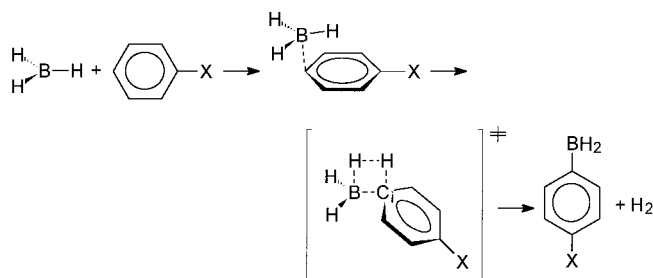


Figure 2. The transition structure of the benzene-borane dehydrogenation optimized at the B3LYP/6-311 ++ G** level.



Scheme 4. Dehydrogenation between BH₃ and monosubstituted benzene derivatives.

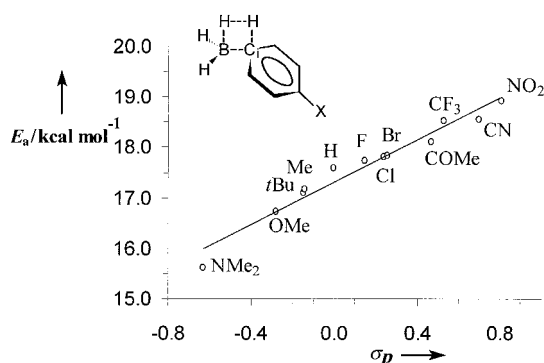


Figure 3. Correlation of the electronic activation energies (E_a) and the Hammett σ_p values for dehydrogenations between borane and monosubstituted benzene derivatives (correlation coefficient: 0.96, $y = 2.1x + 17.3$; Table 3, Scheme 4).

Analysis of the B-C_{ipso} bond lengths and of the C_{ipso} partial charges explains the decrease of the activation barriers with increasingly stronger electron-donating substituents X (Table 4): more negative partial charges at C_{ipso} (e.g. -0.34 for NMe₂ versus -0.26 for NO₂) result in more stable bonds to

Table 4. Selected bond lengths (B-C_i, C_i-H, H-H [Å]) and natural bond orbital (NBO) partial charges q [a.u.] of the transition structures in borane-arene dehydrogenations (Scheme 4).^[a]

X	B-C _i	C _i -H	H-H ^[c]	$q(C_i)^{[d]}$	$q(H_C)^{[d]}$
NMe ₂	1.710	1.431	1.093	-0.341	+0.341
OMe	1.721	1.421	1.106	-0.324	+0.344
<i>t</i> Bu	1.727	1.417	1.114	-0.297	+0.345
Me	1.728	1.417	1.114	-0.299	+0.345
H ^[b]	1.733	1.413	1.120	-0.290	+0.346
F	1.733	1.409	1.121	-0.310	+0.347
Cl	1.735	1.406	1.126	-0.294	+0.348
Br	1.735	1.405	1.127	-0.287	+0.348
COMe	1.739	1.403	1.134	-0.268	+0.347
CF ₃	1.743	1.399	1.138	-0.274	+0.348
CN	1.744	1.396	1.142	-0.267	+0.349
NO ₂	1.747	1.392	1.147	-0.260	+0.350

[a] B3LYP/6-311 ++ G** optimized structures. [b] See Figure 2. [c] Bond lengths in the leaving H₂ molecule. [d] B3LYP/6-31G**//B3LYP/6-31G**.

the electrophilic boron center,^[22] which lead to shorter B-C_{ipso} bonds (1.710 Å for NMe₂ versus 1.747 for NO₂, Table 4).

In conclusion, relatively small activation barriers (≤ 30 kcal mol⁻¹) are predicted for dehydrogenation reactions between borane and hydrocarbons that occur via four-center transition states. Even lower activation barriers and more exothermic reactions should result for donor-substituted arenes. These different reactivities, which can be tuned by the substituents, could provide the basis for selective and directed functionalizations of hydrocarbons.

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Multilevel Molecular Electronic Species: Electrochemical Reduction of a [2 × 2] Co^{II} Grid-Type Complex by 11 Electrons in 10 Reversible Steps**

Mario Ruben, Esther Breuning, Jean-Paul Gisselbrecht,* and Jean-Marie Lehn*

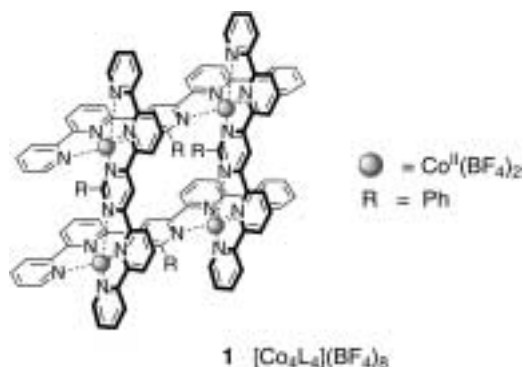
The search for high-density information storage devices has stimulated an increasing interest in molecules exhibiting multistable behavior.^[1] Multistability can be achieved by several means by exploiting changes in intrinsic molecular properties such as spin state,^[2] conformation,^[3] or redox state.^[4] For instance, fullerenes and nanotubes have attracted wide interest in view of their potential in the design of new materials and electronic devices based on their unusual electrochemical behavior (reversible single-electron reductions and semiconducting properties).^[5] Multicenter transition metal complexes with different redox states are very attractive candidates for the design of multilevel electronic systems. Thus, for instance, polynuclear metal complexes of polypyridine ligands present several reversible multielectron steps.^[6]

We have described recently a new class of polypyridine-derived, multinuclear metal complexes of the [2 × 2] M₄^{II} grid-type (M = transition metal),^[7, 8] which have been found to present a range of interesting structural^[7] and physicochem-

[*] Prof. Dr. J.-M. Lehn, Dr. M. Ruben, E. Breuning
Laboratoire de Chimie Supramoléculaire
ISIS-Université Louis Pasteur
4, rue Blaise Pascal, 67000 Strasbourg (France)
Fax: (+33)388-411 020
E-mail: lehn@chimie.u-strasbg.fr
Dr. J.-P. Gisselbrecht
Laboratoire d'Electrochimie et de Chimie-Physique du Corps Solide
UMR 7512,CNRS-Université Louis Pasteur
4, rue Blaise Pascal, 67000 Strasbourg (France)
Fax: (+33)388-611 553
E-mail: gissel@chimie.u-strasbg.fr

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ical (electrochemical,^[7a] magnetic^[9]) properties. Here we report on the exceptional electrochemical and spectroelectrochemical properties of one specific member of this family, complex **1**, [Co₄L₄](BF₄)₈, which was synthesized from ligand



L (L = 4,6-bis(2',2''-bipyrid-6'-yl)-2-phenylpyrimidine^[10]) and Co(BF₄)₂ · 6H₂O as described before.^[7b] The redox behavior of **1** has been investigated in solution (DMF) by cyclic and steady-state voltammetry (Table 1).

Cyclic voltammetry carried out at –20 °C gives rise to a cyclic voltammogram (CV) consisting of ten well-resolved, completely reversible reduction steps on the available potential window (Figures 1a and b). Nine reduction steps are still

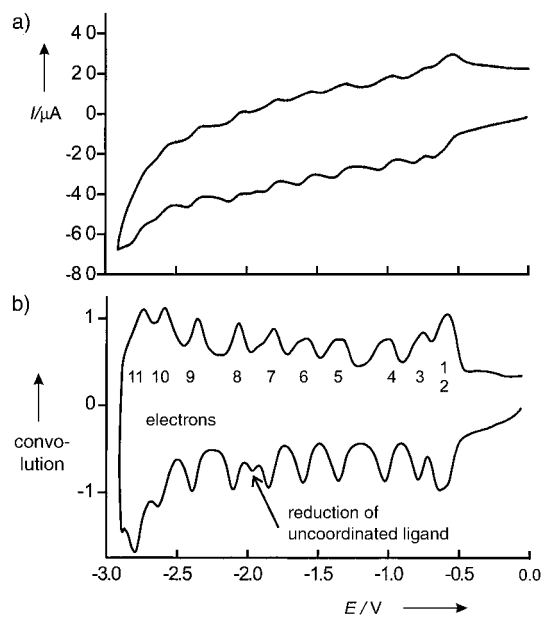


Figure 1. CV (a) and semidifferential convolution of one CV curve (b) of **1** in a 0.1 M solution of Bu₄NPF₆ in DMF at –20 °C on a glassy carbon working electrode at a sweep rate of 1 V s^{–1}.

Table 1. Redox potentials *E* and redox potential separations Δ*E* of **1** observed in a 0.1 M solution of Bu₄NPF₆ in DMF on a glassy carbon working electrode at –20 °C (all potentials given versus ferrocene).

Red. step	1	2	3	4	5	6	7	8	9	10	11
<i>E</i> [V]	–0.59	–0.63	–0.78	–1.02	–1.35	–1.60	–1.85	–2.11	–2.40	–2.61	–2.73
Δ <i>E</i> [V]		0.04	0.15	0.24	0.33	0.35	0.25	0.26	0.29	0.21	0.12

observed at room temperature, one less than at low temperature because of the restricted potential window at that temperature.

Analysis of the peak characteristics shows that the peak potentials are independent of the scan rates (10–1000 mV s^{–1}) and that the cathodic and anodic peak current ratio (*I*_{pc}/*I*_{pa}) for each step is unity. Each peak current is also proportional to the square root of the scan rate, as expected for a reversible process. Thus, subsequent chemical reactions can be excluded. The shape and amplitude of the first reduction peak are indicative of the overlap of two one-electron transfers.

Steady-state voltammetry at the rotating disc electrode gives rise to seven well-resolved reduction waves, further ones being less well defined. The wave amplitudes indicate that the first reduction step involves two electrons, whereas the remaining steps present one electron each. Careful examination of the first reduction step, which presents a log-plot slope of 90 mV (lg[(*I*/*I*_d – *I*)] = *f*(*E*); *I*_d = diffusion current) and twice the amplitude of a one-electron reduction, results in the assignment of two overlapping one-electron steps separated by about 40 mV.^[11]

Based on these results, we can conclude that ten well-resolved, reversible reduction steps involving eleven electrons are observable in **1** at –20 °C. As far as we are aware, this represents the highest reported number of well-characterized, resolved and reversible one-electron reductions on a molecular compound. For example, the fullerenes C₆₀ and C₇₀ exhibit only six reversible single-electron reductions spread over a larger potential window and located at much more negative potentials (from –0.98 V to –3.26 V).^[5]

At –1.95 V, we observe a small additional signal which can be attributed, by comparison, to the reduction of some uncoordinated ligand. This additional signal already appears during the first scan and does not increase on continued scanning. Even the use of pure crystalline material of **1** could not prevent the presence of the additional signal. However, exhaustive electrolysis as carried out by spectroelectrochemical investigations (see below) only slightly increases this signal. Thus, we attribute it to a trace of the uncoordinated ligand present in solution in equilibrium with compound **1** independent of the electrochemical studies. Indeed, investigations concerning the stability of the reduced species indicate that even after 20 repeated scans to –2.73 V, the obtained CV remained superimposable with the first scan. In comparison, the homologous Fe₄^{II} and Zn₄^{II} grid-type compounds as well as the mononuclear [Co(terpy)₂]²⁺ (after threefold reduction; terpy = 2,2':6',2''-terpyridine) showed fast decomposition under identical conditions.^[12]

Basically the same voltammetric pattern is observed in acetonitrile, but with a limited number of observable waves due to the lower solubility of the reduced species of **1** in this solvent. However, an oxidation wave corresponding to the

transfer of four electrons at +1.0 V could be detected only by steady-state voltammetry in acetonitrile. This wave is widely spread out and has a log-plot slope of 300 mV. Such behavior is indicative of a kinetically slow process of the $\text{Co}^{\text{II}}/\text{Co}^{\text{III}}$ redox couple, occurring stepwise on the four metal centers of the complex. Slow electron transfer rates have been observed on encapsulated metal ions in bulky and dendrimeric structures.^[13]

Spectroelectrochemical studies were carried out on **1** up to the seventh reduction step (Figure 2). The evolution of the spectrum during the first four steps is in agreement with ligand-centered reductions: the initial $\pi-\pi^*$ band of the

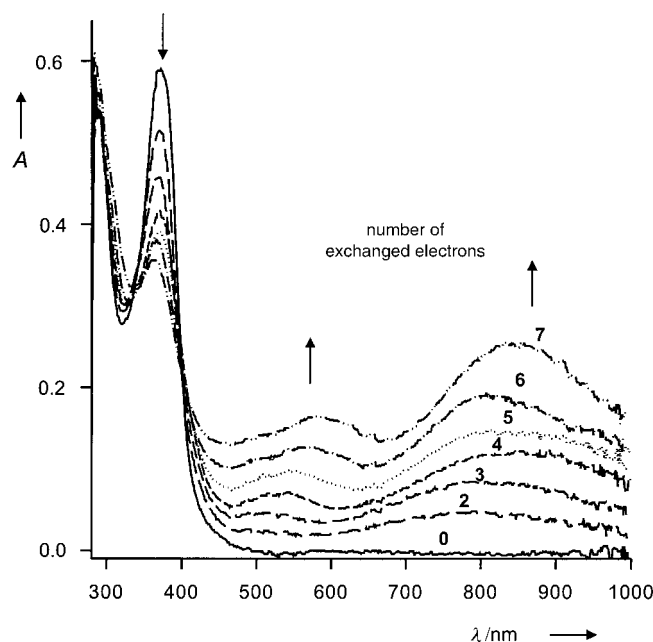


Figure 2. Electronic spectra of **1** and its electrochemically generated reduced forms in a 0.1M solution of Bu_4NPF_6 in DMF at 298 K.

unreduced ligand at 370 nm decreases subsequently and two broad bands appear at 550 nm and around 800 nm. These low-energy bands can be attributed to intraligand $\pi-\pi^*$ transitions from the new SOMOs (monoanion) to the first and the second new LUMOs (monoanion) of the reduced ligands.^[14] The initial spectrum could be recovered by stepwise oxidation of the generated heptaanion, indicative of the high stability of the reduced species.

Semiempirical PM3 molecular orbital calculations^[15] showed that the LUMO of the uncoordinated, neutral ligand is mainly centered on the pyrimidine ring. The double coordination of the pyrimidine in **1** strongly stabilizes this molecular orbital. Thus, the first four reducing electrons should be mainly localized on the bridging pyrimidine rings of the ligands. One notes that, starting with the fifth reduction, the two low-energy bands increase further, but are shifted remarkably to lower energies. Thus, the second series of four electrons is also localized on the ligand (at least up to the observable seventh electron). The red shift of the new intraligand bands can be attributed to an electronic destabilization of the corresponding HOMOs of the ligands, as a

consequence of the two electrons introduced onto the pyrimidine orbital.

In general, the gradual evolution of the intensities of the new bands expresses a proportionality with the number of reducing electrons (only the seventh step is slightly larger because of the involvement of some uncoordinated ligand). The shift of the bands to lower energies in the course of reduction is also an indication for an electronic coupling of the reduced ligands. This is also confirmed by the increasing differences between the values of the first four reduction waves in the CV ($\Delta E(1-2)=0.04$ V; $\Delta E(2-3)=0.15$ V; $\Delta E(3-4)=0.24$ V). Because of the small separation between the first and the second reduction, it can be concluded that the first two reductions concern two parallel ligands and that these electrons are coupled only to a very small extent. The next two reductions occur at the remaining two ligands and the negative charge on the ligands is increasingly delocalized over the complex. We assume that up to twelve electrons could be introduced into compound **1**, but the last one is probably taken up at a potential too negative to be observed.

As far as we are aware, this study presents the highest number of well-resolved, completely reversible reductions of a molecular compound. Additionally, the ten successive reduced species derived from **1** exhibit a remarkable stability in solution. Investigations concerning the reduction behavior of $[2 \times 2]$ Co_4^{I} grid-type compounds deposited as monolayers^[16] on graphite are in progress. They should in particular allow one to examine the potential use of the reported extraordinary electrochemical behavior of this class of compounds in future information storage devices.^[17]

Experimental Section

The electrochemical studies were carried out in a 0.1M solution of Bu_4NPF_6 in DMF in a classical three-electrode cell connected to a computerized electrochemical device AUTOLAB (Eco Chemie B.V. Holland). The working electrode was a glassy carbon disc (3 mm diameter), the auxiliary electrode a platinum wire, and the reference electrode an aqueous Ag/AgCl electrode. The measurements were carried out by cyclic and steady-state voltammetry (rotating-disc electrode). Under our experimental conditions, ferrocene, used as internal standard, was oxidized at +0.45 V versus Ag/AgCl. Spectroelectrochemical studies were carried out in a home-made borosilicate glass cell placed in a diode array UV/vis spectrophotometer HP 8453 (Hewlett Packard). A platinum grid (1000 mesh) was placed in the optical pathway (about 0.1 mm) of the OTTE cell (OTTE = optically transparent thin-layer electrode). The auxiliary electrode was a platinum wire and the reference electrode was an aqueous Ag/AgCl electrode.

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Repetitive Application of Perfluoro-Tagged Pd Complexes for Stille Couplings in a Fluorous Biphasic System**

Siegfried Schneider and Willi Bannwarth*

The recovery and reuse of expensive catalysts is an important topic in catalytic processes. Thus, in homogeneous catalysis the use of liquid biphasic systems has attracted a great deal of attention. Examples include the aqueous biphasic Ruhrchemie/Rhône-Poulenc process,^[1] catalytic reactions in supercritical carbon dioxide,^[2] catalysis in molten salts,^[3] and use of fluorous biphasic systems (FBS).^[4]

In the FBS concept, first reported by Horváth and Rábai,^[5] owing to the perfluorinated ligands on the organometallic catalyst it is soluble in a fluorous phase (e.g. $n\text{-C}_6\text{F}_{14}$, C_7F_{16} , $\text{C}_6\text{F}_{13}\text{Br}$, $\text{CF}_3\text{C}_6\text{F}_{11}$). The reaction is carried out in a two-phase mixture consisting of a perfluorinated solvent and an organic solvent (toluene, hexane, acetonitrile). Such a two-phase system often becomes homogeneous at elevated temperature. Lowering the temperature after reaction leads to reformation of the two phases; the organic phase contains the product, the fluorous phase the catalyst to be reused.

This new concept has been applied to a number of catalytic reactions such as hydroformylation of alkenes,^[5] oligomerization of ethylene,^[6] hydroboration of alkenes,^[7] oxidation of aldehydes,^[8] thioethers,^[8] and alkanes,^[9] epoxidations,^[8, 10–12] Wacker oxidation of alkenes,^[13] rhodium-catalyzed carbenoid reaction,^[14] palladium-catalyzed allylic nucleophilic substitution,^[15] cross-coupling of arylzinc bromides with aryl iodides,^[16] and the Heck reaction.^[17]

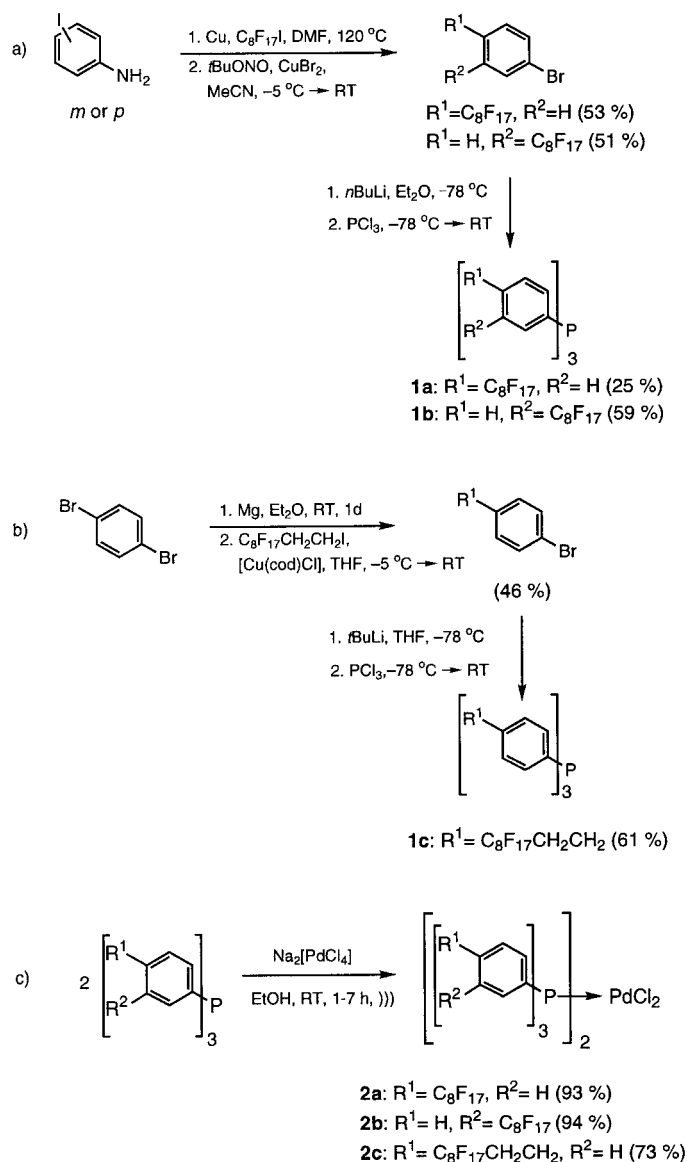
Here, we report on the synthesis of three new fluoro-tagged bis-triphenylphosphanepalladium complexes and their application to the Stille cross-coupling reaction,^[18] with the goal of reusing the catalyst for subsequent cross-couplings.

Fluoro-tagged phosphanes **1a–c** were synthesized with slight modifications of the original procedures^[16, 19] (Scheme 1 a, b). Replacement of the iodine substituent of *m*- or *p*-iodoaniline by the perfluoro tag followed by bromination of the diazonium intermediate delivered the corresponding bromobenzene with the fluoro tag in the *m*- or *p*-position, respectively. Br–Li exchange and reaction with PCl_3 yielded the desired phosphanes **1a** and **1b**. To the best of our knowledge, **1b** has not been reported in the literature. Phosphane **1c** was prepared starting from 1,4-dibromobenzene (Scheme 1 b). Reaction of the corresponding Grignard compound with $\text{C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{I}$ was applied to introduce the perfluoro tag. Br–Li exchange and reaction with PCl_3 led to

[*] Prof. Dr. W. Bannwarth, Dipl.-Chem. S. Schneider^[+]
Universität Freiburg
Institut für Chemie und Biochemie
Albertstrasse 21, 79104 Freiburg (Germany)
Fax: (+49) 761-203-8705
E-mail: willi.bannwarth@organik.chemie.uni-freiburg.de

[+] Present address: Byk-Gulden-Pharmaceuticals
Byk-Gulden-Strasse 2, 78467 Konstanz (Germany)

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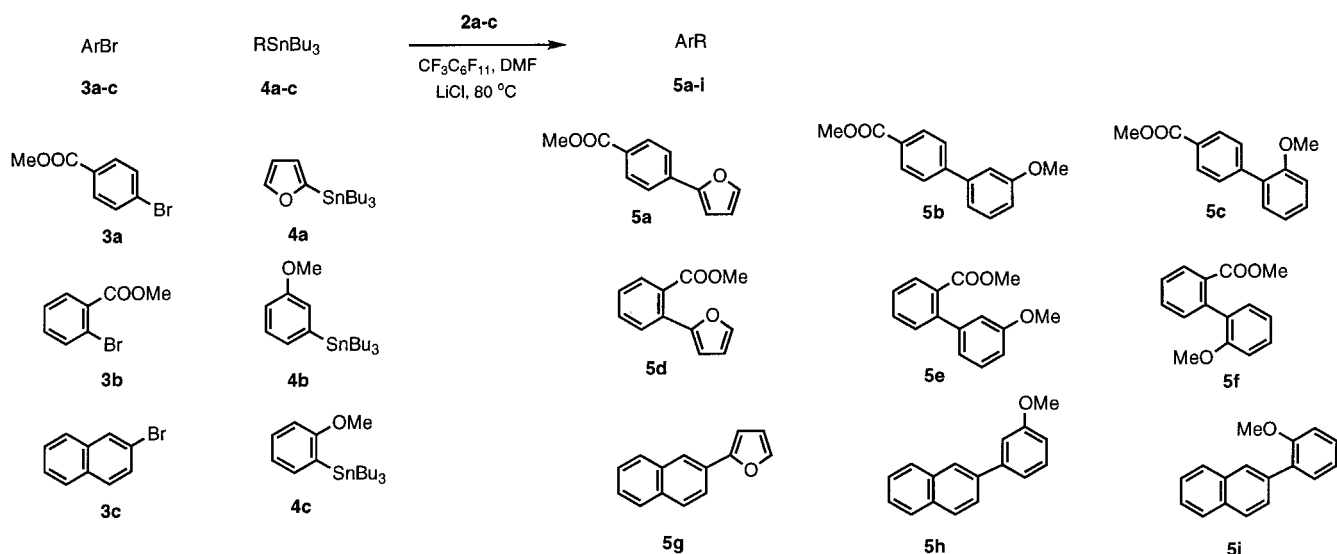
Scheme 1. Synthesis of the ligands **1a–c** and the complexes **2a–c**. cod = 1,5-cyclooctadiene.

phosphane **1c**. The Br–Li exchange was carried out with *t*BuLi since reaction with *n*BuLi led to a severe decrease in the yield of **1c**.

The perfluoro-tagged phosphanes **1a–c** are much more sensitive than unsubstituted triphenylphosphane towards oxidation by air as demonstrated by kinetic studies (³¹P NMR). The oxidation sensitivity decreased in the order **1a** > **1b** > **1c**. The rate of oxidation was dependent on the position of the fluoro tag (cf. **1a** and **1b**), the *p*-isomer **1a** is oxidized faster than the *m*-isomer **1b**. These observations are in agreement with results obtained with trifluoromethyl-substituted triphenylphosphanes.^[20]

The perfluoro-tagged Pd complexes **2a–c** were prepared according to Scheme 1c.^[21] Complex formation was carried out at room temperature with a mixture of Na₂[PdCl₄] and the corresponding fluoro-tagged triphenylphosphane in ethanol under sonication (1–7 h). All three complexes were not air-sensitive and were easy to handle.

The perfluoro-tagged Pd complexes were evaluated in Stille couplings according to Scheme 2. Treatment of three different aryl bromides (**3a–c**) with three different tin compounds (**4a–c**) under standardized conditions in the presence of 1.5 mol % of catalyst **2a–c** led to the desired products **5a–i**. The reactions were carried out in a mixture of DMF and perfluoromethylcyclohexane according to Figure 1. This approach led to an easy recycling of the catalyst which was then reapplied to the same type of Stille coupling under identical conditions. The results are outlined in Table 1. The yields are either based on yields of isolated products or alternatively, if yields were lower, on ¹H NMR spectroscopically determined yields (by comparison of signal intensities of expected product and of the pertinent aryl bromide and the tin compound as starting materials). The results obtained indicate that the perfluoro-tagged Pd complexes **2a–c** were generally suitable for Stille couplings in a solvent system consisting of perfluoromethylcyclohexane in DMF. The yields from the C–C coupling reactions were high for all three complexes except when the stannane **4c** was applied as starting material. This was probably due to steric hindrance encountered during



Scheme 2. Stille coupling with the catalysts **2a–c**.

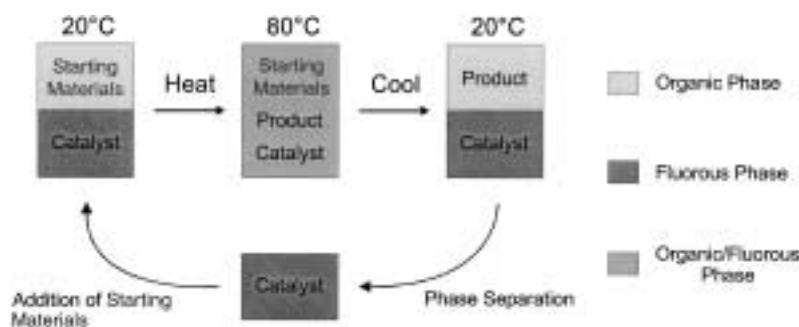


Figure 1. Principle of recycling of the perfluoro-tagged catalysts.

Table 1. Yields of Stille coupling products obtained by employing perfluoro-tagged Pd complexes **2a–c** (reaction times and yields of second and third run in parentheses).

Entry	ArBr	Stannane	Cat.	<i>t</i> [h]	Product	Yield [%]
1a	3a	4a	2a	3 (8, 8)	5a	94 (88, 82)
1b			2b			86 (88, 88)
1c			2c			98 (79, 84)
2a	3a	4b	2a	3 (8, 8)	5b	86 (73, 84)
2b			2b			90 (55, 78)
2c			2c			81 (78, 81)
3a	3a	4c	2a	16 (24)	5c	71 (35)
3b			2b			68 (26)
3c			2c			68 (50)
4a	3b	4a	2a	3 (8, 8)	5d	68 (36, 37)
4b			2b			66 (26, 13)
4c			2c			81 (75, 61)
5a	3b	4b	2a	3 (8, 8)	5e	98 (71, 88)
5b			2b			98 (87, 63)
5c			2c			94 (93, 96)
6a	3b	4c	2a	24 (24)	5f	82 (Spur)
6b			2b			55 (Spur)
6c			2c			70 (70)
7a	3c	4a	2a	3 (8, 8)	5g	90 (89, 76)
7b			2b			84 (78, 79)
7c			2c			91 (93, 95)
8a	3c	4b	2a	3 (8, 8)	5h	74 (67, 55)
8b			2b			63 (62, 32)
8c			2c			74 (64, 66)
9a	3c	4c	2a	20 (24, 24)	5i	49 (43, 44)
9b			2b			39 (10, 10)
9c			2c			50 (58, 49)

coupling in the *o*-position. Longer reaction times were necessary in these reactions to obtain decent yields.

Furthermore, the complexes can be reused after phase separation according to Figure 1. Longer reaction times were employed in run 2 and 3 to compensate for the loss of activity of the catalysts. When relatively low yields were obtained in the first run, the decrease in the yield for the following runs was more drastic for complexes **2a** and **2b** than for **2c**. Recycling of the complexes was not efficient when stannane **4c** was used.

In a further set of experiments we applied the catalysts **2a–c** in repetitive Stille couplings according to Figure 1 leading to **5a**. Identical reaction times were used for all runs (3 h). As can be deduced from Figure 2 the complexes were recycled five times. All three complexes afforded comparable yields in all the runs and only a slight decrease in yield was observed for all three complexes from run 1 to run 6.

The presented three new perfluoro-tagged Pd complexes are suitable as catalysts in Stille couplings in a fluorous biphasic system. They can be recycled and reused after phase separation so that they can be applied up to six times without significant reduction in yield. For all three complexes the yields in these experiments were nearly identical.

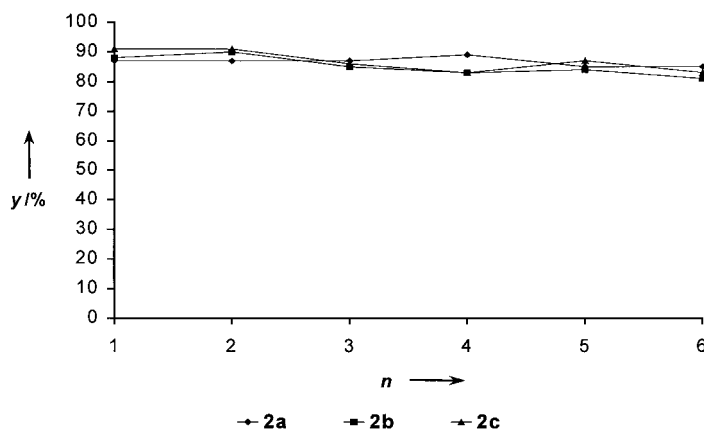


Figure 2. Yields of **5a** after reuse of the catalysts **2a–c**. *n*: number of run; *y*: yield. The yields in the first run are not identical with those in Table 1 because they were determined in a different set of experiments.

Experimental Section

1b: *n*BuLi (6.00 mL of a 1.6 M solution in *n*-hexane) was added over 1 h to a stirred solution of 1-bromo-3-(1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluorooctyl)benzene (5.00 g, 8.70 mmol) in anhydrous Et₂O (30 mL) under argon at -78°C . After the mixture had been stirred for 2 h, PCl₃ (7.25 mL of a 0.40 M solution in diethyl ether) was added dropwise. The mixture was allowed to warm up to room temperature overnight. The mixture was hydrolyzed with a saturated NH₄Cl solution (15 mL) and extracted with diethyl ether (3 $\times$ 20 mL). The combined organic layers were dried over MgSO₄ and evaporated to dryness. The residue was taken up in a small amount of diethyl ether and filtered over silica with *n*-hexane as solvent. After evaporation phosphane **1b** (2.60 g, 59 %) was obtained. M.p. $84-86^{\circ}\text{C}$; ³¹P NMR (80 MHz, CDCl₃): $\delta = -5.7$; ¹H NMR (200 MHz, CDCl₃): $\delta = 7.35$ (d, *J* = 6.2 Hz, 3 H); 7.54–7.65 (m, 9 H); elemental analysis (%) for C₄₂H₁₂F₅₁P (1516.43): calcd: C 33.27, H 0.80; found: C 33.28, H 0.83.

1a: M.p. $92-95^{\circ}\text{C}$; ³¹P NMR (80 MHz, CDCl₃): $\delta = -5.6$; ¹H NMR (200 MHz, CDCl₃): $\delta = 7.41$ (t, *J* = 7.5 Hz, 6 H); 7.60 (d, *J* = 7.8 Hz, 6 H); elemental analysis (%) for C₄₂H₁₂F₅₁P (1516.43): calcd: C 33.27, H 0.80; found: C 33.55, H 0.97.

1c: M.p. $89-91^{\circ}\text{C}$; ³¹P NMR (80 MHz, CDCl₃): $\delta = -7.1$; ¹H NMR (200 MHz, CDCl₃): $\delta = 2.24-2.50$ (m, 6 H, CH₂), 2.87–2.96 (m, 6 H, CH₂), 7.17–7.37 (m, 12 H, aryl-H).

2a: A suspension of the phosphane **1a** (1.05 mmol) and Na₂[PdCl₄] (0.500 mmol) in ethanol was sonicated for 7 h under argon. The color changed from red-brown to yellow. The yellow precipitate was filtered off, washed with water, ethanol, diethyl ether, and dried (Yield: 93 %). M.p. $186-187^{\circ}\text{C}$; ³¹P NMR (80 MHz, CDCl₃): $\delta = 23.6$; elemental analysis (%) for C₈₄H₂₄F₁₀₂P₂PdCl₂ (3210.20): calcd: C 31.43, H 0.75, Cl 2.21; found: C 31.20, H 0.80, Cl 2.06.

The complexes **2b** and **2c** were prepared analogously. **2b**: Yield: 94 %. M.p. $134-136^{\circ}\text{C}$; ³¹P NMR (80 MHz, CDCl₃): $\delta = 25.0$; elemental analysis (%) for C₈₄H₂₄F₁₀₂P₂PdCl₂ (3210.20): calcd: C 31.43, H 0.75, Cl 2.21; found: C 31.52, H 0.81, Cl 2.19.

2c: Yield 73 %. M.p. $182-184^{\circ}\text{C}$; ³¹P NMR (80 MHz, CDCl₃): $\delta = 22.0$; ¹H NMR (200 MHz, CDCl₃): $\delta = 2.06$ (m, br. 12 H); 2.59 (m, br. 12 H); 7.06 (m, br.

12H); 7.81 (m, br. 12H); elemental analysis (%) for $C_{96}H_{48}F_{102}P_2PdCl_2$ (3378.50): calcd: C 34.13, H 1.43, Cl 2.10; found: C 33.91, H 1.46, Cl 2.37.

General coupling procedure (**5a**): To a suspension of catalyst **2c** (10.1 mg, 0.003 mmol) in perfluoromethylcyclohexane (1.2 mL) was added under argon a solution of methyl 4-bromobenzoate **3a** (43.0 mg, 0.200 mmol), tributylstannylfuran **4a** (85.7 mg, 0.240 mmol), and LiCl (8.5 mg, 0.200 mmol) in DMF (1 mL). The mixture was heated at 80 °C for 3 h. After cooling to room temperature the two phases were separated and the fluorine phase was washed with DMF (3 × 1 mL). The fluorine phase containing the catalyst was removed and used as such for the next run. For isolation of product **5a** water (7.5 mL) was added to the DMF layer. After extraction with diethyl ether (4 × 2 mL), the combined diethyl ether solutions were evaporated. The residue was taken up in diethyl ether (1 mL) and aqueous KF solution (5 mL) was added. After the mixture had been stirred overnight, it was extracted with diethyl ether (4 × 2 mL), and the combined diethyl ether layers were passed through a plug consisting of Alox (2 g; neutral, Act. II-III) and silica (4 g). The filtrate was evaporated yielding the desired product **5a** (36.8 mg; 91 %).

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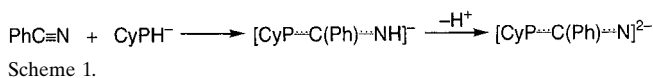
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A Li₁₀ Cage Containing the [C₆H₁₁P≡C(Ph)≡N]²⁻ Ion: Umpolung in the Oligomerization of a Nitrile**

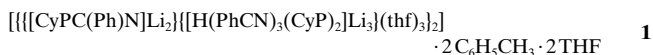
Neil Feeder, Yvonne G. Lawson, Paul R. Raithby, Jeremy M. Rawson, Alexander Steiner, Jody A. Wood, Anthony D. Woods, and Dominic S. Wright*

Studies of oligomeric imido alkali metal complexes $[(R')RC\equiv NLi]_n$, which are readily prepared by the equimolar reactions of organoalkali metal reagents R'M (M = Li–Cs) with organic nitriles (RC≡N), have been fundamental in the development of structural models for a host of related alkali metal complexes.^[1] In contrast to the wealth of knowledge concerning the reaction characteristics of organoalkali metal reagents with nitriles,^[1,2] only a few studies have investigated the analogous addition reactions with alkali metal phosphides RR'PM.^[3] Surprisingly, although alkali metal complexes containing $[R^1P\equiv C(R^2)\equiv NR^3]^-$ monoanions have been prepared and structurally characterized previously, to our knowledge there are no reports of dianions of the type $[R^1P\equiv C(R^2)\equiv N]^{2-}$. Our interest in the latter stems from their potential application as precursors for the synthesis of main group heterocycles.

Accordingly, we carried out the reaction of CyPHLi (Cy = C₆H₁₁) with PhC≡N, followed by deprotonation of the presumed intermediate anion $[CyP\equiv C(Ph)\equiv NH]^-$ ^[3b] with *n*BuLi (Scheme 1; see Experimental Section). The product of



this reaction, however, is the cage complex **1** containing not only the desired dianion $[CyP\equiv C(Ph)\equiv N]^{2-}$ but also the

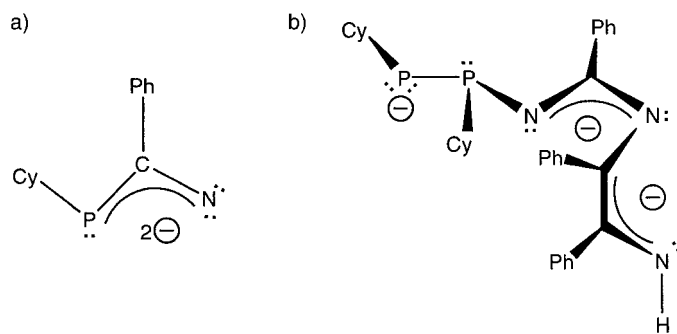


trianion $[H(PhCN)_3(CyP)_2]^{3-}$, in which C–C bond formation between two PhCN molecules has occurred (Scheme 2). The observation of umpolung (polarity reversal) in the reaction forming this trianion contrasts with the normal mode of oligomerization of nitriles, which involves “head-to-tail” C–N

[*] Dr. D. S. Wright, N. Feeder, Dr. Y. G. Lawson, Dr. P. R. Raithby, Dr. J. M. Rawson, J. A. Wood, A. D. Woods
Department of Chemistry
University of Cambridge
Lensfield Road, Cambridge CB2 1EW (UK)
Fax: (+44) 1223-336362
E-mail: dsw1000@cus.cam.ac.uk

Dr. A. Steiner
Department of Chemistry
University of Liverpool
Crown Street, Liverpool L69 7ZD (UK)

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Scheme 2. The bonding within a) the dianion, and b) the trianion present in **1**.

bond formation (as occurs in the cyclotrimerization of PhCN to triazines or lithiotriazines in the presence of organolithium reagents^[2b,c]). The formation of a C–C bond in the trianion prompted us to investigate whether a single-electron transfer (SET) mechanism is involved, which competes with the anion mechanism responsible for the formation of the $[\text{CyP}\equiv\text{C}(\text{Ph})\equiv\text{N}]^{2-}$ ion. Similar competition is well known in reactions of ketones with Grignard reagents,^[4] and the reactions of $\text{Ph}_2\text{C}=\text{O}$ with a range of lithium amides occur through the $[\text{Ph}_2\dot{\text{C}}-\text{O}]^-$ radical anion (with the formation of C–C coupled by-products).^[5] A further clue to the participation of radicals in the reaction forming **1** is the rapid development of a sustained (> 2 h), deep green color upon addition of *n*BuLi to the mixture of PhCN and CyPHLi. Prior to the addition of *n*BuLi, the orange mixture of PhCN and CyPHLi was ESR silent. However, on addition of *n*BuLi the ESR spectrum of the green reaction mixture indicated the possibility of a P-centered radical in low concentration (doublet, $g=2.00$ G, $a_p=33$ G). This radical (or others too short-lived to be observed) could be involved in single-electron reduction of PhCN, with the combination of two $[\text{Ph}\dot{\text{C}}=\text{N}]^-$ radicals resulting in the observed C–C coupling in the trianion of **1**.^[6] The room-temperature ^{31}P NMR spectrum of the initial mixture of PhCN and CyPHLi shows the presence of unreacted CyPHLi ($\delta=-111.9$) and $[\text{CyP}]_4$ ($\delta=-68.3$). However, the major product is the monoanion $[\text{CyP}\equiv\text{C}(\text{Ph})\equiv\text{NH}]^-$,^[3b] the chemical shift involved ($\delta=59.4$) being similar to that found for related anions (e.g., $\delta=34.3$ for $[\text{PhP}\equiv\text{C}(\text{Me})\equiv\text{NH}]^-$ ^[3b] and as high as 65.2 in similarly substituted 1-phosphallyl anions^[7]). Interestingly, the $[\text{CyP}\equiv\text{C}(\text{Ph})\equiv\text{N}]^{2-}$ ion is also a minor product at the initial stage of the reaction ($\delta=192.8$). After the second stage of the reaction is complete (i.e., the green paramagnetic intermediate has faded) the resonance signal of the monoanion has almost disappeared and the resonance signal of the dianion dominates the spectrum; additional resonances ($\delta=38.4$ and 19.4 (ratio 1:1)) are tentatively assigned to the P–P portion of the trianion of **1**. Unfortunately, owing to the very low solubility of **1** once isolated, full confirmation of these ^{31}P NMR assignments has not been possible. However, the appearance of a singlet resonance at $\delta=200.0$ for **1** in DMSO confirms our assignment for the dianion.

The unambiguous characterization of **1** by NMR and elemental analysis was hindered by extensive desolvation of

the complex once isolated under vacuum. The true nature and complexity of this species only became apparent following the structural characterization. Although crystals of **1** are relatively weakly diffracting, the essential structural features of the complex are determined unambiguously.^[8] Centrosymmetric molecules of **1** are composed of two $[\text{CyP}\equiv\text{C}(\text{Ph})\equiv\text{N}]^{2-}$ ions and two $[\text{H}(\text{PhCN})_3(\text{CyP})_2]^{3-}$ ions associated by ten Li^+ ions into an elaborate cage structure (Figure 1 a). In addition, there are two THF molecules and two toluene molecules present in the lattice. The bond lengths found in the $[\text{CyP}\equiv\text{C}(\text{Ph})\equiv\text{N}]^{2-}$ ion of **1** (P(1)–C(1) 1.83(1), C(1)–N(1) 1.27(1) Å) are similar to those observed previously in $[\text{R}^1\text{P}\equiv\text{C}(\text{R}^2)\equiv\text{NR}^3]^-$ monoanions (P–C ca. 1.75–1.79, C–N 1.30–1.35 Å^[3b-d]). The N–C–P angle in the dianion (122.0(7)°) is significantly smaller than that observed for the most closely related $[\text{PhP}\equiv\text{C}(\text{Me})\equiv\text{NH}]^-$ monoanion (132.0(3)°),^[3b] but similar to that observed for silylated monoanions chelated to Group 2 ions.^[3d] The bond lengths within the $[\text{H}(\text{PhCN})_3(\text{CyP})_2]^{3-}$ ion indicate that the charge is located essentially in three distinct regions of the chain (Scheme 2). Although shorter than the value expected for a P–P single bond (ca. 2.21 Å^[9]), the P–P bond length in the $[\text{CyP}]_2$ unit [2.170(4) Å] is similar to that in the $[\text{tBuPPtBu}(\text{H})]^-$ ion (2.150(5) Å),^[10] suggesting that the negative charge is more or less concentrated on the terminal P atom. A further negative charge is delocalized over the N(2)–C(2)–N(3) fragment of the chain; the C–N bond lengths within this unit (C(2)–N(2) 1.32(1), C(2)–N(3) 1.35(1) Å) are typical of those found in amidines.^[11] The final negative charge is found within the C(3)–C(4)–N(4) unit; the C–C (1.40(1) Å) and C–N (1.38(1) Å) bonds involved are similar to those present in aza-allyl complexes.^[12] There appears to be little additional delocalization between the amidine and aza-allyl units, judging by the large C(3)–N(3) bond length (1.45(1) Å; cf mean 1.35 Å for the other C≡N bonds in the trianion) and the acute angle at N(3) (115.8(7)°). In addition, the nonplanar geometry of the chain precludes alignment of the p orbitals between these units.

Each of the five Li^+ ions of the asymmetric unit of **1** has a distinct coordination environment (Figure 1 b). Overall, the connectivities of the three- and four-coordinate Li^+ ions present reflect the charge distributions found in the dianion and trianion (Scheme 2). The coordination of Li(1) is particularly noteworthy. In addition to its bonding to the P(1) center of a dianion (2.45(2) Å^[13]) and the amidine N(2) center (1.93(2) Å) of a trianion, there is an allylic interaction with C(3) (2.41(2) Å) and the *ipso*-C (C(61)) and *ortho*-C (C(66)) centers of the attached phenyl ring (each ca. 2.52 Å).

In summary, the results reported here show that nucleophile-induced oligomerization of organic nitriles does not always occur by head-to-tail C–N bond formation. The presence of the two different anions in **1** can be ascribed to competing nucleophilic and SET mechanisms. However, clearly further mechanistic studies will be required to elucidate the exact mechanism and the extent to which radicals are involved. The $[\text{CyP}\equiv\text{C}(\text{Ph})\equiv\text{N}]^{2-}$ ion in **1** is the first example of such a dianion to be structurally characterized.

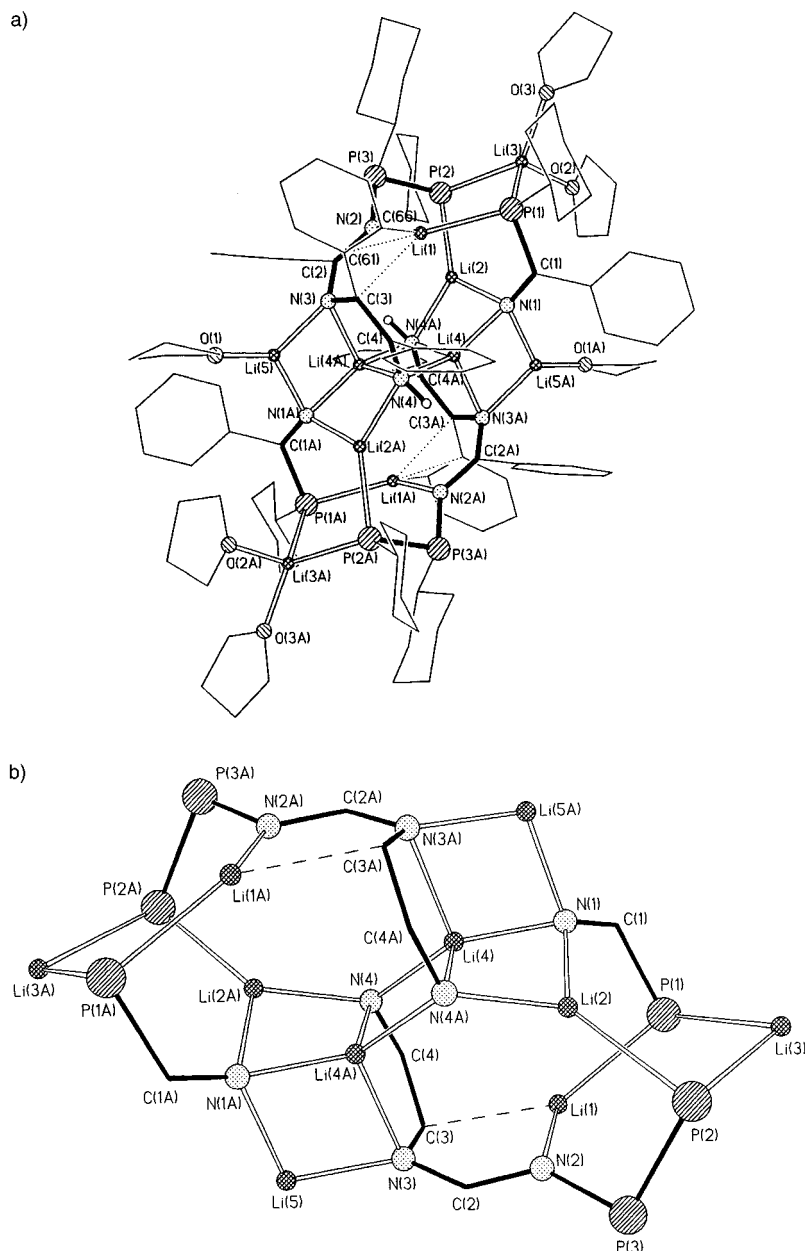


Figure 1. a) Molecular structure of **1**. Hydrogen atoms (except those terminating the trianion chain) and lattice-bound THF and toluene molecules have been omitted for clarity. Key bond lengths [Å] and angles [°]: Li(1)–P(1) 2.45(2), Li(1)–N(2) 1.93(2), Li(1)–C(3) 2.41(2) Li(1)–C(61,66) 2.52 Å (av), Li(2)–N(1) 2.03(2), Li(2)–P(2) 2.50(2), Li(2)–N(4A) 2.11(2), Li(3)–P(1) 2.62(2), Li(3)–P(2) 2.58(2), Li(3)–O(2,3) 1.96 (av), Li(4)–N(4) 2.09(2), Li(4)–N(4A) 2.06(2), Li(4)–N(1) 1.94(2), Li(4)–N(3A) 2.14(2), Li(5)–N(1A) 2.03(2), Li(5)–N(3) 2.01(2), Li(5)–O(1) 1.92(2), P(1)–C(1) 1.83(1), N(1)–C(1) 1.27(1), N(4)–C(4) 1.38(1), C(4)–C(3) 1.40(1), C(3)–N(3) 1.45(1), N(3)–C(2) 1.35(1), C(2)–N(2) 1.32(1), N(2)–P(3) 1.750(8), P(3)–P(2) 2.170(4); P(1)–C(1)–N(1) 122.0(7), N(4)–C(4)–C(3) 122.7(8), C(4)–C(3)–N(3) 118.9(8), C(3)–N(3)–C(2) 115.8(7), N(3)–C(2)–N(2) 125.2(8), C(2)–N(2)–P(3) 121.9(7), N(2)–P(3)–P(2) 102.3(3). b) Core structure of **1** showing the connectivities of the Li⁺ ions.

Experimental Section

1: *n*BuLi (4.70 mL, 7.0 mmol, 1.5 mol L^{−1} in hexanes) was added to a solution of CyPH₂ (0.93 mL, 7.0 mmol) in toluene (10 mL) at −78 °C. A light yellow precipitate of [CyPHLi] was produced by stirring the mixture at room temperature (ca. 30 min). Addition of PhCN (0.72 mL, 7.0 mL) to the mixture at −78 °C gave an immediate red coloration. Stirring at room temperature (ca. 40 min) gave a red-brown solution with a brown suspension, which was completely dissolved by the addition of THF (2.0 mL). Addition of *n*BuLi (4.70 mL, 7.0 mmol, 1.5 mol L^{−1} in hexanes) to

this solution at −78 °C gave a deep orange-red solution. As the temperature was allowed to increase to about 0 °C, a deep green coloration began to develop. Stirring for about 2 h at room temperature led to gradual fading of the green intermediate and finally a deep red solution was obtained. The solvent was removed under vacuum and the glassy red oil was dissolved in toluene (5 mL) and THF (0.5 mL). Storage of the red solution (5 °C, up to two weeks) gave small light orange crystals of **1**. Elemental analysis and the ¹H NMR spectrum reveal that only traces of THF and toluene are present in the complex once isolated under vacuum (15 min, 10^{−1} atm). Yield 0.16–0.23 g (12–17%, based on PhCN consumed, for unsolvated complex). M.p. 225–230 °C (decomp). IR (NaCl, Nujol): $\tilde{\nu}$ = 3374 (vw; =N–H str.), 3050–3023 (w; aryl C–H), 1599 (w.sh), 1594 (m), 1570 (w; C≡N str.), 1480 (s; C≡C str.), other bands at 1203 (w), 1069 (m), 1037 (s), 885 (m), 831 (m), 782 (s), 764 (vs), 719 (vs) cm^{−1} (air-exposure led to an increase in intensity of the =N–H band and the appearance of Li–OH stretching frequency at 3678 cm^{−1}). ¹H NMR (400.13 MHz, [D₆]DMSO, +25 °C): δ = 8.0–7.0 (overlapping m, 20H; Ph), 1.70–0.90 (overlapping m, 33H; Cy). elemental analysis: calcd for unsolvated **1**: C 70.0, H 6.7, N 7.1; found: C 68.5, H, 6.2, N, 8.0. **Caution**: ignites extremely violently in acids, therefore no P analysis could be obtained.

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weakly and the data is relatively poor). Final $R1 = 0.096$ [$I > 2\sigma(I)$] and $wR2 = 0.265$ (all data).^[14] All four of the C atoms of the THF molecule bonded to Li(5) are disordered over 50:50 sites. The H atom attached to N(4), which is inferred from the IR spectrum of **1**, was located in the final difference map. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-144824. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

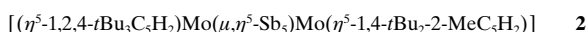
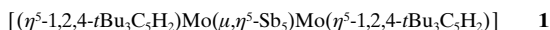
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Stabilization of a Pentastibacyclopentadienyl Ligand in the Triple-Decker Sandwich Complexes $[(\eta^5\text{-1,2,4-}t\text{Bu}_3\text{C}_5\text{H}_2)\text{Mo}]_2\text{-(}\mu\text{-}\eta^5\text{-Sb}_5\text{)}$ and $(\eta^5\text{-1,2,4-}t\text{Bu}_3\text{C}_5\text{H}_2)\text{-Mo}(\mu\text{-}\eta^5\text{-Sb}_5)\text{Mo}(\eta^5\text{-1,4-}t\text{Bu}_2\text{-2-MeC}_5\text{H}_2)^{**}$

Hans Joachim Breunig, Neil Burford, and Roland Rösler*

Triple-decker sandwich complexes containing *cyclo*-($\eta^n\text{-E}_n$) ligands ($n = 5, 6$) are well known for $E = \text{P}, \text{As}$.^[1–3] Although elemental pnictogens (white phosphorus, P_4 , and yellow arsenic, As_4) are the most obvious reagents and have been used in many cases, organophosphorus^[4] and organoarsenic^[1] rings, and $[(\text{CO})_5\text{CrPCL}_3]$ ^[5] are also viable sources of phosphorus and arsenic elemental ligands. Since an Sb_4 molecule is

not available for synthetic purposes, other starting materials have been used for the preparation of complexes with substituent-free antimony ligands: both metallic antimony^[6a] and *cyclo*- Cp^*Sb_4 ^[6b] ($\text{Cp}^* = \text{Me}_5\text{C}_5$) gave complexes containing Sb_2 fragments. *cyclo*- $t\text{Bu}_4\text{Sb}_4$ ^[7] proved to be a versatile reagent in the synthesis of complexes containing both Sb_2 and *cyclo*- Sb_3 ligands.^[8] Compounds containing *cyclo*- Sb_5 ligands were observed as decomposition products in the mass spectra of clusters containing Sb_2 and Sb_3 fragments but have not yet been identified in the condensed phase.^[8b] We report here on the stabilization of the pentastibacyclopentadienyl ligand *cyclo*-($\eta^5\text{-Sb}_5$) as the middle-deck in the triple-decker sandwich complexes **1** and **2**.



Complexes **1** and **2** are formed when a mixture of *cyclo*- $t\text{Bu}_4\text{Sb}_4$ and $[\text{Cp}''(\text{CO})_3\text{MoCH}_3]$ ($\text{Cp}'' = \eta^5\text{-1,2,4-}t\text{Bu}_3\text{C}_5\text{H}_2$)^[9] is heated in decaline. It is not surprising that a by-product of the thermal reaction is $[(\text{Cp}''\text{Mo}(\text{CO})_2)_2]$ ($\text{Mo} \equiv \text{Mo}$) (**3**). Unexpected, however, is the formation of the complex **2** by replacement of one of the *tert*-butyl groups in position 1 or 2 on one of the cyclopentadienyl rings in the compound **1** by a methyl group. The triple-decker sandwich complexes **1** and **2** could not be separated by chromatography. After chromatographic separation from the reaction mixture an air-sensitive, dark green, microcrystalline solid containing both **1** and **2** was obtained in about 18% overall yield. The two complexes are very soluble in hydrocarbons, giving emerald-green solutions that are air-sensitive. These solutions immediately change color to brown when exposed to air. A few relatively large single crystals of **2** were grown from a solution of the mixture in hexane. The identity of the compounds was confirmed by elemental analysis and by ^1H NMR and IR (**3**) spectroscopy and mass spectrometry. The mass spectra suggested a mole ratio of 4:1 (**1:2**) in the mixture. The complexes **1** and **2** have a remarkable thermal stability, the mixture melting without significant decomposition at 331–333 °C and subliming at 260 °C/10^{–3} Torr.

The crystal structure of **2** was determined by X-ray crystallography.^[10] The molecule has a typical triple-decker sandwich structure (Figure 1), with the bulky cyclopentadienyl rings almost parallel to each other (dihedral angle 5.3°) and to the Sb_5 ring (3.2° each). The Sb_5 middle deck is very slightly folded towards Mo(2), with Sb(2) lying 20 pm out of the plane formed by the other four antimony atoms. The Sb–Sb bonds (275.59(11) and 276.56(9) pm) involving Sb(2) are shorter than the single bonds in *cyclo*- $t\text{Bu}_4\text{Sb}_4$ (Sb–Sb 281.7(2)–282.1(2) pm)^[11a] or *cyclo*-($(\text{Me}_3\text{Si})_2\text{CH}$) $_4\text{Sb}_4$ (282.1(1)–287.8(1) pm),^[11b] and similar to those in the clusters $[\text{Cp}(\text{CO})_2\text{MoSb}_3]$ (273.45(10)–278.05(11) pm) and $[\text{Cp}^*(\text{CO})_2\text{MoSb}_3]$ (273.97(9)–276.82(8) pm).^[8a] They are considerably longer than an Sb–Sb double bond (264.2(1) pm in $\{2,4,6\text{-}[(\text{Me}_3\text{Si})_2\text{CH}]_3\text{C}_6\text{H}_2\}_2\text{Sb}_2$).^[12] The Sb–Sb bonds involving Sb(5) are longer (284.24(9) and 285.04(9) pm), their lengths falling in the normal range for Sb–Sb single bonds. The Sb(3)–Sb(4) bond length has an intermediate value (279.78(8) pm). A

[*] Dr. R. Rösler, Prof. Dr. N. Burford
Dalhousie University, Department of Chemistry
Halifax, NS, B3H 4J3 (Canada)
Fax: (+1) 902-494-1310
E-mail: rroesler@is.dal.ca

Prof. Dr. H. J. Breunig
Fachbereich 2 der Universität Bremen
Institut für Anorganische und Physikalische Chemie
Postfach 330440, 28334 Bremen (Germany)

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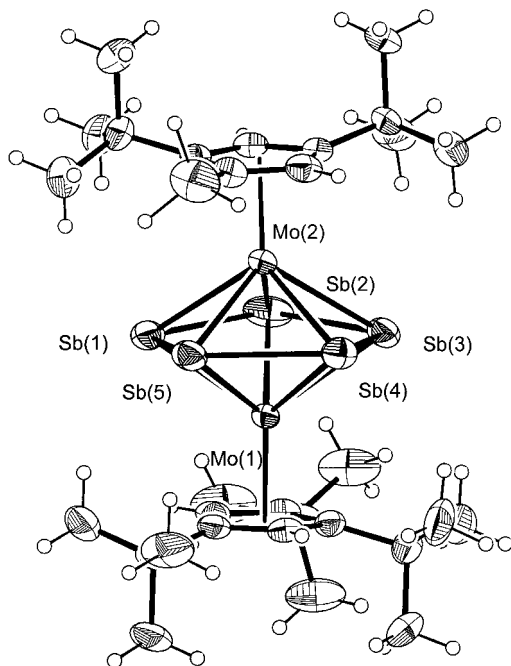


Figure 1. Molecular structure of **2** in the crystal. Selected bond lengths [pm] and angles [°]: Sb(1)–Sb(2) 275.59(11), Sb(2)–Sb(3) 276.56(9), Sb(3)–Sb(4) 279.78(8), Sb(4)–Sb(5) 284.24(9), Sb(1)–Sb(5) 285.04(9), Mo(1)–Mo(2) 296.40(8), Mo(1)–Sb 277.40(9)–290.23(8), Mo(2)–Sb 272.76(8)–283.92(9), Mo–C_{centroid} 201.1 (av); Sb(1)–Sb(2)–Sb(3) 108.06(3), Sb(2)–Sb(3)–Sb(4) 110.13(3), Sb(3)–Sb(4)–Sb(5) 105.82(2), Sb(4)–Sb(5)–Sb(1) 107.63(3), Sb(5)–Sb(1)–Sb(2) 107.86(2), Mo(1)–Sb–Mo(2) 62.43(2)–65.19(2), Sb–Sb–Mo 57.40(2)–63.28(2).

similar but folded five-membered ring is present in an Sb₅^{5−} Zintl ion, whose Sb–Sb bonds are on average 5 pm longer than those in **2**.^[13]

Although the Sb₅ ring in **2** is not as regular as the pnictogen rings in most of the analogous P and As complexes,^[3] its distortion is less dramatic than for [CpMo(μ,η^5 -As₅)MoCp],^[1] where an As₂+As₃ arrangement was observed (As–As 238.9(2)–276.2(3)). This is contrary to predictions, based on theoretical calculations, of relatively stronger Jahn–Teller distortions for an Sb₅ ligand in a complex of type **2** compared to its P and As analogues.^[14] The pronounced deformation of the arsenic middle deck in [CpMo(μ,η^5 -As₅)MoCp] is likely due to the strong intermolecular interactions involving the As₂ fragments, and not to the Jahn–Teller distortion. In the solid state, the molecules of [CpMo(μ,η^5 -As₅)MoCp] associate to dimers over As⋯As contacts (301, 319, 320, 321 pm; sum of van der Waals radii for arsenic is 400 pm); one of these interactions is the shortest intermolecular As⋯As contact known to date. The two molecules of a dimer lie with the pseudo-C₅ axes perpendicular to each other, the As₂ units building a distorted As₄ tetrahedron. Long intermolecular Sb⋯Sb contacts^[15] (397.2, 404.9, 411.0, and 432.6 pm; sum of van der Waals radii for antimony is 440 pm) are responsible for undulating chains of molecules throughout the lattice of **2** and probably determine the slight distortion of the Sb₅ ring.

The Mo(1)–Mo(2) distance (296.40(8) pm) in **2** is considerably longer than that in [CpMo(μ,η^5 -As₅)MoCp] (276.4(2) pm) and corresponds to that of a Mo–Mo single

bond.^[1] The Sb–Mo bond lengths range between 272.76(8) and 290.23(8) pm, and are comparable to other Sb–Mo bond lengths in complexes with substituent-free antimony ligands: 280.8 pm (av) in [[CpMo(CO)₂]₂Sb₂],^[6a] 287.88 pm (av) in [Cp*Mo(CO)₂Sb₃],^[8a] and 290.06 pm (av) in [Cp*Mo(CO)₂-Sb₃].^[8a] An indication for the steric strain in the molecule is the strong bending (12.5–17.4°) of the *tert*-butyl groups outside the Cp plane. Both cyclopentadienyl rings are twisted (14.7 and −9.1°) around the Mo(1)–Mo(2) axis with respect to the Sb₅ ring.

Complex **2** (and very likely, **1**) has 27 valence electrons (3 × 5 + 2 × 6, cf. P and As analogues).^[2b, 3] The broadening of the signals in the ¹H NMR spectrum of **1** and **2**, as well as the absence of signals in the ¹³C NMR spectrum are in agreement with the clusters being paramagnetic.

The observations on the stability of complexes with Sb_n ligands^[8] suggest that, in contrast to the P and As analogues, an efficient steric protection is a condition for the isolation of triple-decker sandwich complexes containing the *cyclo*-Sb₅ ligand. Although such clusters possess a remarkable thermal stability in the gas phase, without efficient steric protection by bulky cyclopentadienyl groups they are likely to suffer decomposition in a condensed phase, with formation of metallic antimony. The same trend is shown by the molecules P₄, As₄, and Sb₄. It is to be seen if appropriate steric protection will enable the isolation of a cluster containing a *cyclo*-Sb₆ ligand, and if bismuth can be involved in a similar chemistry.

Experimental Section

1–3: All operations were carried out with careful exclusion of air in an argon atmosphere or in vacuo. *cyclo*-tBu₄Sb₄ (1.88 g, 2.63 mmol) and [Cp*Mo(CO)₃MoCH₃] (1 g, 2.33 mmol) were stirred under reflux in decaline (60 mL) for about 3 h. The solvent was removed in vacuo and the residue extracted with hexane (3 × 100 mL). The green-yellow extracts were combined, Al₂O₃ (8 g) was added and the solvent was removed under reduced pressure. The residue was placed on a chromatography column (3 × 20 cm Al₂O₃ neutral, particle size 0.063–0.200 mm, degree of activity II/Brockmann). An emerald-green fraction was eluted with hexane/toluene (99/1). Removal of the solvent in vacuo and recrystallization of the residue from hexane at −30 °C gave 265 mg of a microcrystalline green solid containing both **1** and **2**. Dissolution in toluene followed by slow evaporation in vacuo produced a mixture of a microcrystalline powder with larger crystals, which were selected for X-ray crystallography and shown to be **2**. **1 + 2:** M.p. 331–333 °C; elemental analysis (%): calcd for C₃₄H₅₈Mo₂Sb₅, C 32.22, 30.39, H 4.61, 4.29; found: C 32.18, H 4.63; ¹H NMR (250 MHz, C₆D₆, 25 °C): δ = 0.91 (br. s), 1.35 (br. s), 1.38 (br. s), 3.39 (br. s), 3.58 (br. s), 3.62 (br. s), 3.77 (br. s), 29.04 (br. s); MS (EI, 70 eV): *m/z* (%): 1266 (100) [**1**, M⁺], 1224 (25) [**2**, M⁺], 1195 (20) [**1**, M⁺ − C₅H₁₁], 1153 (10) [**2**, M⁺ − C₅H₁₁], 1122 (20), 57 (80) [tBu⁺]. With hexane/toluene (9/1) was eluted a yellow-brown fraction which left behind, after removal of the solvent, **3** as a dark brown solid. Crystals were obtained by cooling a solution in hexane to −30 °C. Yield: 205 mg (22.8 %). **3:** M.p. 185–190 °C; elemental analysis (%): calcd for C₃₈H₅₈Mo₂O₄: C 59.22, H 7.58; found: C 60.48, H 7.64; ¹H NMR (250 MHz, C₆D₆, 25 °C): δ = 1.25 (s, 9H; tBu), 1.43 (s, 18H; tBu), 5.23 (s, 2H; C₅H₂); ¹³C NMR (63 MHz, C₆D₆, 25 °C): δ = 31.4, 32.1, 33.8, 34.3 (tBu), 96.4, 118.3, 123.2 (C₅H₂), 242.4 (CO); IR (Nujol): $\tilde{\nu}$ = 1943, 1878, 1819, 1802 cm^{−1} (C=O); MS (EI, 70 eV): *m/z* (%): 770 (80) [M⁺], 742 (60) [M⁺ − CO], 714 (40) [M⁺ − 2 CO], 686 (30) [M⁺ − 3 CO], 650 (43), 57 (100) [tBu⁺].

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- [10] X-ray crystal structure analysis of **2**: Bruker P4/RA/SMART 1000 CCD diffractometer, Mo_{Kα} radiation, $\lambda = 71.073$ pm, scan mode ϕ/ω , $T = 193(2)$ K, absorption correction SADABS, method of structure solution: direct methods, program used for structure solution SHELXS-97,^[16] method of refinement full-matrix least-squares at F^2 , refinement program SHELXL-97, hydrogen atoms geometrically positioned and refined with a riding model. Crystal dimensions $0.11 \times 0.17 \times 0.22$ mm³, crystal system monoclinic, space group $P2_1/n$, $a = 1320.30(6)$, $b = 1002.10(5)$, $c = 2834.96(12)$ pm, $\beta = 95.429(1)^\circ$, $V = 3.7340(3)$ nm³, $Z = 4$, $\rho_{\text{calc}} = 2.180$ Mg m⁻³, $\theta_{\text{max}} = 26.40^\circ$, 18091 measured reflections, 7653 independent intensities ($R_{\text{int}} = 0.0343$), absorption coefficient 4.230 mm⁻¹, number of free parameters 372, final R ($I > 2\sigma(I)$), $R_1 = 0.0474$, $wR_2 = 0.0916$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-146815. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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Efficient Chemical Synthesis of the Two Anomers of ADP-L-glycero- and D-glycero-D-manno-Heptopyranose Allows the Determination of the Substrate Specificities of Bacterial Heptosyltransferases**

Alla Zamyatina, Sabine Gronow, Clemens Oertelt, Michael Puchberger, Helmut Brade, and Paul Kosma*

Lipopolysaccharides (LPS) of gram-negative bacteria are essential outer membrane components. The lipid moiety and the adjacent first sugar residues, named lipid A and the inner core region, respectively, are of utmost importance for growth and viability.^[1, 2] Thus, the development of inhibitors of the early biosynthetic steps in the assembly of LPS are promising alternatives in antibacterial drug design.

Whereas the biosynthesis of the 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) and the lipid A region of LPS is well understood, that of the heptose region containing L-glycero- or D-glycero-D-manno-heptose, or both, is not known in detail. This concerns the biosynthesis of the sugars, their nucleotide derivatives, and the characterization of heptosyltransferases.^[3–5] Although it is known that some bacterial heptosyltransferases use ADP-heptose as a substrate, the configuration of the side chain and the anomeric carbon is not known for the physiological sugar nucleotide.^[3, 6–8]

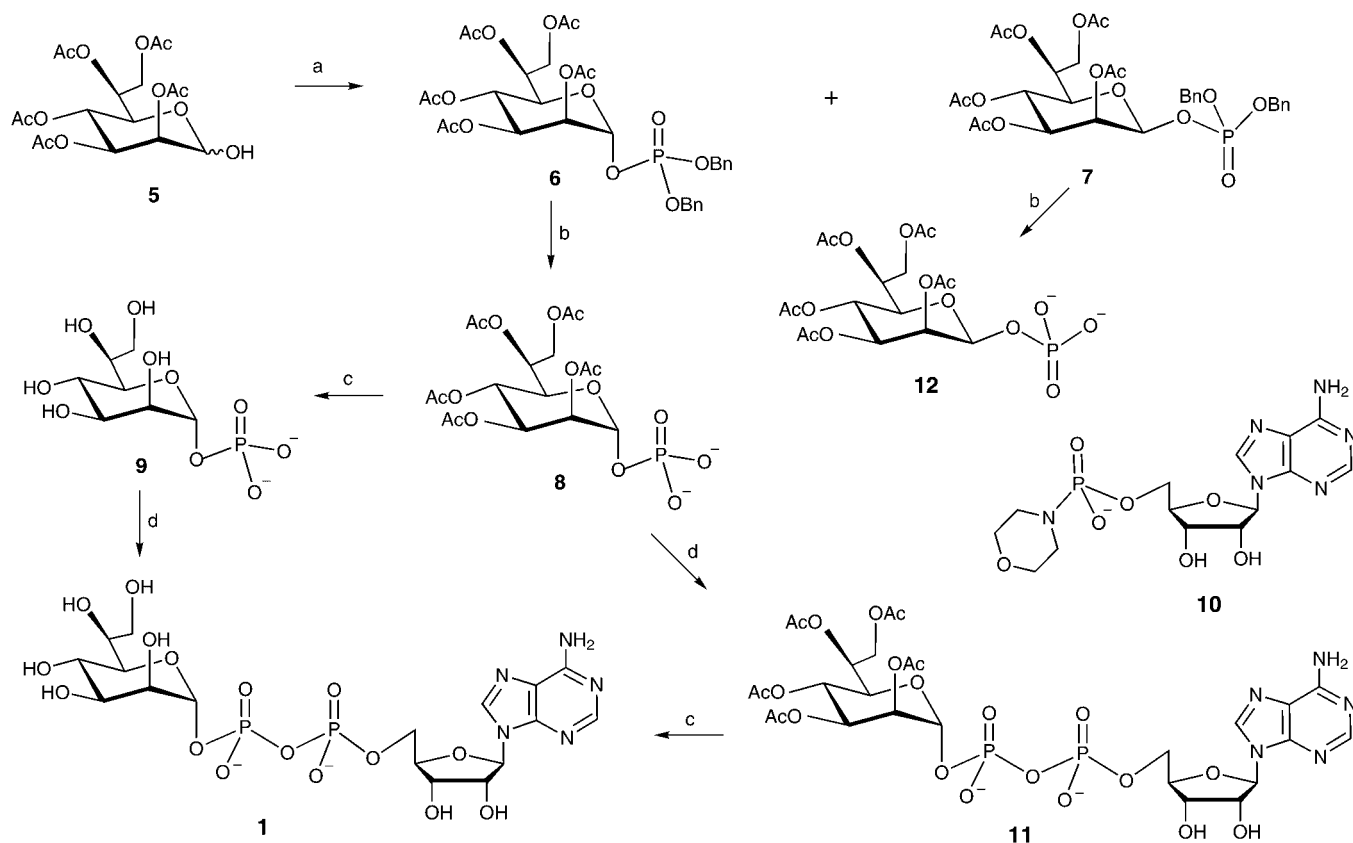
For the synthesis of the glycopyranosyl 1-phosphates,^[9] L-glycero-D-manno-heptopyranose (**5**)^[10] was subjected to phosphorylation^[11] followed by oxidation to give the anomeric phosphate triesters **6** and **7** in approximately 9:1 ratio and in 94% yield (Scheme 1). Alternatively, treatment of **5** with *N*-tetrazolylbisbenzylphosphoramidite increased the formation of the β -anomer **7**.^[12] By slow addition of phosphorylating reagent in the presence of an excess of 4-dimethylaminopyridine (DMAP), the thermodynamically and kinetically controlled α - and β -anomeric phosphite triesters, respectively, were obtained. Subsequent in situ oxidation gave **6** (56%) and **7** (34%), respectively. Cleavage of the protecting groups by hydrogenolysis to furnish **8** and **12** followed by deacetylation gave α - and β -heptose 1-phosphates (**9** and **13**, respectively) in near quantitative yields.

Yields reported for chemical syntheses of sugar nucleoside diphosphates through the coupling of a sugar 1-phosphate with nucleoside monophosphoromorpholidate^[13] are mostly moderate, not exceeding 25–30%, although improvements have been published.^[14] Target compound **1** was prepared first

[*] Prof. P. Kosma, Dr. A. Zamyatina, Dr. M. Puchberger
Institute of Chemistry
University of Agricultural Sciences
Muthgasse 18, 1190 Vienna (Austria)
Fax: (+43) 1-36006-6059
E-mail: pkosma@edv2.boku.ac.at
Dr. S. Gronow, Dipl.-Chem. C. Oertelt, Prof. H. Brade
Research Center Borstel
Center for Medicine and Biosciences
Parkallee 22, 23845 Borstel (Germany)

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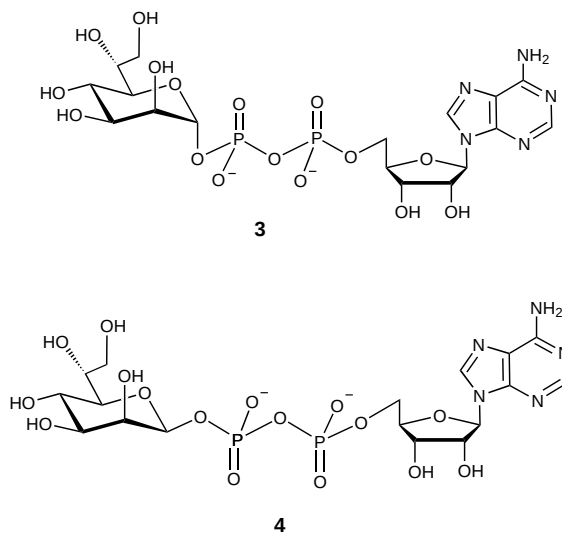
Scheme 1. a) Method A: $(\text{BnO})_2\text{NiPr}_2$, 1*H*-tetrazole, CH_2Cl_2 , then *t*BuOOH (**6**: 85%; **7**: 9%) or Method B: DMAP, tetrazolyl-NP(OBn)₂, $\text{CH}_2\text{Cl}_2/\text{MeCN}$ (1/1), then *t*BuOOH (**6**: 56%; **7**: 34%); b) Pd/C, H_2 , MeOH, Et_3N , 99%; c) MeOH/ $\text{H}_2\text{O}/\text{Et}_3\text{N}$ (3.5/1.5/0.5), RT, 98%; d) 4'-morpholine-*N,N'*-dicyclohexylcarboxamidinium salt of **10**, pyridine (**9**→**1**: 15%; **8**→**11**: 90%).

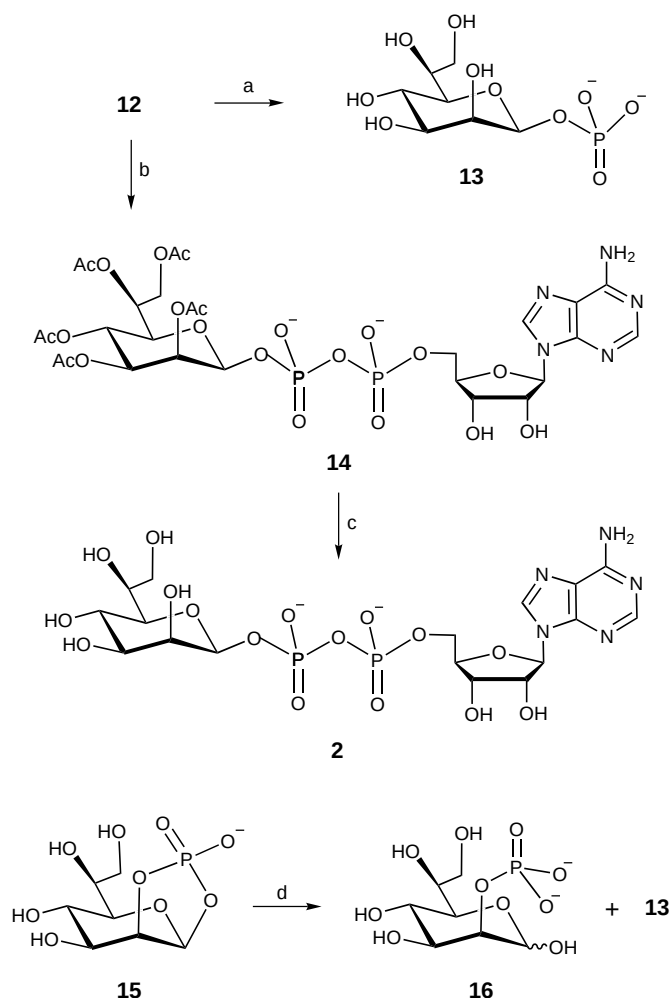
by coupling AMP-morpholidate (**10**) to the tributyl- or trioctylammonium salt of α -heptosyl 1-phosphate (**9**) as described for the synthesis of GDP-fucose.^[15] The salts of **9** were only partially soluble in pyridine and homogeneous reaction conditions could not be achieved. ADP-L-glycero- α -D-manno-heptopyranose (**1**) was isolated in only 15% yield with recovery of **9** in 60% yield. On the other hand, coupling of the soluble montriethylammonium salt of the *O*-acetylated heptosyl phosphate **8** with **10** afforded ADP-per-*O*-acetyl-L-glycero- α -D-manno-heptopyranose (**11**) in 90% yield after purification by anion-exchange chromatography. Subsequent deacetylation of **11** with MeOH/ $\text{H}_2\text{O}/\text{Et}_3\text{N}$ directly gave ADP-heptose **1** in 99% yield after lyophilization. The overall yield for the preparation of **1**, from **5**, was 76%.

The β -configured ADP-heptose **2** was prepared in a similar way (Scheme 2). Coupling of the β -heptosyl 1-phosphate **12** to AMP-morpholidate (**10**) gave a high yield (90%) of the protected ADP-heptose **14**. Attempts to cleave the acetate groups from **14** with aqueous NH_3 or with MeOH/ $\text{H}_2\text{O}/\text{Et}_3\text{N}$ at pH 12–13, however, led to the formation of the 1,2-cyclic phosphate **15** with release of AMP. The structure of **15** was determined from the ^{31}P NMR ($\delta = 17.8$) and ^{13}C NMR signals ($\delta = 79.5$) assigned to C-2, and from subsequent treatment of **15** with 1M NaOH, which gave an approximately 1:1 mixture of heptose 2-phosphate (**16**) and β -heptose 1-phosphate (**13**). Deacetylation of **14** with MeOH/0.1M aq. $\text{Et}_3\text{NH}^+\text{HCO}_3^-/\text{Et}_3\text{N}$ (pH 10–11) at -30°C gave **2** as the major component (80%). Anion-exchange chromatography of the crude mate-

rial on a Bio-Rad High Q resin led to the separation of **15**, and residual AMP was removed by gel chromatography on a Superdex peptide column. Diphosphate **2** slowly decomposed at room temperature at pH 5 (30% decomposition after 10 days).

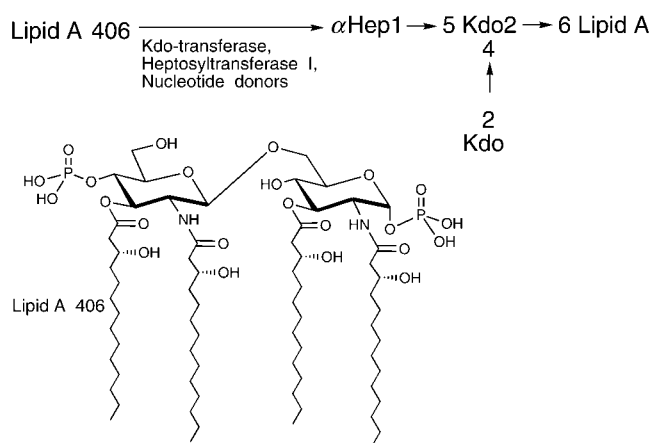
The ADP-heptoses **3** and **4** with D-glycero-D-manno-configuration were synthesized by phosphitylation with tetrazolyl-NP(OBn)₂ in good overall yields (50% for **3** and 24% for **4**, based on the initial quantity of penta-*O*-acetyl-D-glycero-D-manno-heptose^[16]).





Scheme 2. a) Pd/C, H₂, MeOH, Et₃N, 98%; b) 4'-morpholine-*N,N'*-dicyclohexylcarboxamidinium salt of **10**, pyridine, 90%; c) MeOH/0.1M Et₃NH⁺HCO₃⁻/Et₃N (3/4/0.15), -30 °C, 80%; d) 1M NaOH.

Compounds **1**–**4** were subjected to a coupled in vitro reaction, in which two Kdo residues and one heptose were transferred sequentially by Kdo transferase and heptosyltransferase I of *E. coli*, respectively, using synthetic lipid A 406^[17] as an acceptor (Scheme 3). Both enzymes were used as recombinant proteins expressed in a gram-positive host devoid of LPS-biosynthesis genes. Reaction products were detected after separation by thin-layer chromatography and blotting with monoclonal antibody S36-20, which recognizes Rd₂-type LPS.^[18] Since this antibody does not bind to LPS without heptose nor to LPS with two heptose residues, the data show that a single heptosyl residue had been transferred. Similar results were obtained with a natural donor from a bacterial extract^[19] (Figure 1, lane 1) and with synthetic ADP-L-glycero-β-D-manno-heptose (**2**; lane 2). Incubation of the reaction mixture with ADP-D-glycero-β-D-manno-heptose (**4**) also led to product formation, however, at least tenfold higher concentrations were needed in comparison with **2** (lane 3). Neither ADP-L-glycero-α-D-manno-heptose (**1**) nor ADP-D-glycero-α-D-manno-heptose (**3**) were substrates for heptosyltransferase I (lanes 4 and 5). Heptosyltransferase II exhibited the same sugar nucleotide specificity (data not shown).



Scheme 3. In vitro assay with synthetic lipid A 406, recombinant Kdo transferase, and recombinant heptosyltransferase I from *E. coli*.

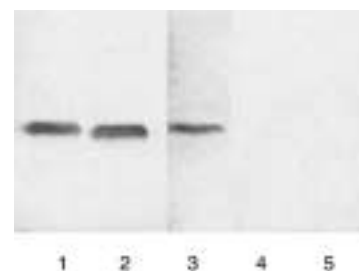


Figure 1. Immunoblot of reaction products of heptosyltransferase I of *E. coli* with ADP-L-glycero-D-manno-heptose of unknown anomeric configuration from a natural extract^[19] (lane 1), ADP-L-glycero-β-D-manno-heptose (**2**; 0.1 mM; lane 2), ADP-D-glycero-β-D-manno-heptose (**4**; 1 mM; lane 3), ADP-L-glycero-α-D-manno-heptose (**1**; 1 mM; lane 4) or ADP-D-glycero-α-D-manno-heptose (**3**; 1 mM; lane 5) as sugar nucleotide donors. Reaction products were separated by thin-layer chromatography, blotted, and visualized with a monoclonal antibody which recognizes Rd₂-type LPS.^[18]

The data show unequivocally that ADP-L-glycero-β-D-manno-heptose represents the physiological sugar nucleotide donor for heptosyltransferases from *E. coli*. ADP-D-glycero-β-D-manno-heptose also serves as a substrate, which is in accordance with the finding that mutants of ADP-L-glycero-D-manno-heptose-6-epimerase (gmhD) incorporate D-glycero-D-manno-heptose.^[20] The synthetic approach using partially acetylated sugar phosphates for the coupling step gives excellent yields, allows efficient purification and might be applicable in the synthesis of other sugar nucleoside diphosphates as well. This should facilitate studies of glycosyltransferases in general, and in particular biosynthetic studies of LPS which contain L-glycero- and D-glycero-D-manno-heptoses.

Experimental Section

Compound **1** (Et₃NH salt): [α]_D²⁰ = +4.5 (*c* = 1 in H₂O); ¹H NMR (300 MHz, D₂O, pD 5): δ = 8.51 (s, 1H; H-8^{Adc}), 8.26 (s, 1H; H-2^{Adc}), 6.14 (d, ³*J*(1,2) = 6.0 Hz, 1H; H-1^{Rib}), 5.51 (dd, ³*J*(1,2) = 1.9, ³*J*(1,P) = 7.4 Hz, 1H; H-1^{Hep}), 4.75 (m, 1H; H-2^{Rib}), 4.52 (dd, ³*J*(2,3) = 5.1, ³*J*(3,4) = 3.5 Hz, 1H; H-3^{Rib}), 4.39 (m, 1H; H-4^{Rib}), 4.22 (m, 2H; H-5a,5b^{Rib}), 4.02 (dd, ³*J*(2,3) = 3.2 Hz, 1H; H-2^{Hep}), 3.99 (m, 1H; H-6^{Hep}), 3.94–3.80 (m, 3H; H-3, H-4, H-5^{Hep}), 3.73 (dd, ²*J*(7a,7b) = 11.6, ³*J*(6,7a) = 6.2 Hz, 1H; H-7a^{Hep}), 3.64 (dd, ³*J*(6,7b) = 7.1 Hz, 1H; H-7b^{Hep}), 3.19 (q, 6H; CH₂N), 1.26 (t, 9H; Me);

^{13}C NMR (75.47 MHz, D_2O , pD 5): δ = 156.50 (C-6^{Adc}), 153.64 (C-2^{Adc}), 150.15 (C-4^{Adc}), 140.92 (C-8^{Adc}), 119.71 (C-5^{Adc}), 97.62 (C-1^{Hep}, $^2J(\text{C-1,P})$ = 6.0 Hz), 87.92 (C-1^{Rib}), 84.95 (C-4^{Rib}, $^3J(\text{C-4,P})$ = 9.0 Hz), 75.36 (C-2^{Rib}), 73.09 (C-5^{Hep}), 71.43 (C-3^{Rib}), 71.35 (C-3^{Hep}), 71.19 (C-2^{Hep}), 69.63 (C-6^{Hep}), 66.97 (C-4^{Hep}), 66.25 (C-5^{Rib}, $^2J(\text{C-5,P})$ = 5.0 Hz), 63.49 (C-7^{Hep}), $^1J(\text{C-1,H-1})$ = 174.0 Hz; ^{31}P NMR: δ = -10.7 (d, P^{Rib} , $^2J(\text{P,P})$ = 21.1 Hz), -13.1 (d, P^{Hep}); negative ion MALDI-TOF MS: m/z : found 618.31, calcd 618.36 (for $\text{C}_{17}\text{H}_{27}\text{N}_5\text{O}_{16}\text{P}_2$ [$\text{M} - \text{H}]^-$); elemental analysis: calcd for $\text{C}_{17}\text{H}_{27}\text{N}_5\text{O}_{16}\text{P}_2 \cdot \text{N}(\text{C}_2\text{H}_5)_3 \cdot 0.5\text{H}_2\text{O}$ (756.605): C 37.87, H 5.99, N 11.52; found: C 38.08, H 6.13, N 11.07.

Compound **2** (Et_3NH salt): $[\alpha]_{\text{D}}^{20}$ = -31 (c = 0.3 in H_2O); ^1H NMR (300 MHz, D_2O , pD 4.7): δ = 8.60 (s, 1H; H-8^{Adc}), 8.37 (s, 1H; H-2^{Adc}), 6.18 (d, $^3J(1,2)$ = 5.8 Hz, 1H; H-1^{Rib}), 5.23 (dd, $^3J(1,2)$ = 1.0, $^3J(1,\text{P})$ = 8.5 Hz, 1H; H-1^{Hep}), 4.75 (m, 1H; H-2^{Rib}), 4.50 (dd, $^3J(2,3)$ = 4.9, $^3J(3,4)$ = 3.8 Hz, 1H; H-3^{Rib}), 4.42 (m, 1H; H-4^{Rib}), 4.25 (m, 2H; H-5a,5b^{Rib}), 4.07 (dd, $^3J(2,3)$ = 3.2 Hz, 1H; H-2^{Hep}), 3.95 (m, 1H; H-6^{Hep}), 3.82 (dd, $^3J(3,4)$ = $^3J(4,5)$ = 9.7 Hz, 1H; H-4^{Hep}), 3.87–3.67 (m, 2H; H-7a,7b^{Hep}), 3.67 (dd, 1H; H-3^{Hep}), 3.37 (dd, $^3J(5,6)$ = 1.7 Hz, 1H; H-5^{Hep}), 3.20 (m, 6H; CH_2N), 1.28 (t, 9H; Me); ^{13}C NMR (75.47 MHz, D_2O , pD 5): δ = 156.75 (C-6^{Adc}), 153.99 (C-2^{Adc}), 150.23 (C-4^{Adc}), 140.89 (C-8^{Adc}), 119.80 (C-5^{Adc}), 96.89 (C-1^{Hep}, $^2J(\text{C-1,P})$ = 4.8 Hz), 87.84 (C-1^{Rib}), 84.88 (C-4^{Rib}, $^3J(\text{C-4,P})$ = 8.7 Hz), 76.04 (C-5^{Hep}), 75.34 (C-2^{Rib}), 73.72 (C-3^{Hep}), 71.73 (C-2^{Hep}), 71.49 (C-3^{Rib}), 69.71 (C-6^{Hep}), 66.66 (C-4^{Hep}), 66.35 (C-5^{Rib}, $^2J(\text{C-5,P})$ = 5.8 Hz), 63.40 (C-7^{Hep}); $^1J(\text{C-1,H-1})$ = 163.3 Hz; ^{31}P NMR: δ = -10.8 (d, P^{Rib} , $^2J(\text{P,P})$ = 20.8 Hz), -12.8 (d, P^{Hep}); negative ion MALDI-TOF MS: m/z : found 618.63, calcd 618.36 (for $\text{C}_{17}\text{H}_{27}\text{N}_5\text{O}_{16}\text{P}_2$ [$\text{M} - \text{H}]^-$).

Enzymatic *in vitro* assays to test the transfer of the α - and β -configured ADP-heptoses **1–4** by heptosyltransferase I of *E. coli* were basically performed as described in refs. [6a, 21].

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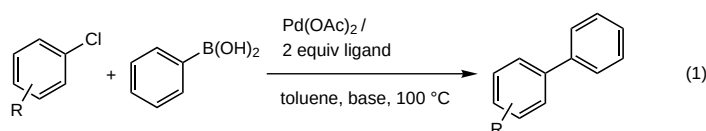
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A New Highly Efficient Catalyst System for the Coupling of Nonactivated and Deactivated Aryl Chlorides with Arylboronic Acids**

Alexander Zapf, Andreas Ehrentraut, and Matthias Beller*

Dedicated to Professor Othmar Stelzer on the occasion of his 60th birthday

Substituted biphenyls are central components of fine chemicals for a diverse range of applications. In particular pharmaceuticals^[1] and herbicides^[2] with biaryl structures are of general interest. In addition, biaryls are applied as chiral ligands in catalysis,^[3] as liquid crystals,^[4] or organic conductors.^[5] The most common method used for linking the central aryl–aryl bond is the palladium- or nickel-catalyzed coupling of aryl halides or aryl pseudohalides with arylboronic acids [Suzuki reaction, Eq. (1)].^[6] This method has



the advantage over other alternatives in that stoichiometric amounts of heavy metals are not required (unlike the Ullmann and Stille couplings that use copper and tin, respectively). From an industrial viewpoint the chemically

[*] Prof. Dr. M. Beller, Dr. A. Zapf, Dipl.-Chem. A. Ehrentraut
Institut für Organische Katalyseforschung an der Universität Rostock
e. V. (IfOK)
Buchbinderstrasse 5–6, 18055 Rostock (Germany)
Fax: (+49) 381-466-9324
E-mail: matthias.beller@ifok.uni-rostock.de

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inert, but cheap and readily accessible aryl chlorides are particularly important as starting materials for transition metal catalyzed C–C and C–X coupling.^[7] Therefore it is not surprising that activation of chloroarenes has developed into one of the most intensely studied areas of homogeneous catalysis in the last decade.^[7] In Suzuki reactions of non-activated and deactivated chloroarenes^[8] in the last three years Indolese^[9] and Miyaura et al.^[10] have achieved significant success with nickel catalysts, and the groups of Fu,^[11] Buchwald,^[3b, 12] Guram,^[13] Nolan,^[14] Herrmann,^[15] as well as ourselves^[16] have achieved similar success with palladium catalysts.

Despite the good yields obtained in many Suzuki reactions of chloroarenes, generally, comparatively large amounts of catalyst are required (1–3 mol %).^[17] The application of such amounts of homogeneous catalysts make industrial applications almost impossible. Given the current price of palladium, this leads to catalyst costs of > 100 € per kg product;^[18] that is, the availability and the price advantage of chloroarenes over other haloarenes as substrates no longer plays a role!

We describe herein a new catalyst system, with which we have achieved the coupling of nonactivated and deactivated aryl chlorides highly efficiently in good yields with generally only 0.005 mol % palladium and thus under industrially viable conditions.

As a starting point for the development of new more efficient catalyst systems, we chose the synthesis of diadamantylphosphanes. Adamantylphosphanes, which are sterically demanding, basic ligands, have hitherto been little examined in catalysis.^[19] Among a variety of diadamantylalkylphosphanes tested in initial experiments with chloroarenes, the diadamantyl-*n*-butylphosphane (BuPAD₂) proved to be extremely reactive. In order to compare the new ligands with the best catalyst systems known to date for the Suzuki reaction, we chose the reaction of 4-chlorotoluene with phenylboronic acid. In all cases the reaction conditions optimized by Buchwald et al.^[12b] for Suzuki reactions were employed (palladium source: palladium(II) acetate; P: Pd = 2:1; solvent: toluene; base: potassium phosphate; 100 °C). As Table 1 shows, simple triphenylphosphane did not lead to an active catalyst (< 5 % yield; Table 1, entry 1). With Buchwald's 2-(dicyclohexylphosphanyl)biphenyl the reaction proceeds very well (93 %) in the presence of at least 0.05 mol % palladium (Table 1, entry 5);^[12b] however, it

Table 1. Influence of the ligand on the coupling of 4-chlorotoluene and phenylboronic acid.

Entry	PR ₃	Pd [mol %]	Yield [%]	TON
1	PPh ₃	0.1	5	50
2	PhPCy ₂	0.1	23	230
3 ^[a]	(<i>o</i> -tol)PCy ₂	0.1	49	490
4 ^[a]	(<i>o</i> -anisyl)PCy ₂	0.1	42	420
5 ^[12b]	(<i>o</i> -biph)PCy ₂	0.05	93	1860
6	(<i>o</i> -biph)PCy ₂	0.01	47	4700
7	PCy ₃	0.1	23	230
8	PrBu ₃	0.01	92	9200
9	PrBu ₃	0.005	41	8200
10	BuPAD ₂	0.01	94	9400
11	BuPAD ₂	0.005	87	17400

[a] P/Pd = 4:1.

reaches its limits at 0.01 mol % palladium (47 %; Table 1, entry 6) and gives the desired product in only 16 % yield in the presence of 0.005 mol % catalyst. The comparison with the electronically similar, but sterically significantly less demanding ligands dicyclohexylphenylphosphane as well as 2-(dicyclohexylphosphanyl)toluene and -anisole (Table 1, entries 2, 3, and 4, respectively)^[7a] illustrates the importance of the right Tolman angle. Even the steric demand of the standard ligand tricyclohexylphosphane is not sufficient for this reaction (Table 1, entry 7). On the other hand, tri-*tert*-butylphosphane, introduced by Fu et al.^[11] for the Suzuki reaction, is a simple, but efficient ligand for the coupling of 4-chlorotoluene (92 % at 0.01 mol % Pd; 41 % at 0.005 mol % Pd; Table 1, entries 8 and 9, respectively). With diadamantyl-*n*-butylphosphane, we obtained even significantly improved turnover numbers (Table 1, entries 10 and 11). Thus, our catalyst system represents the most productive catalyst system described to date for the arylation of chloroarenes (87 % yield at 0.005 mol % Pd; TON = 17400). The reaction with the new ligand system is already complete after a few hours; to allow a better comparison it was left to run for 20 hours. Already after four hours 4-methylbiphenyl is obtained in 74 % yield, which corresponds to a turnover frequency of 3700 per hour; thus also the activity of the new system is unparalleled.

In the presence of alternative bases that are even more suitable for an industrial reaction, for example potassium carbonate, 4-methylbiphenyl was obtained in a respectable yield of 74 % in the presence of 0.01 mol % Pd(OAc)₂ and 0.02 mol % BuPAD₂ (TON = 7400).

Other chloroarenes led to results similar to those for 4-chlorotoluene, essentially independent of their steric and electronic properties (Table 2). *ortho*-substituents are toler-

Table 2. Suzuki coupling of different aryl chlorides with phenylboronic acid in the presence of Pd(OAc)₂/2 BuPAD₂.

Entry	R	Pd [mol %]	Yield [%]	TON
1	4-Me	0.005	87	17400
2 ^[a]	4-Me	0.005	74	14800
3	2-Me	0.005	85	17000
4	2,6-Me ₂	0.005	68	13600
5	H	0.005	80	16000
6	2-F	0.005	96	19200
7	2-F	0.001 ^[b]	55	55000
8	4-MeO	0.005	64	12800
9	3-MeO	0.005	58	11600
10	2-CN	0.005	100	20000
11	2-CN	0.001 ^[b]	69	69000
12	"3-N" ^[c]	0.005	99	19800

[a] 4 instead of 20 h. [b] Pd:P = 1:4. [c] 3-chloropyridine.

ated, and even the strongly sterically hindered 2,6-dimethyl-substituted chlorobenzene was coupled to approximately 70 % yield (TON = 13600; Table 2, entry 4). Only strongly electron-donating groups (e.g. methoxy groups) led to a drop in the yield to about 60 % (Table 2, entries 8 and 9). Heteroaryl chlorides, such as 3-chloropyridine, are likewise converted highly efficiently to the corresponding biaryls (99 %; Table 2, entry 12).

A further reduction of the amount of catalyst by a factor of 5 to 10 ppm Pd (based on the chloroarene), was tested, for

example, for the reactions of 4-chlorotoluene, 2-fluorochlorobenzene, and 2-chlorobenzonitrile. The new catalyst system reached its limits for such low palladium concentrations. Nevertheless 2-fluorobiphenyl and 2-cyanobiphenyl were obtained in satisfactory yields of 55 and 69%, respectively (with corresponding TONs of 55000 and 69000, respectively; Table 2, entries 7 and 11, respectively).

Diadamantyl-*n*-butylphosphane/palladium(II) acetate represents the as yet most efficient catalyst system for the coupling of chloroarenes with arylboronic acids. It is significantly better in terms of the productivity and activity than the hitherto best known catalyst systems. Even with deactivated aryl chlorides turnover numbers of 10000–20000 are achieved for good to excellent yields. The described catalyst system as well as similar systems are currently being tested in other catalytic C–C and C–X coupling reactions.

Experimental Section

Procedure for 4-methylbiphenyl: In an ACE pressure tube 4-chlorotoluene (3.0 mmol), phenylboronic acid (4.5 mmol), tripotassium phosphate (6.0 mmol), hexadecane (100 μ L; as internal GC standard), the ligand, and palladium(II) acetate were suspended in dry toluene (6 mL) in an argon atmosphere. The pressure tube was sealed and suspended in a preheated oil bath at 100 °C. After 20 h the mixture was allowed to cool to room temperature and the solids were dissolved by addition of dichloromethane (10 mL) and dilute sodium hydroxide (10 mL). The organic phase was analyzed by gas chromatography. To isolate the product, the organic phase was washed with water and saturated brine, and dried. The solvent was removed by distillation and the residue was purified by chromatography with hexane/ethyl acetate (9:1; v/v) over silica gel 60. ^1H NMR (400 MHz, CDCl_3 , 21 °C): δ = 7.63 (m, 2H), 7.54 (m, 2H), 7.47 (m, 2H), 7.36 (m, 1H), 7.29 (m, 2H), 2.44 (s, 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CDCl_3 , 21 °C): δ = 141.1, 138.3, 137.0, 129.5, 128.7, 127.0, 126.9, 126.9, 21.1; MS (70 eV, EI): m/z (%): 168 (100) [M^+], 152 (15) [$M^+ - \text{CH}_3$].

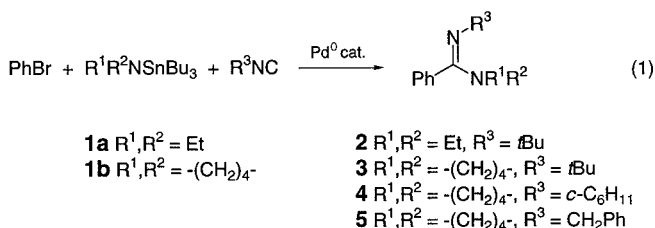
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A Palladium-Catalyzed Synthesis of Amidines from Aryl Halides**

C. Gustaf Saluste, Richard J. Whitby,* and Mark Furber

The advent of high-throughput screening of compounds for biological activity has created a demand for synthetic methods that allow diverse arrays of organic compounds to be synthesized quickly. Palladium-catalyzed elaborations of aryl halides, for example by Suzuki couplings with arylboronates,^[1] or Buchwald/Hartwig coupling with amines^[2] are important tools. Extension of palladium-catalyzed reactions to three-component couplings by incorporation of a carbenoid fragment would be valuable. Such reactions incorporating carbon monoxide are well known,^[3] but use of isoelectronic (and structurally variable) isocyanides is virtually unexplored.^[4] One exception is the coupling between bromobenzene, *tert*-butyl isocyanide, and tributylstannyl(diethyl)amine **1a** (5 mol % [Pd(PPh₃)₄], benzene, 120 °C, 20 h) to afford the amidine **2** in 22 % yield (GC) [Eq. (1)], described by Kosugi et al. in 1986.^[4a] We now report on the optimization of this



reaction and development of a tin-free system to provide a useful convergent synthesis of aromatic and heteroaromatic amidines. Amidines are important constituents of many biologically active compounds.^[5]

We initially optimized the coupling of bromobenzene with *N*-tributylstannylpyrrolidine (**1b**) (1.3 equiv) and *tert*-butyl isocyanide (1.5 equiv).^[6, 7] High yields of the amidine **3** could be obtained by using (dibenzylideneacetone)dipalladium(0)–chloroform adduct ([Pd₂(dba)₃]·CHCl₃; 2.5 mol %) as the palladium source and 1,1′-bis(diphenylphosphanyl)ferrocene (dppf, 10 mol %) as the ligand in a variety of solvents (toluene, benzene, THF, DMF) at 109 °C for 20 h [Eq. (1)]. Under these conditions other isocyanides (*c*-C₆H₁₁NC, PhCH₂NC, BuNC, PhNC) gave minimal conversions. Moderate yields of the amidines **4** and **5** [Eq. (1)] could be obtained by slow addition (13 h) of cyclohexyl isocyanide (42 %) or benzylisocyanide (36 %), respectively.

Tin is toxic and best avoided in preparations of compounds for biological application so we next investigated the development of a tin-free system. Using the [Pd₂(dba)₃]·CHCl₃/dppf/toluene conditions developed above the coupling of bromobenzene, *tert*-butyl isocyanide, and pyrrolidine (5 equiv), failed. As found for the direct coupling of amines and aromatic halides^[2] addition of the inorganic base Cs₂CO₃ (1.3 equiv) allowed the reaction to proceed to give amidine **3** in quantitative yield [Eq. (2), Table 1, entry 1]. Initial

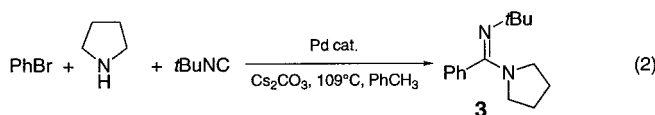


Table 1. Optimization of tin-free conditions for amidine formation^[a].

Entry	Pyrrolidine [equiv]	Ligand	Pd source	L:Pd	<i>t</i> ^[b] [h]	Yield ^[c] [%]
1	5	dppf	[Pd ₂ (dba) ₃]·CHCl ₃	2:1	6	100
2	5	dppf	PdCl ₂	2:1	3	100
3	5	dppf	PdCl ₂	1:1	2	100
4	5	–	PdCl ₂	–	20	54
5	5	dppf	PdCl ₂	4:1	20	100
6	5	PPh ₃	PdCl ₂	4:1	20	28
7	5	(2-furyl) ₃ P	PdCl ₂	4:1	20	36
8	5	<i>t</i> Bu ₃ P	PdCl ₂	4:1	20	11
9	5	(C ₆ F ₅) ₂ PPh	PdCl ₂	4:1	20	0
10	5	BINAP	PdCl ₂	2:1	6	77
11	5	dppe	PdCl ₂	2:1	3	100
12	5	dppp	PdCl ₂	2:1	3	100
13	1.5	dppf	PdCl ₂	2:1	5	100
14	1.1	dppf	PdCl ₂	2:1	8	86
15	0.9	dppf	PdCl ₂	2:1	8	63 ^[d]
16	1.5	dppf	PdCl ₂	1:1	15	83
17	5	dppf	Pd(OAc) ₂	2:1	2	100
18	5	dppf	Pd(OAc) ₂	1:1	2	100
19	5	–	Pd(OAc) ₂	–	20	56
20	1.5	dppf	Pd(OAc) ₂	2:1	2	100
21	1.5	dppf	Pd(OAc) ₂	1:1	5	100

[a] Conditions: Toluene, 109 °C, *t*BuNC (1.5 equiv), Pd (5 mol %), dry Cs₂CO₃ (1.3 equiv). [b] Time for complete or maximum conversion. [c] Yield of **3** based on PhBr by GC using an internal standard of *n*-tridecane. [d] Yield based on pyrrolidine.

variability in results was traced to the dryness of the commercial anhydrous Cs₂CO₃ used. Carefully dried material gave consistent and high-yielding results. A significant increase in rate was observed on changing the palladium source to the much cheaper, and more easily handled PdCl₂ (Table 1, entry 2). Lowering the ligand:palladium ratio to 1:1 gave almost equivalent results (Table 1, entry 3), and even in the absence of a phosphane ligand the reaction gave **3** in 54 % yield (Table 1, entry 4). Using a ligand:palladium ratio of 4:1 slowed the reaction (Table 1, entry 5). Triphenylphosphane, tri(2-furyl)phosphane, tri(*tert*-butyl)phosphane, bis(pentafluorophenyl)phenylphosphane, *rac*-2,2′-bis(diphenylphosphanyl)-1,1′-binaphthyl (BINAP), 1,2-bis(diphenylphosphanyl)ethane (dppe) and 1,3-bis(diphenylphosphanyl)propane (dppp) were tried as ligands (Table 1, entries 6–12). Only the bidentate ligands were effective, dppe and dppp proving comparable to the more expensive but easily handled dppf.

[*] Prof. R. J. Whitby, C. G. Saluste

Department of Chemistry
Southampton University
Southampton, SO17 1BJ (UK)
Fax: (+44)23-8059-3781
E-mail: rjw1@soton.ac.uk

Dr. M. Furber
AstraZeneca Charnwood,
Department of Medicinal Chemistry
Bakewell Road, Loughborough, LE11 5RH (UK)

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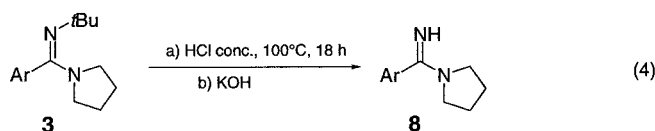
With the PdCl₂/dppf conditions we examined the effect of reducing the amount of pyrrolidine. With a ligand:palladium ratio of 2:1 the reaction reached 100% conversion after 5 h with 1.5 equivalents of pyrrolidine, and respectable yields were obtained with 1.1 or even 0.9 equivalents (Table 1, entries 13–15). The latter results are useful if the amine component is expensive. With only one equivalent of dppf per palladium and 1.5 equivalents pyrrolidine the reaction took 15 h to reach 83% yield (Table 1, entry 16, only 20% after 5 h). Reactions using PdCl₂ had a significant initiation period so we examined the use of Pd(OAc)₂ which is soluble in toluene. Using five equivalents of pyrrolidine the reactions quickly reached full conversion when using either two or one equivalents of dppf per palladium (Table 1, entries 17 and 18), and as with PdCl₂, even in the absence of a phosphane ligand gave a reasonable yield of **3** (Table 1, entry 19). Lowering the amount of amine to 1.5 equivalents still gave high-yielding and fast conversions with only one equivalent of ligand per palladium (Table 1, entry 21) providing our most cost effective conditions to date.

Extension to isocyanides other than *tert*-butyl isocyanide has had limited success. Our best result is a 39% yield of **4** obtained by slow addition (8 h) of cyclohexyl isocyanide (1.5 equiv) to bromobenzene, pyrrolidine (5 equiv), Cs₂CO₃ (1.3 equiv), PdCl₂ (5 mol %), dppf (10 mol %), in toluene at 109 °C.

With high-yielding conditions in hand we undertook the synthesis and isolation of a range of aryl amidines **6** [Eq. (3), Table 2]. In all cases the conversions were clean by GC, but there were losses on isolation of pure materials. In general we found the best work-up procedure to be extraction of the strongly basic amidine into dilute aqueous acetic acid. After washing with diethyl ether addition of concentrated KOH solution allowed extraction of the amidine. Removal of

solvent and Kugelrohr distillation gave analytically pure amidines in good overall yield. The results show that electron-rich, electron-poor, and heteroaromatic halides work well. The amine may be primary or secondary, alkyl- or aryl-substituted, and cyclic or acyclic. Calculations, and ¹³C NMR shifts of the *tert*-butyl groups indicate that the amidines derived from primary amines exist in the tautomeric form **7**.

In order to provide access to *N*-unsubstituted amidines we investigated methods for removal of the *tert*-butyl group in **3** and found that refluxing concentrated hydrochloric acid was effective [Eq. (4)].^[8] The deprotection could be carried out on the crude product from the palladium-catalyzed coupling providing **8** in 60% yield from bromobenzene.



Overall we have developed a simple and efficient intermolecular three-component synthesis of aromatic and heteroaromatic *N*-*tert*-butyl amidines from aryl and heteroaryl halides which should find use in pharmaceutical discovery.

Experimental Section

6g: Dry Cs₂CO₃ (0.85 g, 2.6 mmol), dppf (0.111 g, 0.20 mmol), 4-bromoanisole (0.375 g, 2.0 mmol), and pyrrolidine (0.71 g, 10 mmol) were added to a 20 mL Schlenk tube under argon. Dry, degassed toluene (10 mL) was added, followed by *t*BuNC (0.34 mL, 3.0 mmol), and finally PdCl₂ (17.7 mg, 0.10 mmol) was added against a flow of argon. The tube was stoppered and heated with stirring at 109 °C for 20 h. After the mixture had been cooled to room temperature, diethyl ether (20 mL) was added and the resulting mixture filtered to give a dark brown solution which was extracted with dilute acetic acid (2.5% in water, 6 × 5 mL). The combined aqueous extracts were washed with diethyl ether (2 × 10 mL), then treated with a concentrated KOH solution (30 mL) in the presence of diethyl ether (20 mL). The ethereal layer was collected and the aqueous layer extracted with diethyl ether (3 × 30 mL). The combined diethyl ether phases were dried over MgSO₄ before evaporation. Kugelrohr distillation (100–110 °C/1 Torr) gave the amidine **6g** as a pale yellow oil which crystallized to a white solid (0.43 g, 83%). M.p. 56–57 °C; ¹H NMR (300 MHz, CDCl₃): δ = 7.12 (2H, d, *J* = 8.8 Hz), 6.87 (2H, d, *J* = 8.8 Hz), 3.83 (3H, s), 3.11 (4H, brs), 1.78 (4H, m), 1.02 (9H, s); ¹³C NMR (75 MHz, CDCl₃): δ = 159.19 (s), 156.70 (s), 131.65 (s), 129.67 (d), 113.35 (d), 55.32 (q), 52.82 (s), 47.61 (t), 33.09 (q), 25.46 (t); MS (APCI): *m/z* (%): 261 (100) [*M*+H⁺]; IR (neat): $\tilde{\nu}$ = 2958 (s), 2921 (m), 1597 (s), 1509 (m), 1375 (s), 1354 (s), 1245 (s), 1186 (m), 1221 (s), 1025 (m) cm⁻¹; elemental analysis (%) calcd for C₁₆H₂₄N₂O: C 73.81, H 9.29, N 10.76; found: C 73.83, H 9.50, N 10.95.

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Table 2. Palladium-catalyzed synthesis of amidines.

ArBr	Amine R ¹ R ²	6/7	Yield (6/7) ^[a] [%]	M.p. (6/7) [°C]	B.p. (6/7) ^[d] [°C]
C ₆ H ₄ Br	–(CH ₂) ₄ –	6a	78, 58 ^[b]	49–50	
C ₆ H ₄ Br	Bu H	7b	55, 29 ^[b]		90–100
C ₆ H ₃ Br	Ph H	7c	45, 40 ^[c]	115–117	
C ₆ H ₃ Br	Et Et	6d	61		80–90
<i>p</i> -Me ₂ NC ₆ H ₄ Br	–(CH ₂) ₄ –	6e	74	91–92	
<i>p</i> -Me ₂ NC ₆ H ₄ Br	–(CH ₂) ₂ O(CH ₂) ₂ –	6f	76	125–127	
<i>p</i> -MeOC ₆ H ₄ Br	–(CH ₂) ₄ –	6g	83, 79 ^[c]	56–57	
<i>p</i> -MeOC ₆ H ₄ Br	–(CH ₂) ₂ O(CH ₂) ₂ –	6h	76	79–80	
<i>p</i> -MeCOC ₆ H ₄ Br	–(CH ₂) ₄ –	6i	61	76–78	
<i>p</i> -MeCOC ₆ H ₄ Br	Bu H	7j	49	74–76	
<i>p</i> -MeCOC ₆ H ₄ Br	–(CH ₂) ₂ O(CH ₂) ₂ –	6k	62	102–104	
3-bromopyridine	–(CH ₂) ₄ –	6l	71	28–29	
3-bromopyridine	–(CH ₂) ₂ O(CH ₂) ₂ –	6m	57		110–115

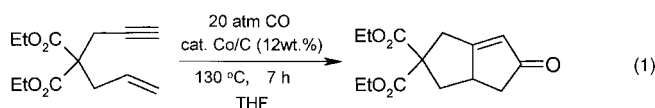
[a] Conditions: Amine (5 equiv), *t*BuNC (1.5 equiv), Cs₂CO₃ (1.3 equiv), PdCl₂ (5 mol %), dppf (10 mol %), toluene, 109 °C, 3–24 h. [b] Conditions: As [a] but amine (1 equiv), Pd(OAc)₂ (5 mol %), dppf (5 mol %). [c] Conditions: As [b] but amine (1.5 equiv). [d] At 1 Torr.

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Cobalt on Charcoal: A Convenient and Inexpensive Heterogeneous Pauson–Khand Catalyst**

Seung Uk Son, Sang Ick Lee, and Young Keun Chung*

Although much success has been achieved in the field of transition metal mediated (or catalyzed) synthesis of cyclopentenones from readily available substrates,^[1] industrial applications are surprisingly still undeveloped. In order to develop large-scale processes, it is extremely important and prerequisite to heterogenize homogeneous systems. Recently we published^[2] the first heterogeneous catalyst system for the Pauson–Khand reaction based on cobalt on mesoporous silica. Polymer-supported cobalt carbonyl complexes have been reported^[3] as catalysts of the intramolecular Pauson–Khand reaction, but the catalytic activity was low. The cobalt on a mesoporous material system is a quite effective catalyst for the intramolecular Pauson–Khand reaction, but displays low activity in the intermolecular Pauson–Khand reaction. Furthermore, mesoporous silica is not freely available. In view of the drawbacks of cobalt on mesoporous materials, much work has focused on finding more suitable supports. Herein we report on a new catalyst system based on cobalt on charcoal (Co/C). This heterogeneous catalyst system exhibits an excellent catalytic performance for intra- and intermolecular Pauson–Khand reactions [Eq. (1)]. The catalyst system is quite stable and can be reused. Recently, catalyst systems



[*] Prof. Y. K. Chung, S. U. Son, S. I. Lee
 School of Chemistry and Center for Molecular Catalysis
 Seoul National University, Seoul 151-747 (Korea)
 Fax: (+82) 2-889-1568

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based on transition metals on charcoal have attracted much attention.^[4]

The catalyst system was prepared by thermal decomposition of $[\text{Co}_2(\text{CO})_8]$ in the presence of commercially available charcoal bone in THF under reflux.^[5] The catalytic activity of Co/C strongly depends on the amount of cobalt on charcoal (wt %). When the cobalt loading on charcoal was less than 7.5 wt %, no catalytic reaction was observed. The optimum cobalt loading on charcoal was about 12 wt %. The IR spectra of the supported catalysts show no carbonyl absorptions, confirming a complete decomposition of the metal carbonyl. X-ray powder diffraction (XRD) patterns of the supported catalysts revealed peaks of hexagonal close packed (hcp) metallic cobalt, as in the bulk phase.^[6] A transmission electron microscopic (TEM) study (Figure 1) shows that cobalt atoms are not distributed homogeneously in the charcoal surface, but form metallic cobalt particles (100–1000 nm). The black Co/C shows ferromagnetism and is easily recovered by filtration or by using a magnet.

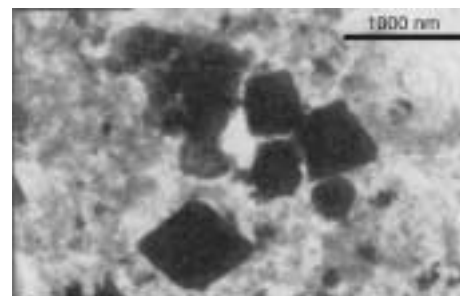


Figure 1. Transmission electron micrograph of Co/C.

The scope of application of this catalyst was examined with a number of substrates both in inter- and intramolecular variants of the Pauson–Khand reaction. Several representative results obtained under standard conditions are given in Table 1. The intramolecular cycloaddition proceeded smoothly with monosubstituted olefins regardless of the substitution pattern of alkynes (Table 1, entries 1–4), although the internal olefin substrate (Table 1, entry 5) needs a relatively long reaction time. Heteroatom-bridged enynes produced aza- (Table 1, entry 4) and oxobicyclic compounds (Table 1, entries 3 and 5) from the corresponding substrates. In most cases, the yields of the intramolecular reactions are almost quantitative. For intermolecular cycloadditions with the Co/C system the substrate structure has little influence on the results. Satisfactory results are obtained with simple terminal alkynes (Table 1, entries 6 and 7), a conjugated alkyne (Table 1, entry 8), and a diyne (Table 1, entry 10). In contrast, an *n*-alkyl halide substituted alkyne (Table 1, entry 9)^[7] was not a suitable substrate for the Co/C system. In the case of the diyne substrate (entry 10), a double intermolecular Pauson–Khand reaction^[8] occurred and no [2+2+2] cycloaddition product was observed.

The most important advantage of heterogeneous catalysis over its homogeneous counterpart is the possibility of recovering the catalyst after reaction by simple filtration. For the intramolecular Pauson–Khand reaction according to

Table 1. Pauson–Khand reaction with various substrates.^[a]

Entry	Substrate	Product	<i>p</i> [atm]	<i>t</i> [h]	Yield ^[b] [%]
1			20	18	98
2			20	7	93
3			20	18	98
4			20	7	98
5			30	24	61
6			30	18	98
7			30	18	98
8			30	18	98
9			30	18	22
10			30	48	89

[a] 0.1 g Co/charcoal (12 wt. %) catalyst and 1.26 mmol (entries 1–5) or 1.64 mmol (entries 6–10) substrate were used. TsH₃CC₆H₄SO₂. [b] Yield of isolated product.

Equation (1), the catalyst system could be reused 10 times without loss of activity (Table 2); the maximum reusability

Table 2. Test for the reusability of the catalyst. The reaction given in Equation (1) was selected as the test reaction.^[a]

Runs	Catalyst	Yield [%] ^[b]
1	Co/charcoal (12 %)	91
2	recovered from 1	98
3	recovered from 2	95
4	recovered from 3	97
5	recovered from 4	98
6	recovered from 5	95
7	recovered from 6	98
8	recovered from 7	98
9	recovered from 8	98
10	recovered from 9	95

[a] 0.1 g catalyst and 1.26 mmol substrate were used. [b] Yield of isolated product.

has not been tested. The reusability can be understood when we consider almost no bleeding of cobalt from the charcoal surface. Elemental analysis (by inductively coupled plasma atomic emission spectroscopy (ICP-AES)) of the reaction mixture after completion of the reaction showed that less than 0.1 ppm of cobalt species was bled.

We have screened graphite and mesoporous carbon as a support. Interestingly, the catalytic activities of cobalt on graphite and mesoporous carbon are almost the same as that of cobalt on charcoal even though they have quite different surface areas and structures. Furthermore, XRD and TEM studies on cobalt on graphite and mesoporous carbon likewise show peaks for hexagonal close packed metallic cobalt similar in intensity to those for cobalt on charcoal. Thus, it seems likely that the catalytic activity depends on the state of metallic cobalt on the support and not on the nature of the carbon support.

In conclusion, cobalt on charcoal has been developed as an inexpensive, heterogeneous, and highly effective catalyst for intra- and intermolecular Pauson–Khand reactions. The reusability of the catalyst and the experimental simplicity are especially attractive and should encourage the use of this catalyst system among synthetic chemists and in industrial applications.

Experimental Section

The enyne shown in Equation (1) (0.30 g, 1.26 mmol) and Co/C (0.10 g, 12.2 wt % Co) in THF (15 mL) was heated under carbon monoxide (20 atm at room temperature) at 130 °C for 7 h. After workup and column chromatography, the corresponding cyclopentenone derivative was obtained 92 % yield.

Characterization of the product of entry 10 in Table 1: IR (NaCl): $\tilde{\nu}$ = 1690 (s; $\nu(\text{CO})$) cm^{-1} ; m.p. 51 °C; ^1H NMR (300 MHz, CDCl_3): δ = 7.16 (s, 2H), 6.28 (m, 2H), 6.20 (m, 2H), 2.90 (br.s, 2H), 2.70 (br.s, 2H), 2.66 (br.s, 2H), 2.27 (m, 2H), 2.15 (t, J = 7.3 Hz, 4H), 1.49 (q, J = 7.4 Hz, 4H), 1.37 (q, J = 7.3 Hz, 2H), 1.36 (d, J = 9.2 Hz, 2H), 1.19 (d, J = 9.2 Hz, 2H); elemental analysis (%): calcd: C 83.29, H 7.89; found: C 83.36, H 7.97.

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(CuI)₂P₈Se₃: An Adduct of D₃-Symmetrical P₈Se₃ Cage Molecules with Cu₂I₂ Rhomboids**

Arno Pfitzner,* Sara Reiser, and Tom Nilges

Copper(I) halides have been established as a preparative tool for neutral and low charged molecules of the group 15 and 16 elements. Thus, several adducts of polymers, either of phosphorus or chalcogens, could be prepared in a crystalline state and characterized.^[1] By this approach, the β -P₄Se₄ cage, which was not previously accessible, was synthesized in (CuI)₃P₄Se₄. This compound is an adduct of a neutral phosphoselenide cage with copper iodide.^[2] Lowering the annealing temperature of the starting materials, copper iodide, phosphorus, and selenium, yields (CuI)P₄Se₄,^[3] in which phosphorus and selenium are arranged as polymers consisting of P₄Se₃ cages, analogous to norbornane, bridged by selenium atoms. This finding is consistent with the behavior of free equimolar mixtures of phosphorus and selenium, that, at low temperatures, form a polymer^[4] which depolymerizes at higher temperatures.^[5] The depolymerization processes in phosphorus–selenium melts were subject of a series of experiments.^[5] By means of NMR techniques, it was shown for P–Se mixtures of 48 % P that, at about 375 °C, the depolymerization and the formation of oligomers becomes significant.^[6] However, to date only four further phosphoselenide binary compounds have been crystallographically characterized. These compounds are P₂Se₅,^[7] P₄Se,^[8] P₄Se₅,^[9] and P₄Se₃.^[10] Therefore, information on the molecular structures of phosphoselenides is rather limited as compared to phosphosulfides.^[11] The major problem that hinders the preparation of phosphoselenides seems to be their tendency to form amorphous polymers at ambient temperatures. Since oligomeric and polymeric P₄Se₄ can be obtained in copper iodide at different temperatures, we were encouraged to explore the system CuI–P–Se for the existence of further adducts of either oligomeric or polymeric phosphoselenides with copper iodide. Herein, we report the synthesis and the structural characterization of (CuI)₂P₈Se₃.

The adduct (CuI)₂P₈Se₃ was obtained as a red crystalline material from the reaction of stoichiometric mixtures of CuI, P, and Se (CuI:P:Se = 2:8:3). The crystal structure was determined from single crystals.^[12] It consists of planar Cu₂I₂ rhomboids and P₈Se₃ phosphoselenide cages. It has been recently discussed that this building unit can adapt to a given coordination necessity due to the flexibility of both the χ (Cu–I–Cu) and the χ (I–Cu–I) angles.^[3] Typical measures for these rhomboids are, for example, the distance $d(\text{Cu–Cu})$ and the angles mentioned above. For (CuI)₂P₈Se₃, the following

[*] Priv.-Doz. Dr. A. Pfitzner, Dipl.-Chem. S. Reiser, Dipl.-Chem. T. Nilges
Universität Siegen, Anorganische Chemie
57068 Siegen (Germany)
Fax: (+49) 271-740-2555
E-mail: pfitzner@chemie.uni-siegen.de

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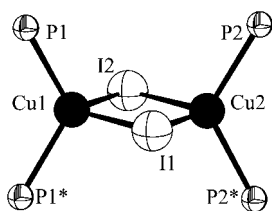


Figure 1. Labeling scheme and coordination pattern of the Cu_2I_2 rhomboids and copper-coordinated P atoms in $(\text{CuI})_2\text{P}_8\text{Se}_3$ (symmetry generated atoms are marked by a star). The bond distances $d(\text{Cu}-\text{I})$ are slightly longer for I1 than for I2, due to the larger angles $\angle(\text{I}-\text{Cu}-\text{P})$ for I2.

each copper atom to two phosphorus atoms. Due to the arrangement in the crystal structure, the angle $\angle(\text{I}-\text{Cu}-\text{P})$ is larger for I2 than for I1. Thus, repulsion by the bulky P_8Se_3 molecules can be regarded as the reason for the slight elongation of the Cu-I1 bonds.

The most remarkable feature of the crystal structure of $(\text{CuI})_2\text{P}_8\text{Se}_3$ are the novel P_8Se_3 cage molecules. Their D_3 symmetry is crystallographically reduced to C_2 symmetry, as shown in Figure 2. The slight deviations from ideality are due to the crystal structure arrangement. The P_8Se_3 cages under discussion can be derived from a P_8 cube by inserting selenium into three edges related by a threefold axis of the cube. The deviations from the ideal D_3 symmetry become obvious when the lengths and angles within this covalent molecular arrangement are discussed. The threefold axis is defined by P1 and P1', which are symmetry related. P1 has three covalent bonds to the phosphorus atoms P2, P3, and P4 (Figure 2). The distances $d(\text{P}-\text{P})$ for P1 range

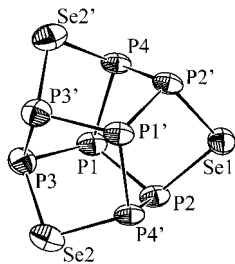


Figure 2. Labeling scheme of the P_8Se_3 molecules in $(\text{CuI})_2\text{P}_8\text{Se}_3$. The cages are coordinated to copper through P1/P1' and P2/P2'. The molecular D_3 symmetry is lowered in the crystal structure and only one C_2 axis remains. Atoms marked by ' are generated by this twofold axis. Ellipsoids are drawn at a 80% probability level.

from 2.226 to 2.246 Å and the angles $\angle(\text{P}-\text{P}-\text{P})$ are in the narrow range from 104.35° to 105.06°. In contrast, P2, P3, and P4 have only two covalent bonds to other phosphorus atoms. In addition, each of them is covalently bonded to one selenium atom. The averaged bond length $\bar{d}(\text{P}-\text{P}) = 2.23$ Å is only slightly smaller than the averaged bond lengths $\bar{d}(\text{P}-\text{Se}) = 2.26$ Å. The bond angles $\angle(\text{P}-\text{Se}-\text{P})$ of 93.13° (Se1) and 98.96° (Se2) are typical for phosphoselenides.

Figure 3 shows a section of the crystal structure of $(\text{CuI})_2\text{P}_8\text{Se}_3$. Each P_8Se_3 cage is coordinated by four copper ions belonging to four different Cu_2I_2 rhomboids by Cu1-P1 (P1') and Cu2-P2 (P2') bonds. The crystal structure consists of alternating layers of the phosphoselenide cages and of the Cu_2I_2 rhomboids. These layers are oriented along (001). The single cages are well separated due to their coordination to

values are found: $d(\text{Cu}-\text{Cu}) = 3.039$ Å, $\angle(\text{Cu}-\text{I}-\text{Cu}) = 69.36^\circ$ (I1) and 71.79° (I2), $\angle(\text{I}-\text{Cu}-\text{I}) = 109.39^\circ$ (Cu1) and 109.46° (Cu2). A slight deviation of an ideal rhomboid is present since both copper atoms and both iodine atoms of each rhomboid are crystallographically inequivalent. Thus, the bond length $d(\text{I1}-\text{Cu})$ is about 0.08 Å longer than the corresponding bond length $d(\text{I2}-\text{Cu})$. As shown in Figure 1, the reason for this bond length difference is because of the coordination of

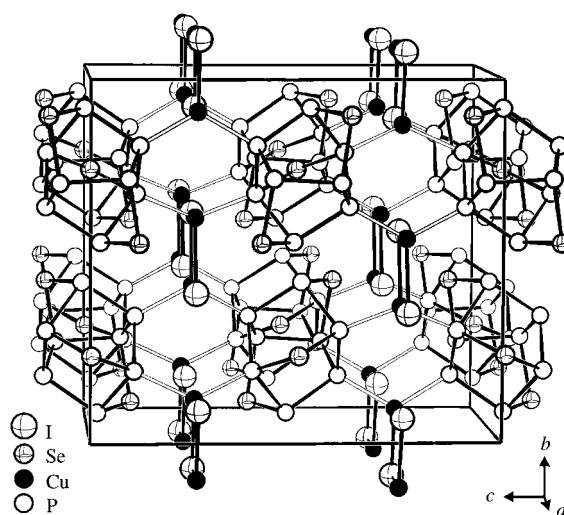


Figure 3. Section of the crystal structure of $(\text{CuI})_2\text{P}_8\text{Se}_3$. The P_8Se_3 cages and the Cu_2I_2 rhomboids are arranged in layers parallel to (001).

copper iodide. The shortest interlayer and intralayer distances between different P_8Se_3 units are about 3.8 Å.

The P_8Se_3 cage molecules are closely related to P_{11}^{3-} polyanions, the so-called "ufosane", which are present in Na_3P_{11} and K_3P_{11} .^[8, 16] The negatively charged two-coordinate phosphorus atoms of P_{11}^{3-} are replaced by selenium, consistent with the Zintl–Klemm concept. Thus, the polyanion P_{11}^{3-} is transformed to the neutral heteroatomic molecule P_8Se_3 . The most important function of copper iodide is to support the crystallization and, in addition, to hinder the polymerization of the cage molecules.^[1] If this concept holds, one might expect a large number of new adduct compounds of phosphoselenides with copper iodide when the impressive group of polyphosphide ions^[8] is regarded.

Experimental Section

$(\text{CuI})_2\text{P}_8\text{Se}_3$ was synthesized in quantitative yield by the reaction of stoichiometric amounts of CuI (>98%, Merck), red phosphorus (99.999%, Hoechst), and grey selenium (99.999%, Chempur). CuI was purified by recrystallisation from an aqueous HI solution prior to use.^[1] The reaction mixture of CuI, P, and Se ($\text{CuI}:\text{P}:\text{Se} = 2:8:3$) was sealed in dried and evacuated quartz glass ampoules, heated slowly to 600 °C, and then cooled to 350 °C. A pure microcrystalline product containing small dark red crystals was obtained after an annealing period of four weeks. The purity of the samples was checked by X-ray powder diffraction (flat sample in transmission geometry, Siemens D5000, $\text{CuK}\alpha_1$, $\lambda = 1.54051$ Å, Si as an external standard). Thermal analyses were performed with a DTA L62 apparatus (Linseis) with Al_2O_3 as reference material. $(\text{CuI})_2\text{P}_8\text{Se}_3$ has a melting point of 408 ± 2 °C.

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The Structure of the Fe^{IV}O₄⁴⁻ Ion**

Mark T. Weller* and Andrew L. Hector

Most of the complex oxide chemistry of iron is restricted to the oxidation states 2 and 3 though tetravalent, pentavalent, and hexavalent iron compounds can be formed in combination with Group 1 and 2 cations. Hexavalent iron is well known as the red-purple tetrahedral ferrate(vi) ion, FeO₄²⁻, in compounds such as Cs₂FeO₄^[1] and Na₂FeO₄.^[2] The FeO₄³⁻ ion, containing iron(v), has also been structurally characterized in K₃FeO₄.^[3] Iron(iv) compounds include the alkali metal iron oxides such as Sr₂FeO₄,^[4] Sr₃Fe₂O₇,^[5] SrFeO₃,^[6] and CaFeO₃,^[7] which contain distorted FeO₆ octahedra sharing vertices; unusual electronic properties are exhibited by some of these d⁴ materials due to a spin-pairing mechanism.^[8] The existence of a phase of the stoichiometry Ba₂FeO₄, reported by Scholder et al.^[9] as adopting the β -Ca₂SO₄ structure on the basis of similarities in the powder X-ray diffraction patterns, has not been substantiated. Fe³⁺ adopts octahedral coordination to oxygen in most of its complex oxides though the tetrahedral FeO₄⁵⁻ ion is known in the compound Na₅FeO₄.^[10]

Scholder et al.^[9] reported the synthesis of a sodium iron(iv) oxide of the stoichiometry Na₄FeO₄ but no structural data exist for this material, which might be expected to contain tetrahedral FeO₄⁴⁻ by analogy with other Na₄MO₄ (M = Ti, Cr, Co, Ge) compounds.^[11–14] Indeed the structure of the ferrate(iv) ion has not been described to date. Iron(iv) is isoelectronic with manganese(III) and both ions exhibit Jahn–Teller distortions, due to their high-spin d⁴ configurations. The observation of unusual colossal magnetoresistive properties in some Mn^{III} and Fe^{IV} compounds^[15] is associated with structural distortions in these d⁴ species. Herein we report for the first time the geometry of the FeO₄⁴⁻ ion as determined in sodium ferrate(iv).

The structure of Na₄FeO₄ consists of discrete FeO₄⁴⁻ ions separated by sodium ions as shown in Figure 1. Each unit cell contains two ferrate(iv) ions related by a center of symmetry,

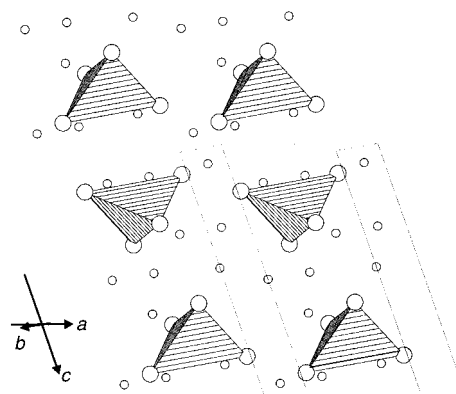


Figure 1. Unit cell of Na₄FeO₄. FeO₄⁴⁻ units are shown as polyhedra and sodium atoms as spheres.

and the sodium ions coordinate to four (Na1, Na2, and Na3) or five (Na4) oxygen atoms at 2.3–2.5 Å, though Na1 has two and Na3 has one longer, additional interactions to an oxygen atom. The most remarkable feature of the structure is that the FeO₄⁴⁻ tetrahedron is strongly distorted (Figure 2). The unit is squashed along one C₂ axis to give the ion approximately D_{2d} symmetry, rather than perfect tetrahedral, and two of the

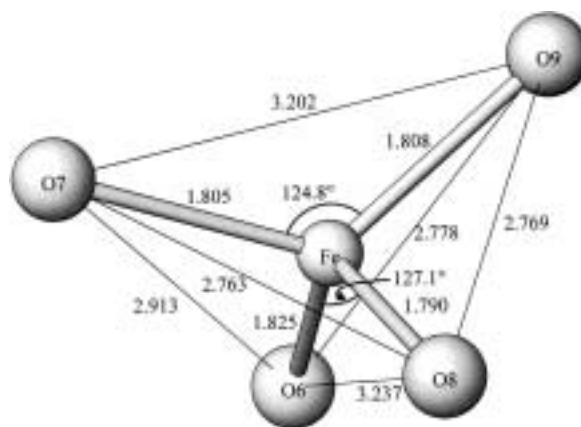


Figure 2. Geometry of the FeO₄⁴⁻ ion. Other bond angles [°] not shown for clarity are O6–Fe–O7 106.7(2), O6–Fe–O9 99.8(2), O7–Fe–O8 100.5(2), O8–Fe–O9 100.6(2).

[*] Prof. M. T. Weller, Dr. A. L. Hector
 Department of Chemistry
 University of Southampton
 Highfield, Southampton SO17 1BJ (UK)
 Fax: (+44) 2380 593592
 E-mail: mtw@soton.ac.uk

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O-Fe-O bond angles open out to 125° and 127°, respectively. Such behavior is well known for d⁹ systems, for example tetrahedral copper(II) in species such as CuCl₄²⁻ in Cs₂CuCl₄ and, much more rarely, d⁴-centered tetrahedra.^[16] The only previous example of a d⁴ oxotetrahedral unit is in the MnO₄⁵⁻ ion present in Na₅MnO₄, where squashed tetrahedral geometry is also adopted with two O-Mn-O bond angles between 130° and 135°.^[17] The d⁴ Fe^{IV} ion in Na₄FeO₄ evidently displays similar Jahn–Teller-originated distortion. In terms of the unit cell this flattening of the tetrahedron in comparison with other MO₄⁴⁻ ions in Na₄MO₄ compounds results in a contraction along the *c* direction (e.g. Na₄GeO₄ 8.58 Å, Na₄FeO₄ 8.24 Å) as the tetrahedra can approach each other more closely.

The average Fe–O distance in the ferrate(IV) ion of 1.807 Å is in line with that expected from other FeO₄ⁿ⁻ ions (FeO₄²⁻ 1.647, FeO₄³⁻ 1.720, FeO₄⁵⁻ 1.889 Å). However all these other ferrate ions are close to perfect tetrahedra.

Experimental Section

Black Na₄FeO₄ was synthesized from FeO (Aldrich, 99.9%) and Na₂O₂ (Aldrich, 97%) (1:2) by heating the mixture at 400 °C for 48 h. The mixture was pelletized, placed in a gold crucible, and sealed under vacuum into a Pyrex tube. All reagents and products were handled in a dry nitrogen glove box. The powder X-ray diffraction pattern (Siemens D5000, Cu_{Kα1} radiation) of the crystalline product showed no evidence of starting materials or other known compounds from the Na-Fe-O system. Time-of-flight neutron diffraction data were collected for 2 h on the POLARIS high-flux diffractometer at the ISIS facility. IR (nujol, Perkin-Elmer Spectrum One): $\tilde{\nu}$ = 600 (br), 799 cm⁻¹ (br); Fe content (gravimetric with 8-hydroxyquinoline); found: 27.4%; calcd: 26.4%; Fe oxidation state (iodometric): 3.97 ± 0.05.

2θ reflection positions were extracted from the neutron diffraction data and indexed by using the DICVOL91 program.^[18] Successful indexing was performed with a triclinic cell of dimensions and volume similar to those reported for other Na₄MO₄ phases though one angle and cell dimension were significantly different indicating a compression of the unit cell along one direction. A Le Bail extraction using this cell and the high-resolution back scattering neutron diffraction data produced an excellent fit (χ^2 ca. 1) demonstrating this as the correct unit cell. Structural models were developed from the published data on Na₄GeO₄ (P1)^[14] and Na₄CoO₄ (P1)^[13] and these were refined by using the GSAS program suite.^[19] After

Table 1. Refined atomic coordinates for Na₄FeO₄ and the parameter *B*_{iso} for the profile fit (esds are given in parentheses).^[a]

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> _{iso} [Å ²]
Na1	0.4061(11)	0.7871(10)	0.1306(7)	1.99(10)
Na2	0.7920(9)	0.2657(9)	0.4835(6)	1.11(10)
Na3	0.7521(10)	0.7702(8)	0.3342(6)	1.37(10)
Na4	0.9502(10)	0.2800(9)	0.0679(6)	1.49(10)
Fe5	0.4083(3)	0.2786(4)	0.2482(2)	0.88(4)
O6	0.2429(7)	0.0979(6)	0.3938(4)	1.60(7)
O7	0.1650(6)	0.5031(6)	0.1708(4)	1.24(7)
O8	0.5630(6)	0.4729(6)	0.2998(4)	1.01(6)
O9	0.6901(6)	0.0282(6)	0.1164(4)	1.05(5)

[a] Space Group *P* $\bar{1}$, *a* = 5.76205(11), *b* = 5.76188(10), *c* = 8.24301(15) Å, α = 87.8769(14), β = 71.9681(14), γ = 69.3396(12)°, *V* = 242.690(8) Å³, *R*_{wp} = 0.0205, *R*_p = 0.0366, *R*_i = 0.0305.

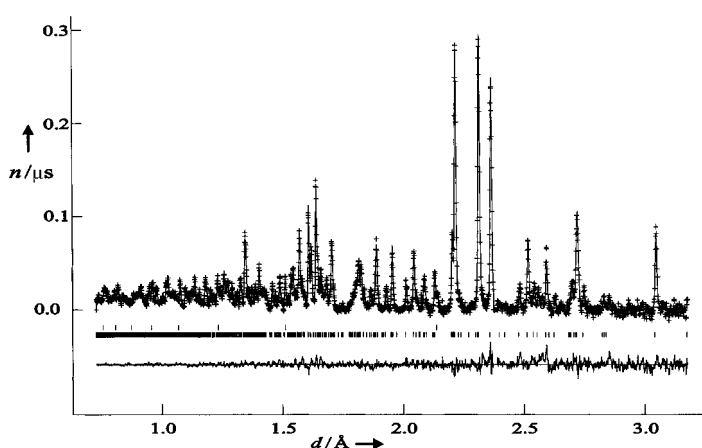


Figure 3. Final profile fit obtained to the powder neutron diffraction data from Na₄FeO₄. Crosses are observed data, upper continuous line the calculated profile and lower continuous line the difference. Tick marks represent peak positions, upper set vanadium from the sample can and lower Na₄FeO₄. *n* is the number of counts.

refinement an excellent fit to the profiles was obtained in *P* $\bar{1}$ and it was found unnecessary to reduce the symmetry to *P*1. Final refined atomic coordinates and profile fit parameters are summarized in Table 1, derived bond lengths and angles of significance relating to the FeO₄ tetrahedron are shown in Figure 2 and the final profile fit achieved is shown in Figure 3.

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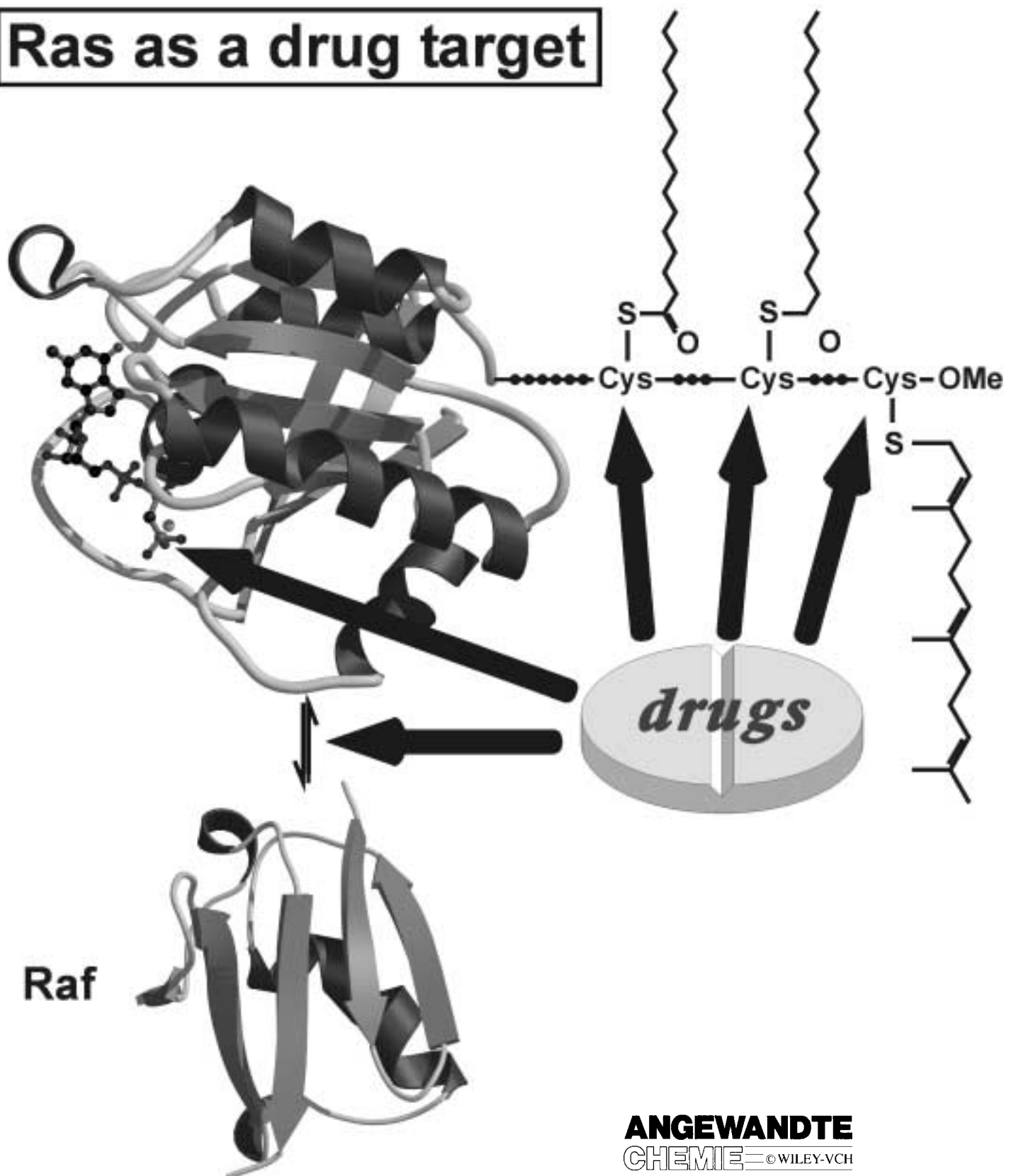
Cover Picture

Kiyohiko Kawai,* Yasuharu Wata, Nobuyuki Ichinose, and Tetsuro Majima

The cover picture shows in red the three-dimensional structure of the Ras protein in a ribbon representation, with its β -strands, α -helices and connecting loops. The protein bound cofactor Guanosine triphosphate (guanine base up, phosphates down) is shown in a space-filling model. Due to its central role in many signal transduction processes and since it is heart-shaped, Ras has been called the beating heart of signal transduction (i.e. H. Bos, Utrecht).



Ras as a drug target



Ras—A Molecular Switch Involved in Tumor Formation

Alfred Wittinghofer* and Herbert Waldmann

Ras, a GTP-hydrolyzing protein, is the product of a proto-oncogene found mutated in about 20–30 % of human tumors. It binds GDP/GTP with high affinity and in the presence of a GTPase-activating protein (GAP) has high GTP-hydrolyzing activity. The proto-oncogenic “normal” Ras functions as a regulated molecular switch cycling between a GDP-bound OFF

and a GTP-bound ON state and is involved in signal transduction pathways controlling cell growth, differentiation, apoptosis, and other events. The oncogenic versions of Ras contain point mutations which block the GTPase activity in the presence and absence of GAP. This process in turn inhibits the cycling of the switch and leads to the accumulation of Ras in the

active form and contributes to tumor formation. Substantial effort has been devoted towards understanding the molecular basis for the switch function of Ras proteins and developing Ras-directed antitumor drugs.

Keywords: antitumor agents • drug research • enzyme inhibitors • GTP hydrolysis • proteins

1. Introduction

Ras is the founding member of a superfamily of proteins called Ras-related GTP-binding proteins. Their common property is a molecular mass of 20–25 kDa and the ability to bind guanine nucleotides. They function as molecular switches by cycling between a GTP-bound active state and a GDP-bound inactive state. They regulate a multitude of different cellular reactions but can be grouped into two major categories: the signaling proteins, which act catalytically and are involved in communication between cells and between different cellular compartments, and a second group which acts stoichiometrically as transport factors regulating the delivery of material between cellular compartments.

In this review we will concentrate on the signaling protein Ras, which is a major regulator of many signal transduction processes, such as those regulating growth, differentiation, cell cycle, and apoptosis, some of which are actually seemingly opposing functions. Although we will outline the biological function of Ras, this review focuses on more structural-biochemical and biophysical aspects of the protein and on how this knowledge is applied to the development of potential antitumor drugs.

For more detailed aspects of Ras biology and for earlier references other reviews should be consulted.^[1–5]

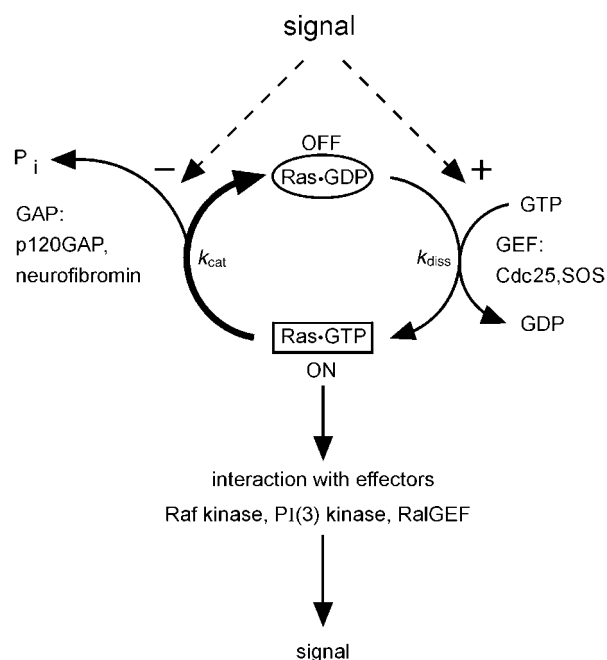
[*] Prof. Dr. A. Wittinghofer, Prof. Dr. H. Waldmann
Max-Planck-Institut für molekulare Physiologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
Fax: (+49) 231-1332199
E-mail: Alfred.Wittinghofer@mpi-dortmund.mpg.de

2. Ras as a Molecular Switch

2.1. Biological Signal Transduction

There are three human *Ras* genes, *H-*, *N-*, and *K-Ras*, the latter of which is further spliced to produce two different variants, *K-Ras4A* and *K-Ras4B*, which are divergent only in the last 25 residues encoded by the fourth exon. The corresponding H-, N-, and K-Ras proteins have a molecular mass of 21 kDa. They are posttranslationally modified by the attachment of lipophilic groups to the C-terminus and this modification is necessary for the biological function of the protein.^[6] Ras is a molecular switch that cycles between the GDP-bound and the GTP-bound state (Scheme 1). Since the switch-on and the switch-off reactions in the cycle of Ras are intrinsically very slow, the regulatory input by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAP) determines the lifetime of the two states. In the resting state Ras is bound to GDP. It is activated by the action of GEF catalyzing the dissociation of GDP, thus facilitating the loading with GTP, which is the more prevalent guanine nucleotide in the living cell. In the GTP-bound state it interacts with effectors, which are defined as proteins that interact specifically with the GTP-bound state and transmit a signal. The signal is terminated by the GTPase reaction on Ras, which is very slow but is accelerated by Ras-specific GAP. Ras in the GDP-bound state is no longer able to interact with the effectors.

The lifetime of the signal transduced by Ras is determined by the lifetime of the active state. If it is artificially extended, the now unregulated biological response may lead to drastic



Scheme 1. Cycling of the Ras molecular switch. Ras is inactive in the GDP-bound form. It can be activated by the action of guanine nucleotide exchange factors (GEFs). In the GTP-bound form it interacts with effectors, which are defined as proteins specifically recognizing the GTP-bound form. To complete the cycle, Ras hydrolyzes GTP to GDP and phosphate (P_i). Ras can in principle also be activated by inhibiting the GTPase reaction.

consequences in the cell. Ras was indeed originally found as the active principle of rodent tumor viruses (the name Ras is derived from *rat sarcoma*) and was called an oncogene, a gene that is able to induce tumors in animals or in cell cultures.^[2, 5] Later, activated forms of Ras were found in human tumors, and it is now estimated that up to 30 % of human tumors carry a mutated Ras oncogene, usually derived from *N*- or *K*-Ras. The activation of the *Ras* gene from a proto-oncogene to an oncogene is the result of a point mutation at either position 12, 13, or 61, the biochemical consequence of which is to render the protein unable to hydrolyze GTP.^[2, 5] Since many other genes involved in the Ras signal transduction pathway are also found as oncogenes in human or animal tumors, Ras itself and the Ras pathway are thus considered to be prime targets for antitumor therapy and indeed great progress has been made in that direction.

In the early 1990s, by using information from studies with mammalian cell lines and from *Drosophila melanogaster*^[7] and *Caenorhabditis elegans*^[8] genetics, a linear signal transduction pathway linking Ras with growth factor receptors and MAP kinase (*mitogen activated protein kinase*) activation was established, and this “Ras pathway” is now considered to be the paradigm for biological signal transduction.^[4, 9] The signal is initiated by the binding of a growth factor such as EGF (*epidermal growth factor*) or PDGF (*platelet-derived growth factor*) to the extracellular domain of their corresponding receptors (Scheme 2). These are transmembrane

Alfred Wittinghofer was born 1943 in Xanten on the Niederrhein. He studied chemistry in Aachen and made his diploma thesis and his Ph.D. in the laboratory of Prof. Dr. H. Zahn at the Deutsche Wollforschungsinstitut in Aachen, where he synthesized the A chain of insulin with a preformed selective intrachain disulfide bridge in the presence of two additional cysteine residues. He spent three years as a post-doc with Dr. R. Hiskey at the University of North Carolina on the preparation of semi-synthetic insulin. He came back to Germany to work a number of years as a postdoc in the Max-Planck-Institut fuer medizinische Forschung in Heidelberg where he studied phosphoryl transfer enzymes. After establishing his own research group there he moved into the field of Ras and other Ras-like GTP-binding proteins on which he has been working ever since. He was appointed Director of the Max-Planck-Institut fuer molekulare Physiologie in 1992.

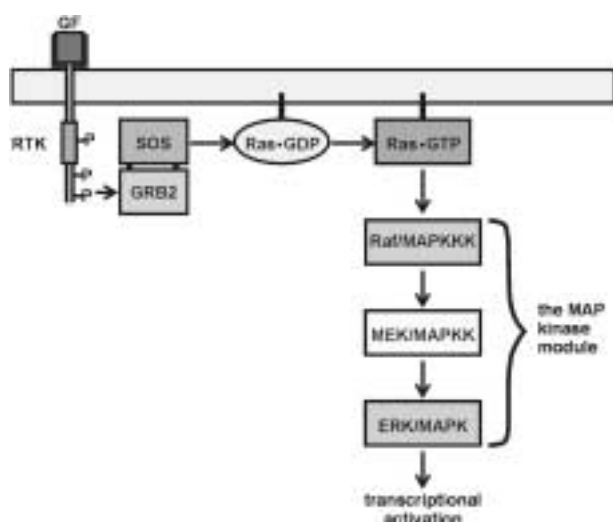


A. Wittinghofer



H. Waldmann

Herbert Waldmann, born in 1957, received his Ph.D. in 1985 (Universität Mainz, H. Kunz). After postdoctoral studies (1985–1986, Harvard University, George Whitesides) and habilitation (1991, Universität Mainz) he accepted a professorship at the Universität Bonn in 1991. In 1993 he moved to the Universität Karlsruhe as Full Professor of Organic Chemistry. In 1999 he was appointed as Director at the Max Planck Institut of Molecular Physiology, Dortmund (Department of Chemical Biology) and as Full Professor of Biochemistry at the University of Dortmund. Herbert Waldmann has been the recipient of the Friedrich Weygand Award for the advancement of peptide chemistry, of the Carl Duisberg Award of the Gesellschaft Deutscher Chemiker, and the Steinhof Award of the Steinhof Foundation. His current research interests include bioorganic chemistry and natural product synthesis as well as biocatalysis, stereoselective synthesis, and combinatorial chemistry. A major focus of the research activities is on the combination of organic chemistry, biophysics, and biology for the synthesis and biological evaluation of peptide and protein conjugates which are involved in biological signal transduction processes. Most recently syntheses of natural products and natural product derived compound libraries on polymeric supports have been investigated by the Waldmann group (for further information see: www.chemie.uni-karlsruhe.de/OC/akwa/ak.htm).



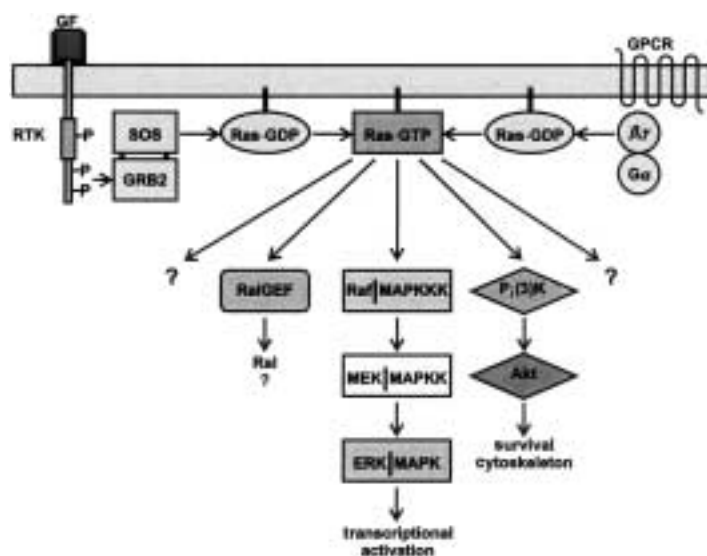
Scheme 2. Simple version of the Ras signal transduction pathway as described in the text. Recruitment of the RasGEF SOS to the plasma membrane by activated, growth factor bound RTKs (receptor tyrosine kinases) leads to activation of Ras. It in turn activates a cascade of protein kinases, MAP kinase kinase kinase (MAPKKK), MAP kinase kinase (MAPKK), MAP kinase (MAPK), whereby the upstream kinases activates the next in-line kinase by phosphorylation. Phosphorylated MAPK (= Erk) is transported into the nucleus to phosphorylate transcription factors which mediate the cellular response to the original signal, the growth factor (GF).

proteins with a cytoplasmic tyrosine-specific protein kinase domain called receptor tyrosine kinases (RTKs). The binding of ligand induces dimerization of the receptor and subsequent transphosphorylation on tyrosine residues in the cytoplasmic part of the receptor, both in the kinase domain and other regions. Phosphorylation activates the receptor and generates binding sites for proteins containing SH2 (src homology 2, where *src* is an oncogene originally isolated from a *Sarcom*) domains, which are protein modules specifically recognizing phosphorylated tyrosine residues in a sequence-specific context. One such protein is Grb2 (growth factor receptor binding), which is complexed to the Ras guanine nucleotide exchange factor RasGEF SOS (from the *Drosophila* gene *son-of-sevenless*). The translocation of SOS to the plasma membrane is believed to activate Ras by bringing both proteins close to each other and thus favoring the interaction.

Once activated Ras interacts with the downstream target or effector, the Ser/Thr-specific protein kinase Raf—itsself originally found as an oncogene (*rapid fibrosarcoma*). The terms upstream and downstream in signal transduction derives from a (now obsolete) concept of a linear hierarchical order of reactions where one reaction leads to (upstream) and one from (downstream) the protein in question. Translocation of Raf kinase to the plasma membrane somehow activates the kinase activity by a process that is not completely understood, but involves allosteric regulation as well as participation of other kinases and regulatory proteins such as 14-3-3.^[1, 10–12] Once activated Raf in turn activates the protein kinase MEK by phosphorylation, and this in turn activates the protein kinase ERK. These three in-line kinases are also called MAP kinase (MAPK, mitogen activated protein kinase), MAP kinase kinase (MAPKK), and MAP kinase kinase kinase (MAKKK), and constitute a so-called MAP kinase module.^[13]

Such kinase modules are found in a large variety in all higher eucaryotic cells and are sometimes (maybe always) bound to so-called scaffold proteins, which help to organize the module and possibly guarantee the specificity of signal transmission.^[14] Ras signaling is terminated by the action of GAP, which may also be recruited to the plasma membrane for more efficient interaction. The prototype p120GAP is in fact an SH2-containing protein and has been shown to be recruited to activated receptors.^[15–19] The signal is also terminated by MAPK phosphorylating SOS, which in turn is no longer able to interact with Grb2.^[20–23]

The elucidation of the linear signal transduction cascade through the MAP kinase module was a landmark achievement performed in several laboratories and was believed to explain the biological function of Ras. However, in recent years it has become clear that the different functions of Ras are unlikely to be explained by a single linear MAP kinase cascade and that different signals upstream and downstream of Ras more likely have to be invoked to explain the complexities of Ras biology (Scheme 3). Ras was shown to



Scheme 3. Involvement of Ras in more than one signal transduction event. Ras is activated by more than one upstream signal and relays the signal to more than one effector. The outcome of the cellular response depends on the number of signals and their integration by the target protein(s), that is, transcription factors.

be activated not only by receptor tyrosine kinases (RPTKs) but also by cytoplasmic tyrosine kinases such as Src and by G-protein-coupled receptors (GPCRs), to name just a few. Similarly it was shown that Ras induces different signals presumably by interacting with more than one effector. In a landmark experiment it was in fact shown by White et al.^[24] that one can dissect different downstream signaling pathways of Ras by introducing mutations into the effector region which block a certain set of pathways without touching others,^[24–28] and that more than one pathway has to be switched on for full transformation by Ras.

In the meantime, by using the newly developed techniques of the two-hybrid analysis, a large number of actual or potential Ras effectors have been identified as molecules which interact with the GTP-bound form—but not the GDP-

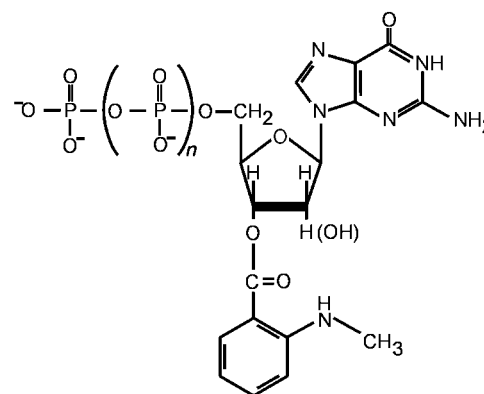
bound form—of Ras. The most important among these are the various isoforms of the lipid kinase phosphatidylinositol-3'-kinase (PI3K)^[29–31] and of RalGEF^[32–34], which is a nucleotide exchange factor (GEF) for the Ras-related protein Ral, of which several isoforms (Rgl, Rlf/RalGEF2^[35, 36]) have been described. Another complication in unraveling the complicated network of interactions is the fact that certain Ras-like proteins such as Rap (four isoforms) and R-Ras (three isoforms) also interact with the same set of effectors in a GTP-dependent manner.

Although it is tempting to speculate that particular pathways are switched on for one particular biological effect, this appears not to be the case. Rather, we may assume that Ras always induces more than one pathway at a time and that the final signal output depends on a multitude of Ras-dependent and Ras-independent parallel and interfering (crossover) signaling pathways which are different in different types of cells. These signals are finally integrated by a complex downstream machinery to produce the cell-type-specific biological response.

2.2. Biochemistry and Structure of Ras

Most of the biochemical and structural studies have been done with H-Ras (Ras, for short) for no obvious reason and its properties will thus be discussed here, but it is clear that the biochemical and structural properties of the other isoforms are similar, even though they are not completely identical in sequence. Ras consists of 189 amino acids with five conserved sequence elements which are also found in other Ras-related proteins and are necessary for binding guanine nucleotides and for the hydrolysis of GTP. The last four amino acids (CVLS) constitute what is called a CAAX box, where C stands for cysteine, A for an aliphatic, and X for any amino acid. CAAX is the signal recognition sequence for the enzyme farnesyltransferase which thioalkylates the cysteine residue with a C15 prenyl group (see Section 3). This modification is the first in a series of posttranslational modifications which is responsible for anchoring Ras into the plasma membrane, a process which is necessary for its biological activity.^[37–40] It has been shown that the first 166 residues of Ras are necessary and sufficient for its biochemical properties^[41] and that the C terminus is thus only necessary for localization in the plasma membrane and is not involved in any interaction. Correspondingly, for technical reasons, many biochemical and structural studies on Ras have been performed with versions with truncated C termini (amino acids 1–166 or 1–172) of the protein.

Ras binds guanine nucleotides with high specificity and affinity. The affinity for GDP/GTP is so high that no equilibrium method can be used for its measurement. The affinity is instead determined by measuring both association and dissociation rate constants, which is most conveniently done by using fluorescently labeled methylantraniloyl (mant- or m- in brief) nucleotides (Scheme 4). These nucleotides have been proven to be very versatile for the study of the biochemistry of Ras proteins.^[42–44] The rates thus measured allow the calculation of an equilibrium dissociation constant



Scheme 4. Examples of fluorescent guanosine nucleotides which are used in the biochemical analysis of Ras proteins. They carry a fluorescent *N*-methylantraniloyl group on the 2' or 3'-position of the ribose group. The 2'-deoxy mGDP ($n=1$) and 2'-dmGTP ($n=2$) nucleotides are shown. In the case of the ribose derivatives, a mixture of 2',3' analogues is commonly used.

in the order of 10 pM for GDP and GTP.^[43–45] The binding affinity is strongly dependent on the presence of Mg^{2+} ions. In their absence the dissociation rate constant increases by several hundredfold with a concomitant increase in the dissociation constant.^[46, 47]

The GTPase reaction is very slow, of the order of 0.02 min^{-1} , and is strongly stimulated by GTPase-activating proteins (see Sections 2.5 and 3.4). Like any other biological phosphoryl transfer reaction it is strictly dependent on the presence of Mg^{2+} ions. The specificity for binding guanine nucleotide is very high, and no other standard nucleotide shows easily measurable binding. The affinity for adenine nucleotides has been estimated from kinetic methods to be lower by at least six orders of magnitude.^[48] Small changes of the guanine ring system led to drastic changes in affinity: xanthosine, which has the 2-amino group replaced by an oxygen atom, binds with a 280-times lower affinity, and an inosine nucleotide diphosphate, which misses the 2-amino group, shows a similarly reduced binding.^[48] The β -phosphate group is necessary for high affinity binding as shown by GMP, which has an affinity six orders of magnitude lower.^[43–48]

The three-dimensional structure of Ras has been determined from two truncated versions of the H-Ras protein that terminate either at position 166^[49, 50] or at 171.^[51, 52] The structure of full-length proteins has never been solved, although crystals of the protein have been reported.^[52] The fold of the protein consists of a six-stranded mixed β -sheet and five α -helices on both sides of the sheet (Figure 1A). This fold is found in all standard GTP-binding proteins, including the much larger heterotrimeric G proteins or biosynthesis elongation factor Tu (EF-TU; the first GTP-binding protein whose structure was solved), and is called the G-domain fold.

Although some of the sequence conservation of GTP-binding proteins (Figure 1B) had been observed before the first three-dimensional structure had been solved^[53] the role of the conserved sequence elements could only be appreciated afterwards. Not surprisingly, they are invariably involved in the binding of guanine nucleotides, hydrolysis of GTP, and/or in the mechanism of conformational change. They have been

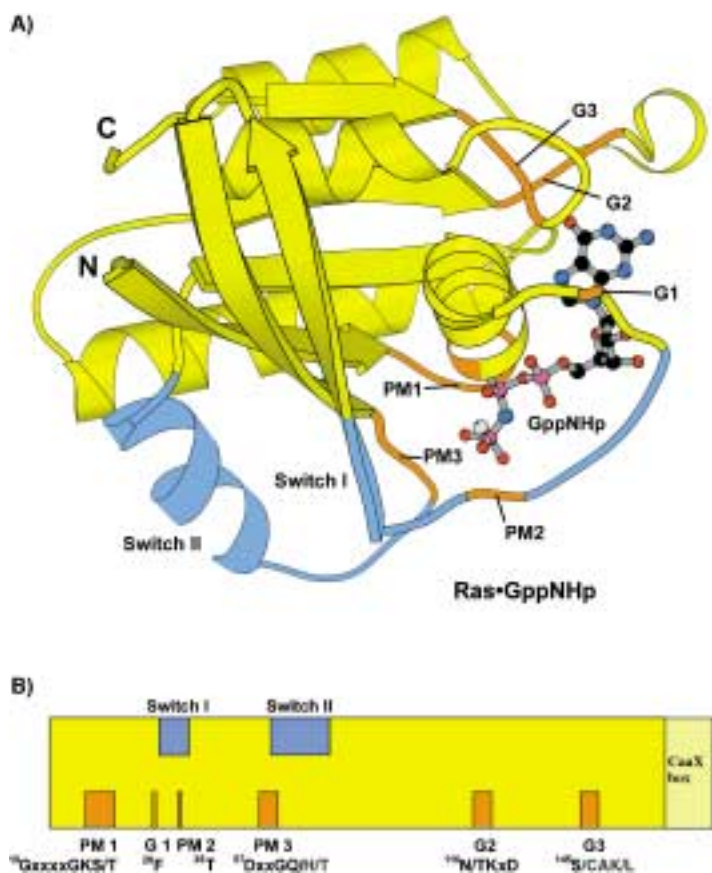
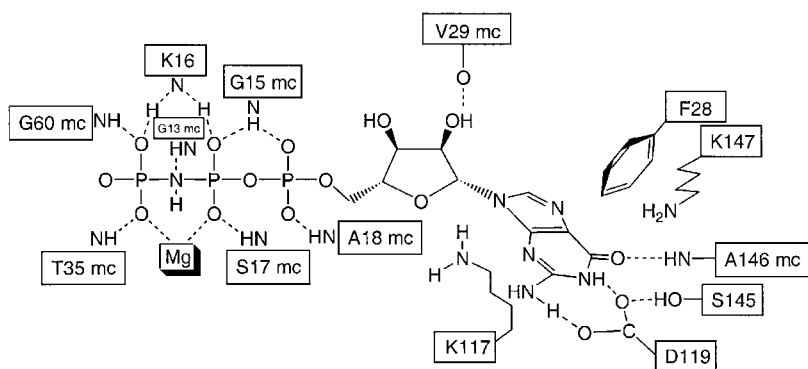


Figure 1. Primary and tertiary structure of Ras. A) A ribbon plot of Ras in the triphosphate form (as GppNHp complex), with the conserved sequence elements of all GTP-binding proteins (brown) located around the nucleotide. The two regions in Ras which change their structure between the GTP- and GDP-bound forms, the Switch I and II regions, are shown in blue. The N and C termini of the polypeptide chain are indicated. B) Linear schematic representation of Ras, with the location of conserved sequence elements, and Switch regions (color coding as in A). The structure in A is only that of the actual G domain which misses the C-terminal extension with the CAAX box which is absolutely essential for the biological function but is not necessary for any of the structural and biochemical properties discussed here.

called the G motifs (G1 to G4)^[54, 55] or, according to their function, PM and G motifs which describe their involvement in the binding of either phosphate and Mg^{2+} ions or the guanine base, respectively.^[56] The network of interactions in the nucleotide binding site is shown in Scheme 5.



Scheme 5. Interactions of the Ras-bound triphosphate analogue GppNHp with selected, usually conserved residues in Ras. mc = peptide main chain.

The PM1 motif $^{10}GxxxxGKS/T$ forms a loop (L1 in Ras), also called the P loop, which is involved in binding the charged phosphate groups. Through its totally conserved lysine it forms a ringlike structure that wraps tightly around the β -phosphate of GDP/GTP. The main-chain nitrogen atoms of residues 13–16 point towards the negatively charged phosphate groups and, together with the side chain of Lys16, create a positively polarized environment for these charges. The serine/threonine residue following the lysine coordinates to the important Mg^{2+} cofactor in both the GDP- and GTP-bound state. Its substitution by Ala or Asn severely modifies the interaction between the protein, metal ion, and nucleotide.^[57–59] As a result of this the affinity to guanine nucleotides is very weak and in turn the protein has a relatively high affinity (relative to GDP/GTP) to guanine nucleotide exchange factors. The GEFs are then sequestered by the mutated Ras proteins. Therefore such a mutation is called dominant negative, because it inhibits signaling through the GEFs.

Although various consensus motifs have been proposed around this conserved threonine (^{35}T) in the PM2 motif, none of these residues except Thr are actually conserved. This threonine is a direct ligand of Mg^{2+} in the GTP-bound state, binds the γ -phosphate of GTP, and is a key residue that triggers the conformational change after GTP hydrolysis.^[60] Mutation of Thr35 to alanine reduces the affinity to nucleotide and the mutant protein can no longer be activated by GAP, probably as a result of the incorrect coordination of Mg^{2+} ions.^[59]

In the $^{57}DxxGQ/H/T$ (PM3) motif the aspartate group is involved in binding Mg^{2+} ions through a water molecule. The glycine residue is coordinated by a main-chain hydrogen bond to the γ -phosphate group, another interaction crucial for the conformational change after GTP hydrolysis. Most GTP-binding proteins contain a Gln residue crucial for GTP hydrolysis. This residue is replaced by Thr in Rap proteins and by His in elongation factors.

The Phe or Tyr residue constituting the G1 motif is only conserved in proteins belonging to the Ras superfamily. It is located perpendicular to the guanine base and creates strong hydrophobic interactions between the protein and base together with the conserved lysine from the G2 motif. Mutating this residue to leucine weakens the nucleotide binding drastically by a corresponding increase in the dissociation rate.

In $^{116}N/TKxD$ (G2) the guanine base of the nucleotide is located in a hydrophobic pocket. Specific hydrophobic contacts and ionic interactions and hydrogen bonds stabilize its binding. Both the Asn and Lys are involved in linking together various subregions of the nucleotide binding site. The aspartate residue is involved in a double hydrogen bond to the guanine base and is the major element responsible for the high specificity of the protein for guanine nucleotides. Many residues in the G2 motif have been mutated in Ras-related proteins. The effect of the mutations is invariably to increase the rate of dissociation of the nucleotides from the protein. Such mutants

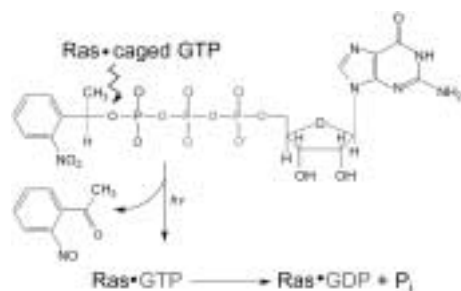
are permanently activated as a consequence of the fast exchange reaction and/or are dominant negative. Mutation of Asp to Asn reduces the affinity for guanine nucleotides several hundredfold, but shows a similarly increased affinity for xanthosine nucleotides,^[45, 61, 62] since binding of the latter preserves the double hydrogen-bond system. These types of mutants have been useful in studying the role of EF-Tu,^[63] Ras,^[45, 64] Rab5,^[65] and FtsY.^[66]

The G3 motif ¹⁴⁵S/CAK/L is, with the exception of the alanine residue, not totally conserved in the Ras superfamily. It seems to serve as a helper function in the binding of the guanine base since its side chains do not participate in any direct interaction with the base. Instead the Lys or Leu residue is involved in stabilizing the position of Phe28 residue (the G1 motif) by a hydrophobic interaction and Ser145 forms a hydrogen bond with Asp119. The nitrogen atom on the Ala146 residue of the main chain makes a strong hydrogen bond with O6 of the base and this interaction is another factor responsible for the specificity of guanine nucleotide binding; adenine would not be accommodated for steric reasons.

The structure of Ras was solved both in the triphosphate and diphosphate form,^[49–52, 67] by using GTP analogues such as GppNHP^[49, 50] and Gpp(CH₂)p^[51] (two non- or slowly hydrolyzing analogues), where the β - γ -bridging oxygen atom is replaced by an NH or CH₂ group. These studies showed that the structural changes are confined to only two small areas which have been defined as Switch I and II.^[51] The structures showed how the conformational change is triggered since both switch regions are bound to the γ -phosphate through two invariant residues: Switch I by Thr35 and Switch II by Gly60.^[60] Removal of the γ -phosphate group in the course of GTP hydrolysis allows these regions to relax and adopt a new conformation in what may be termed a loaded spring mechanism.^[68]

The structure of Ras was also solved by using various analogues of guanine nucleotides such as the fluorescent derivative mantGppNHP^[69] (Scheme 4), and caged GTP,^[67] which carries a photolabile protecting group on the γ -phosphate residue (Scheme 6). Crystals of the Ras·caged GTP complex were obtained and the protecting group was removed by flash photolysis. The Ras·GTP structure then obtained represents a true enzyme·substrate complex.^[67, 70] In an extension of these studies, Ras·GTP was allowed to hydrolyze to Ras·GDP in the crystal.

A number of mutants of Ras were also structurally investigated, most notably oncogenic mutants of Ras, such



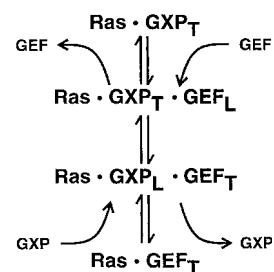
Scheme 6. Structure of caged GTP. Crystals containing Ras·caged GTP are photolyzed and then used to determine the crystal structure of Ras·GTP, which represents the Michaelis–Menten complex of the GTPase reaction.

as G12V, G12R, G12D, and Q61L.^[52, 71, 72] They showed a similar overall structure as the wild-type. Although small changes around the γ -phosphate group were observed in all of these structures the analysis did not provide any consistent explanation for the inability of these mutants to hydrolyze GTP.

2.3. The Switch-On Mechanism

Although Ras can in principle be switched ON by inhibiting the GAP-mediated GTPase reaction^[73] the prevalent mode of activation is by interaction with GEFs. A number of specific GEFs for Ras have been identified which can be grouped into two main categories (for a review, see ref. [74]). The first is SOS, short for the *son-of-sevenless* mutation in *Drosophila*, with two mammalian isoforms which contain polyproline motifs necessary for its interaction with SH3 domains, such as those contained in the adaptor protein Grb2.^[75–79] These exchange factors are activated by recruitment of the SOS·Grb2 complex to tyrosine-phosphorylated receptors. The second class of RasGEF, with three isoforms and several splice variants, contains a number of domains which are believed to bind to and are activated by second messengers, such as Ca²⁺ and Ca²⁺-calmodulin.^[80, 81] A recently identified member of this group is activated by diacylglycerols.^[82, 83] Both types of GEFs contain a Cdc25-like domain, which was first identified in the yeast protein Cdc25. This domain is necessary and sufficient for fast guanine nucleotide exchange.^[84] A second domain, REM (*ras exchange motif*), is found at a variable distance from the CDC25 domain.^[85] As the structure has shown (see below), the latter is mostly responsible for the stability of the catalytic domain.

GEFs increase the dissociation rate dramatically, by several orders of magnitude in the case of Ras and Ran where it has been measured under saturating conditions, by reducing the affinity appropriately. The nucleotide thus comes off in less than a second.^[84, 86, 87] A minimal model for the reaction (Scheme 7) involves GEF approaching the Ras·GDP binary complex and forming a ternary complex.^[84, 86–90] Thereafter the nucleotide binding pocket goes from a tight (GXP_T) to a loose binding conformation (GXP_L), which results in a fast release of nucleotide from the ternary Ras·GDP·GEF complex and formation of the Ras·GEF complex. Ras·GTP is generated by binding GTP (the dominant nucleotide in the cell), formation of a ternary complex, and release of GEF.



Scheme 7. The GEF-catalyzed exchange reaction on Ras. The Ras·GXP_T-nucleotide complex, where the nucleotide is still tightly bound, interacts with GEF to form a ternary complex with loosely (L) bound GEF. This complex undergoes a conformational change to a state with loosely bound nucleotide and tightly bound GEF, which is now able to relax into a nucleotide-free binary complex. The reversal of the reaction reforms the Ras·nucleotide complex, which under the conditions in the cell would mostly be Ras·GTP.

It should be stressed that the guanine nucleotide exchange reaction can in principle proceed in both directions (GDP versus GTP exchange or the reverse), since all the partial reaction steps are very fast in each direction.^[84, 86, 87] In the case of EF-Tu (R. Goody, unpublished results) or Ran,^[86, 87] the reaction is actually faster in the direction where the GDP-bound state is formed. Since GEF merely works as a catalyst, and in the absence of other restraints that drive the reaction towards the GTP-bound state—such as incorporation of the products into the membrane—the outcome of the reaction is solely determined by the relative affinity of the GTP-binding proteins, the concentration of GDP versus GTP in the cell (or in the particular compartment of the cell where the reaction occurs), and the concentration of components that bind either the GDP- or the GTP-bound state.

Very little information on the kinetic mechanism of the GEF reaction has been obtained so far. The existence of a binary complex between GEF and the GTP-binding protein, anticipated from the EF-Tu · EF-Ts complex described earlier, was demonstrated for Ras · Cdc25.^[84, 88–90] It has been postulated that the rate-limiting step of the overall exchange reaction on Ras mediated by Cdc25 is the conformational change of the nucleotide on Ras from a tight to a loose binding conformation.^[84] The rate of this reaction is very similar to that of the second step of the two-step binding reaction of a nucleotide to Ras alone, where the initially formed complex with loosely bound nucleotide ($K_D \approx 10 \mu\text{M}$) isomerizes to a tightly-bound complex ($K_D \approx 10 \text{pM}$).^[43] This mechanism enables a similar rearrangement of the structure when the nucleotide binds to empty Ras or the binary Ras · GEF complex. The studies with Cdc25 have also shown that the affinity of the Cdc25 domain for RasGDP is of the order of several hundred micromolar,^[84] which explains why no futile nucleotide exchange occurs unless the exchange factor is co-localized with Ras in the membrane.

The structure of a Ras · SOS complex has been solved by Boriak-Sjodin et al.^[91] and it gave important insights into the mechanism of Ras activation (Figure 2). The minimal domain of Cdc25, and presumably of SOS, responsible and sufficient for efficient catalytic activity contains 248 residues (Cool and Wittinghofer, unpublished results) and most of the detailed kinetic studies have been performed with fragments of a similar size.^[84, 89, 90] The fragment of SOS used for the crystallization was 485 residues long, which corresponds to residues 564–1049 from human SOS. This fragment also contains the REM motif,^[85] also called CR0 (conserved region 0^[91]). Sequence analysis showed this region to be conserved in Cdc25-like Ras exchange factors, but is found at different distances relative to the catalytic domain in different Ras, Ral, and Rap exchange factors. In the structure this region apparently supports a protruding helical hairpin from the catalytic domain and is probably responsible for its stability, since smaller fragments of SOS, and also probably of Cdc25, tend to be labile. The catalytic domain containing the sequence-conserved regions SCR 1–3 is almost completely α -helical (Figure 2).

The catalytic domain of SOS is described as an oblong bowl, in the center of which binds the Ras. The structure of the Ras · SOS complex shows that the P loop, the Switch I and II

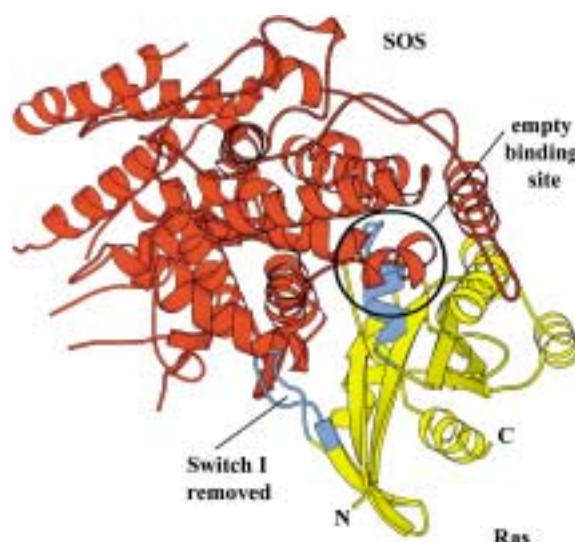


Figure 2. Ribbon representation of the binary Ras · SOS complex, an intermediate in the exchange reaction shown in Scheme 7. Ras is coloured as in Figure 1, SOS is shown in red, and the location of the nucleotide binding site on Ras is indicated.

regions, and the region around $\beta 3$ of Ras are involved in the interaction, as predicted from a number of mutational studies in many laboratories.^[92, 93] The most prominent, although not necessarily most important, interactions between Ras and SOS in terms of binding energy for the binary complex involve Switch I and the protruding helical hairpin. The Switch I region is completely flipped out of its normal position such that the residues, such as Phe28 and Thr35, which contact the base and the phosphate groups are removed from their binding site positions and stabilized by the helical hairpin of SOS. The other important features of the formation of the binary complex involve almost all the residues from the Switch II region forming direct contacts with helices B, D, E, and G from SOS. The Switch II region is very well structured in the Ras · SOS complex and shows features that are incompatible with nucleotide binding. These features are the intrusion of a glutamic acid (Glu942) and a hydrophobic leucine (L938) into the Mg^{2+} and phosphate binding area, occupation of the Mg^{2+} binding site by the carbonyl oxygen atom from Ala59, and the complete rearrangement of the $\beta 3$ - $\alpha 2$ loop such that Gly60 and Lys16 are involved in a tight interaction with Glu62 of Ras. This residue has been shown to be crucial for the exchange reaction with Cdc25 and Sdc25.^[94] Lys16 is particularly important for the binding of phosphates, as it contacts the β -phosphate group in the GDP- and the $\beta\gamma$ -phosphate group in the GTP complex. It is totally invariant in all P-loop proteins,^[95] and its mutation in Ras reduces drastically their affinity to guanine nucleotides.^[96] The structure of the Ras · SOS binary complex shows that the base-binding area on Ras is open, while binding to the phosphate binding area is hindered by many interactions with SOS. Thus, in order for the nucleotide to rebind to Ras, it would have to enter through the base-binding site, and induce a conformational change which releases SOS and also induces tight binding in the phosphate binding area.^[91, 97] Such a structural change might correspond to the rate-limiting first-order reaction identified in kinetic experiments.^[84]

2.4. Interaction with Effectors

Once activated, Ras·GTP interacts with effectors. The first one to be isolated was the protein kinase Raf, of which three isoforms Raf-1, A-, and B-Raf exist. They are 70–80 kDa proteins with a C-terminal kinase domain and an N-terminal regulatory region consisting of many modules which somehow, by a mechanism that is not completely understood, keep the kinase in an inactive state.^[10–12] The best understood module in the regulatory N-terminus is the Ras binding domain (RBD) which consists of 80 residues^[98] and is a stable, independently folding domain.^[99, 100] It is necessary and sufficient for the interaction with Ras in the GTP-bound form. Residues in the effector region whose mutation have been shown to block the biological activity of Ras are at the same time unable to interact with the RBD, thus strengthening the assumption that the isolated domain retains the same properties as in the intact protein.^[98, 101–104] It is believed that the main function of Ras is to recruit Raf, through RBD, to the plasma membrane where in a chain of events that include allosteric interactions, phosphorylations, and interactions with other proteins such as 14-3-3, the protein kinase somehow becomes activated.^[11, 12] Another regulatory domain in the N-terminus of Raf is the cysteine-rich domain (CRD), a zinc-binding domain which is also believed to interact with Ras, as well as with 14-3-3 proteins and lipids, and whose role in activation and/or down-regulation of the kinase activity is disputed.^[105–112]

The structure of the Ras-binding domain of Raf-1 alone has been determined with NMR spectroscopy^[113, 114] and in its complex with the Ras homologue Rap1A by X-ray crystallography.^[115] Rap1A is a close homologue of Ras, with an overall sequence identity of 50% and with almost identical relevant residues in the effector region. The structure showed that RafRBD has the same fold as ubiquitin—apparently a stable fold that can be used for many purposes. The interaction with Ras involves only the Switch I region and not the Switch II region (Figure 3A). An interprotein β -sheet is formed between the two proteins, with some main-chain interactions between the strands, but the majority of interactions are through hydrophilic side chains, whereby Rap is mostly negatively and RafRBD is mostly positively charged. Mutants in the effector region that had been shown to block the biological activity of Ras were indeed involved in forming the interface of the complex. The structure of a complex between RafRBD and a mutant of Rap which even more closely resembles Ras (Raps; Figure 3A) showed that the major difference between the Ras-Raf and Rap-Raf interaction and the reason for weaker affinity between Rap and Raf^[116, 117] is a charge reversal in the effector region: a glutamic acid in Ras and a lysine in Rap1A.

The CRD structure was solved by NMR spectroscopy.^[118] As expected from the conspicuous location of cysteine residues, CRD is a Zn-coordinating protein with high structural similarity to the diacylglycerol binding domain found in the classical protein kinase C.^[119]

RalGEF and its isoforms were also investigated structurally and biochemically in more detail. They are approximately 600–800 residues large and contain at the C terminus a motif

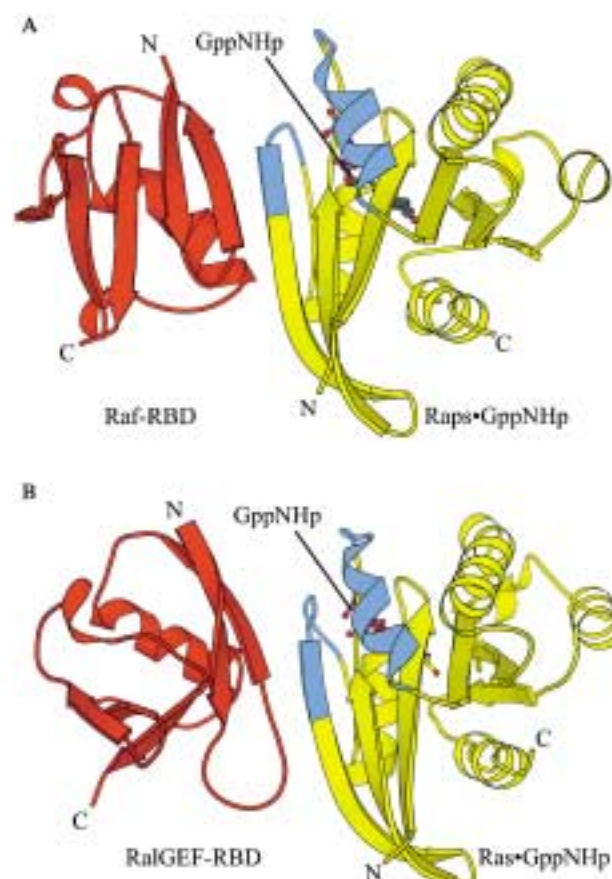


Figure 3. A) Ribbon diagram of the Raps·Raf-RBD complex, where Raps is a close homologue of Ras with identical residues at the interface with the complex, and Raf-RBD is the Ras binding domain of the Ras effector Raf kinase. B) Ribbon diagram of the Ras·RalGEF-RBD complex. Although there is no sequence homology between the two Ras-binding domains from different effectors, they have a similar tertiary structure and interact with Ras in a similar way, albeit using somewhat different residues on Ras. Effectors are colored red.

that binds to Ras·GTP.^[32–34] It was shown that a small 100-residue module from the C terminus is necessary and sufficient to bind to Ras in a GTP-dependent manner.^[116] It was also shown that this Ras binding domain from RalGEF binds with high affinity to Rap·GppNHp and with lower affinity to Ras·GppNHp and that again residue 31 in Rap/Ras is responsible for most of the specificity.^[116] The structure of the domain was solved by NMR spectroscopy^[120] and by X-ray crystallography.^[121] The structures showed RalGEF·RBD to have the same ubiquitin fold as RafRBD, even though it does not show significant sequence homology. The structures of the Ras-binding domains of Rlf^[122] and Rgl^[123] have additionally been determined and, not surprisingly, show the same structural topology.

The structures of complexes of RalGEF-RBD with either a Ras mutant^[124] or Ras itself^[125] were solved by X-ray crystallography, and show the same type of interaction through an interprotein β -sheet as observed in Ras·RafRBD (Figure 3B). However different residues of Ras are involved. This observation was not totally unexpected as residues in the effector region had been identified whose mutation specifically block the interaction with a subset of

effectors without greatly disturbing those of others. Although these partial loss-of-function mutations are not totally specific, it is generally believed that the E37G mutation blocks Ras-Raf interaction, the Ras-T35S mutation blocks the Ras-RalGEF interaction, and Y40C blocks both interactions without disturbing the PI(3)K.^[27, 126–131] The structure shows a weak hydrogen bond between Glu37 of Ras and RalGEF and none between the Ras-E31K mutant and RalGEF. However, E37 shows a strong hydrogen bond with Raf-RBD, which supports the biological data obtained. Tyr40 of Ras forms a considerable part of the binding interface of Ras·RalGEF, thus explaining the loss in affinity, and seems to be indirectly involved in the Ras·Raf-RBD interface (Vetter et al., unpublished results). The specificity of Ras versus Rap binding in the Ral signal transduction pathway seems to be a result of a charge repulsion between Glu31 and a number of acidic residues on RalGEF-RBD.^[124, 125] A mutational analysis shows the distinction between Ras and Rap is abolished by mutation of one of these acidic residues.^[132]

Not much is known about the binding interaction with other effectors. However, it is evident from mutational data that PI(3)K also interacts through the effector region, although in a different mode, as evidenced from partial loss-of-function mutations. Although the Ras binding region and interacting residues on the catalytic p110 subunit on PI(3) kinases were identified, a stable,^[128, 133] independently folding domain could not be expressed. The structure of the p110 catalytic subunit of the PI(3) kinase in the γ isoform shows a Ras binding domain highly homologous to that of Raf and RalGEF. Computer simulations suggest a similar mode of interaction.^[134] However, the structure also shows that the RBD is tightly coupled to the kinase domain, which suggests there is an allosteric control of the enzymatic activity by Ras. AF6, another putative effector,^[135, 136] has been shown to bind in a similar way as Raf and RalGEF, as evidenced from ³¹P NMR data, where AF6 induces a similar conformation of the mobile effector loop on binding to Ras·GppNHP.^[137]

2.5. The Switch-Off Reaction

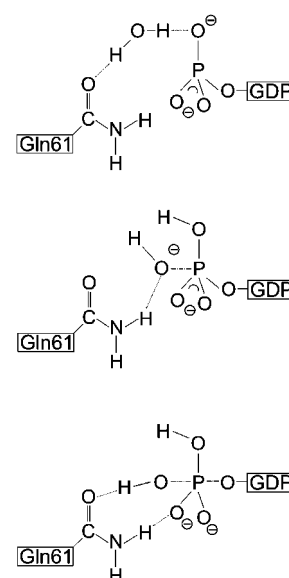
In terms of the physiological and medical relevance the most important aspect of Ras biochemistry is the GTPase reaction, as highlighted by the fact that oncogenic versions of Ras are blocked in both the intrinsic and—even more importantly—in the GAP-mediated GTPase reaction. For this reason its reaction mechanism has been the focus of a heated debate.

In the high-resolution structure of Ras·GppNHP a nucleophilic water molecule was found close to the γ -phosphate group and is considered to represent the attacking nucleophile.^[50] It was also found that Gln61 is close to the γ -phosphate group and that in turn a carboxylate group was in hydrogen-bonding distance from the side chain of Gln61. This observation led to the hypothesis that Gln61 might serve as a general base for activating the nucleophile.^[50] Some experimental evidence apparently supports this view.^[138, 139] However, based on theoretical considerations^[140] and by introducing an unnatural glutamine analogue with an even lower pK_a

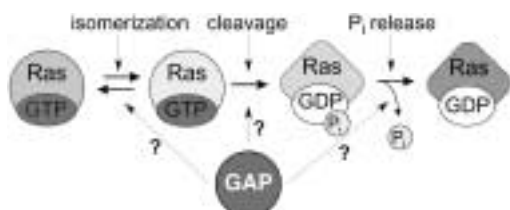
value into the Gln61 position,^[141] as well as from mutational analysis of the carboxylate group^[142] this interpretation appeared unlikely. Model calculations suggested that the γ -phosphate group, with a calculated pK_a value of approximately 3, might itself function as a base (Scheme 8).^[140] Experimental studies by mutagenesis and NMR spectroscopy showed that this suggestion of a substrate-assisted catalysis is likely to be valid. A linear free energy relationship was in fact established between the pK_a value of the γ -phosphate group and the rate of GTP hydrolysis, thus supporting the concept.^[142–144] The same was also postulated for the GTPase reaction of the α subunit of a heterotrimeric G protein.^[145]

Since the slow switch-off by the intrinsic GTPase was considered physiologically less important than the GAP-mediated reaction, the latter mechanism was of considerable interest. GAP proteins were first discovered by Trahey and McCormick in a landmark paper where they showed that while recombinant Ras·GTP in vitro has a half-life of about 30 minutes it is quickly hydrolyzed after microinjection into cells.^[146] The protein responsible was then shown to indeed stimulate the hydrolysis of wild-type but not of oncogenic Ras. Since then several Ras-specific GAPs (RasGAPs) have been isolated and cloned, the most important and best studied are p120GAP^[147, 148] and the product of the neurofibromin gene responsible for the genetically determined disease neurofibromatosis (type I).^[149–152] All RasGAPs are different proteins of varying length and complexity and contain a domain of approximately 330 residues, which alone is able to induce rapid GTP hydrolysis.^[153, 154] It was shown for neurofibromin that a domain of approximately 220 residues seemed to be required and sufficient for inducing GTP hydrolysis.^[155]

For the GAP-mediated GTPase reaction of Ras it was shown by saturating GAP or neurofibromin with increasing concentrations of Ras and treating the data with the Michaelis–Menten equation that the k_{cat} value is between 5–20 s^{−1}, that is, about 10⁵ times higher than the unstimulated reaction.^[154, 156, 157] Since the overall reaction is stimulated by such a large factor it was discussed which of the partial reaction steps was rate limiting and was stimulated by GAP (Scheme 9). Original data seemed to suggest that a conformational change in the intrinsic reaction determined the overall rate^[44] and it was therefore intriguing to assume that GAP was stimulating this conformational change. This assumption would have meant that Ras was in principle a fast GTPase enzyme and just needed GAP to become activated. Data

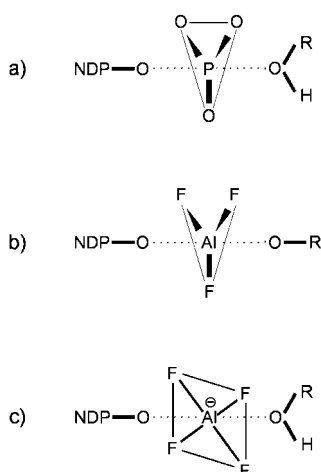


Scheme 8. Proposed substrate-assisted mechanism for the intrinsic GTPase reaction of Ras, where the γ -phosphate group itself activates the attacking nucleophilic water molecule, and Gln61 stabilizes the transition state.



Scheme 9. Schematic overview of the partial steps of the GTPase reaction of Ras. It is now generally believed that the chemical cleavage itself is rate-limiting in the intrinsic GTPase reaction of Ras and is stimulated by GAP.

supporting and questioning this assumption were published.^[158, 159] In another school of thought it was proposed that Ras was an imperfect enzyme and needed the presence of chemical groups on GAP for fast hydrolysis.^[142, 159] Support for this proposal came from studies with aluminum fluoride, which mimicks the transition state of the phosphoryl transfer reaction (Scheme 10) in many enzymatic systems.^[160] It was found that Ras binds aluminum fluoride only in the presence of stoichiometric amounts of GAP and that the presence of an invariant Arg residue on GAP is necessary for this effect.^[161] Furthermore stopped-flow single turnover experiments demonstrated that the catalysis by GAP, but not its binding to Ras, required the presence of this arginine residue, which suggests that catalysis does indeed need the participation of at least one residue from GAP.^[155, 162]



Scheme 10. Aluminum fluoride complexes are believed to be (stable) mimics for the transferred phosphate group in the transition state of (enzymatic) phosphoryl transfer reactions. Both planar AlF_3 and AlF_4^- configurations with two axial oxygen atoms from the nucleophile and the leaving group have been observed in crystallographic structures. NDP = nucleotide diphosphate.

The issue was finally solved independently by solving the structure of the complex between Ras · GDP and GAP334 in the presence of aluminum fluoride.^[163, 164] It indeed shows an arginine residue from GAP penetrating the active site of Ras and contacting the aluminum fluoride, which is supposed to mimic the phosphoryl group to be transferred (Figure 4). This structure was interpreted to mean that the phosphoryl transfer reaction is associative and accumulates negative charge in the transition state of the GTPase reaction, and that the guanidinium side of Arg 789 was neutralizing this negative

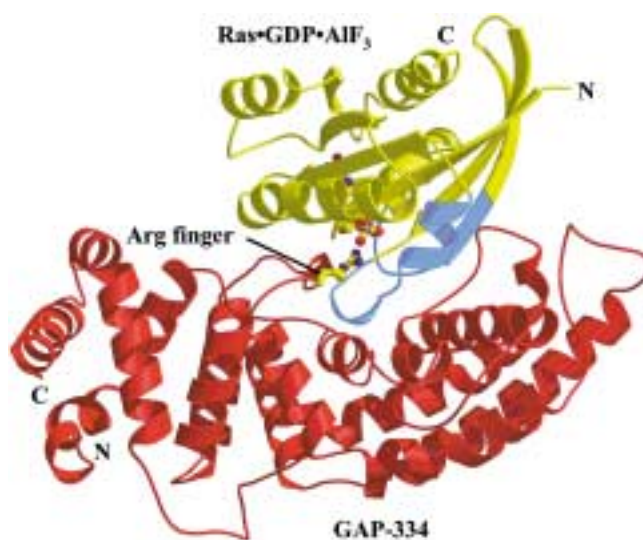


Figure 4. Ribbon plot showing the complex between GAP-333 and Ras-GDP in the presence of aluminum fluoride. The structure represents a mimic of the transition state of the GAP-catalyzed reaction and shows an arginine residue (the arginine finger) contacting AlF_3 , which mimics the transferred phosphate.

charge and thereby stabilizing the transition state of the reaction. This interpretation supported the kinetic data which showed that mutation of this Arg residue reduced the k_{cat} value in both p12GAP and neurofibromin 2000-fold.^[162, 165]

The structure further showed that Gln61 from Ras stabilizes the transition state by making a bidentate hydrogen bond to both the fluoride ion and the attacking water molecule. This observation explained the finding that Gln 61, which is found mutated in many tumors, is also a very important chemical group for catalysis. Its mutation to alanine reduces the catalytic efficiency of the Ras · RasGAP system by five orders of magnitude (Ahmadian et al., unpublished results). The structure also showed why mutations of Gly 12 to any other amino acid inhibits the GAP-mediated GTP hydrolysis, since they would sterically clash with many atoms in the active site of the transition-state mimic. Since Gly 12 mutants have been shown to bind to GAP^[156, 157, 166, 167] we can assume that the ground-state Ras-RasGAP complex is different from the transition-state complex seen in the structure. The mechanism of GTPase activation by GAPs was structurally and biochemically confirmed for the interaction between Rho-type GTP-binding proteins and their specific Rho-GAPs^[168–174] and might thus constitute a universal principle for the GTPase activation of small GTP-binding proteins.^[175]

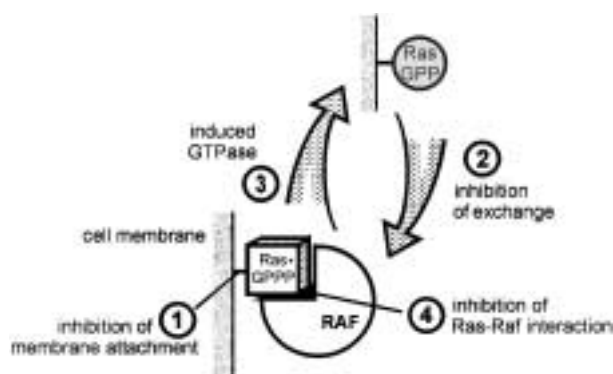
3. The Ras Protein as a Molecular Target for Antitumor Drugs

The crucial role of the Ras proteins in maintaining the regular life cycle of cells and the extensive involvement of mutated Ras in the development of numerous cancers has sparked the idea that influencing the function of Ras might open up new opportunities for the development of alternative antitumor drugs.^[176, 177] Disruption of the aberrant growth

signals of oncogenic Ras proteins provides an example of what is now recognized as “signal transduction therapy”.^[178]

At least four distinct approaches have been pursued for targeting the transforming actions of mutated Ras proteins (Scheme 11):

1. Ras proteins must be localized at the plasma membrane in order to perform their biological function in both the normal and the transformed state of the cell. Covalently attached lipids are the major driving force for membrane localization. Nonlipidated Ras is cytosolic and biologically inactive. Several posttranslational modifications are required at the carboxyl terminus of Ras before the initially biosynthesized precursor protein matures into a biologically active protein localized to the plasma membrane. Interfering with these processing steps might disrupt the aberrant growth signals of oncogenic Ras proteins.
2. Ras proteins are activated by the exchange of GDP for GTP. Prevention of this exchange reaction has been considered by some as an opportunity to block Ras action.
3. Oncogenic mutants of Ras contain mutations that impair their sensitivity to RasGAPs, thus preventing the Ras protein from being switched off again. Compounds that stimulate the intrinsic GTPase activity of oncogenic Ras proteins would convert them back into inactive proteins and terminate the growth-promoting signal.
4. Upon activation, Ras relays the stimulus by activating further proteins such as the Raf, RalGEF, and PI(3) kinase. Thus antagonizing its interactions with downstream effectors would prevent the activated Ras from relaying its signal.

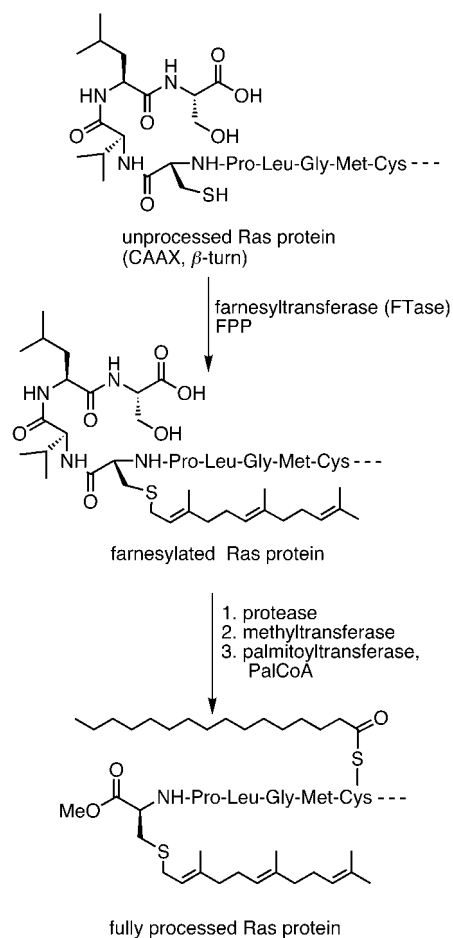


Scheme 11. Schematic view of the possible approaches for developing anti-Ras drugs for use in antitumor therapy (see text).

3.1. Inhibition of Posttranslational Modification of the Ras Proteins

The Ras proteins are synthesized as biologically inactive, cytosolic precursor proteins. They are then modified by several posttranslational processing steps at the carboxyl terminus and thereby converted into biologically active proteins localized at the plasma membrane. The cysteine residue of the C-terminal CAAX sequence (C is cysteine, A is generally an aliphatic amino acid, and X is methionine, serine, alanine, or glutamine) is first enzymatically S-farnesylated, the AAX part is then cleaved off by a specific protease, and

the free C-terminal cysteine is finally converted into a methyl ester (Scheme 12). Additional cysteine residues in the immediate vicinity of the CAAX moiety in H- and N-Ras are lipidated by formation of palmitic acid thioesters. The anchoring of the Ras proteins to the plasma membrane is



Scheme 12. Posttranslational modification of Ras proteins with lipid groups (FPP = farnesylpyrophosphate, PalCoA = palmitoyl CoA).

mediated by the lipid groups. Genetic experiments have shown that farnesylation is essential to Ras function.^[40] Palmitoylation appears not to be an absolute necessity for Ras cell-transforming activity but is important for conferring full biological activity on Ras.^[179] The K-Ras4B protein compensates for the absence of a hydrophobic palmitoyl moiety by having a positively charged lysine-rich region near the CAAX box to enhance membrane interactions with negatively charged phospholipids.

The finding that lipid modification of Ras, in particular farnesylation, is crucial to its biological activity led to the idea that blocking the enzymes responsible for the modification would in turn inhibit Ras function and provide new approaches for developing potent antitumor drugs.

3.1.1. Inhibition of Ras Farnesyltransferase (FTase)

FTase is a heterodimeric protein composed of an α and a β subunit. Zinc and magnesium ions are required for its

Since the discovery that the Ras proteins are farnesylated numerous inhibitors of FTase have been developed. This field has been reviewed in detail and only a brief overview is given here.^[38, 176, 177, 182–184] Initial studies on inhibitor development relied on information about substrate specificity as well as structural studies of the substrate both in solution and bound to the enzyme (obtained from NMR spectroscopic investigations) where the bound ligand Cys-Val-Trp-Met forms a β loop of type III (Scheme 12). The crystal structure of the free enzyme at 2.25 Å resolution^[185] shows the active site consists of two clefts which are at the junction of a bound zinc ion. The zinc ion coordinates the thiol group of cysteine in a ternary complex. One of the clefts was found to be a hydrophilic surface groove near the subunit interface. It could correspond to the site of binding of the Ras protein. The second cleft is a region of aromatic residues and of a size that would accommodate the farnesyl moiety, but not the longer geranylgeranyl pyrophosphate. In addition the structure of the rat enzyme in the complex with farnesyl pyrophosphate was solved at 3.41 Å resolution.^[186] The three-dimensional

CC(C)[C@H](N)CSCCNC(=O)OCCOP(=O)(C1=CC=CC=C1)C(=O)NCC(=O)OCCOS(=O)(=O)CCC(C)=C/C=C/C(=O)N[C@@H](Cc1ccccc1)[C@@H](C(=O)OC)C(=O)OCCC(C)(C)C(=O)N[C@@H](Cc1ccccc1)C(=O)N[C@@H](CC(=O)O)C(=O)O

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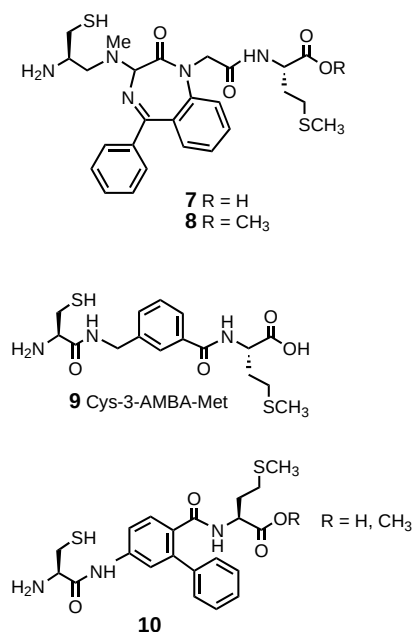
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Peptide analogues were designed on the basis of the CAAX motif in which peptide amide bonds were replaced by amine and ether groups.^[187] In particular the β -turn mimetic **1** (Scheme 13) inhibits the FTase in vitro with an IC₅₀ value of 1.8 nM and shows highly specific activity compared to the inhibition of GGTase I. The less-polar methyl ester prodrug **2** showed better results in vivo and inhibits both farnesylation of the Ras protein and growth of Ras-transformed cells, whilst proliferation of Raf- or Mos-transformed cells was not influenced. Growth of human pancreatic adeno-

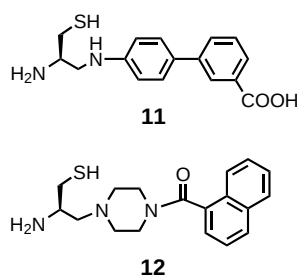
Replacement of the two aliphatic amino acids in the CAAX motif was achieved with benzodiazepines such as **7** and **8** (Scheme 14). The central unit of these peptidomimetics imitates a β -turn and brings the NH_2 terminus of the cysteine analogue and the COOH terminus of the methionine group into spatial proximity; these then can complex the Zn^{2+} ion which is essential for FTase activity.^[195] The free acid **7** inhibits the enzyme with an IC_{50} value of 1 nM, whilst in intact cells the methyl ester **8**, despite its weaker in vitro activity, is significantly more potent because it can penetrate the plasma membrane better as a result of its lower polarity. This property can be used to convert the morphology of H-Ras-transformed cells back into the normal form and to inhibit growth of these cells. The substance shows no effect on Src-transformed and untransformed rat fibroblasts. The inhibitor therefore acts selectively on Ras-transformed cells and does not influence growth of normal cells. This result is noteworthy because farnesylation of the wild-type H-Ras protein is inhibited by the benzodiazepine, and H-Ras is involved in growth of normal fibroblasts. Detailed examination of this unexpected result shows that inhibitor **8** reduces the amount of farnesylated H-Ras protein in both nontransformed and



Scheme 14. FTase inhibitors in which the AA dipeptide was replaced.

H-Ras-transformed cells. However, only in the transformed cells does this lead to the expected reduction of enzyme activity in the Ras cascade: both the amount of phosphorylated Raf and the activities of MEK-1, MEK-2, and MAP kinase were reduced in the tumor cells.^[196] In addition, stimulation of the Ras signal pathway was not influenced by EGF in normal cells or in Src-transformed cells. Normal cells apparently have the ability to activate the signal pathway independently of Ras-inhibitory drugs. In fact, further experiments have shown that farnesylation of the K-Ras protein by the inhibitors is considerably less influenced than that of H-Ras. K-Ras is also a substrate of GGTase I and can therefore be geranylgeranylated if activity of the FTase is reduced. Normal cells and Src-transformed cells, in contrast to H-Ras-transformed cells, are able to compensate for the loss of a Ras protein or an enzyme important for normal cell growth by activation of alternative pathways.

The two aliphatic amino acids have also been replaced by the hydrophobic spacers 3-(aminomethyl)benzoic acid (3-AMBA, **9**, Scheme 14)^[197] and 3- and 4-aminobenzoic acid (3- and 4-ABA) as well as 2-phenylaminobenzoic acid (**10**).^[198, 199] These compounds displayed IC₅₀ values in the nanomolar range, and **10** (R = H) blocked the growth in nude mice of a human lung carcinoma expressing oncogenic Ras.^[200]



Scheme 15. FTase inhibitors in which the AAX tripeptide was replaced.

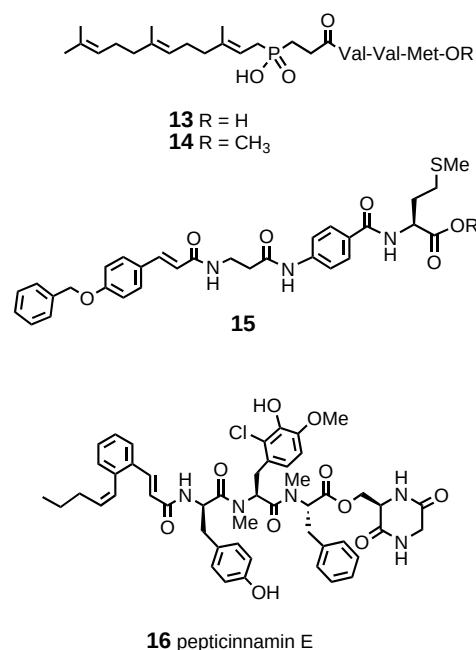
Further design led to the replacement not only of the central AA dipeptide but of the AAX tripeptide. Thus, biphenyl derivative **11**^[201] and piperazine analogue **12**^[202] were developed (Scheme 15). They display IC₅₀ values in the nanomolar range, whereas geranylgeranyltransferase was blocked

with micromolar IC₅₀ values. Both compounds disrupt Ras processing in cells, and piperazine derivative **12** suppresses tumor growth by 75 % at a dose of 40 mg kg⁻¹.

3.1.3. Bisubstrate Inhibitors

The design of FTase inhibitors based on the structure of farnesyl pyrophosphate has been pursued with less intensity as a consequence of the possible nonselective effects of competing with other enzymes, such as squalene synthetase, which also accept farnesyl pyrophosphate as a substrate.^[38, 176, 177, 182–184]

However, bisubstrate inhibitors incorporating a farnesyl and a CAAX mimetic are very promising since one can expect that they will display enhanced activity and selectivity. The phosphinic acids **13** (R = H) and **14** (R = Me, Scheme 16) are examples of bisubstrate analogues. Indeed, **13** is an effective



Scheme 16. Bisubstrate inhibitors of FTase.

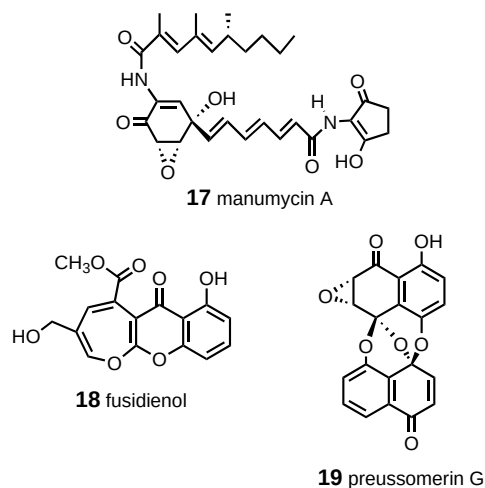
in vitro inhibitor and the prodrug **14** has activity in H-Ras- and—to a lesser extent—K-Ras-transformed cells.^[203] Prodrug **14** also inhibits growth of malignant cells of the neurofibromatosis type I which presumably contain overactive wild-type Ras as a result of the absence of active neurofibromin GAP.^[204] These highly promising results are tempered, however, by the lower bioavailability and in vivo activity in comparison to the peptidomimetics. Recently, benzyloxycinnamoylamide **15** and two analogues thereof were reported as bisubstrate inhibitors of yeast FTase,^[205] where the benzyloxycinnamoyl group mimics the farnesyl moiety. These compounds displayed IC₅₀ values in the low micromolar range.

The natural product peptidocinnamin E (**16**) is also considered a bisubstrate inhibitor of FTase. The successful synthesis of peptidocinnamin E sets the stage for preparing analogues of

this peptidic compound and to delineate the important structural parameters responsible for its FTase-inhibiting activity. Initial studies revealed that the central tripeptide part and, in particular, the absolute configuration of the central chlorinated amino acid are decisive for the inhibitory activity.^[206] The bisubstrate inhibitors developed so far (as well as some of the CAAX mimetics, see Section 3.1.2) have the advantage that they do not contain a free cysteine SH group and are thus more stable.

3.1.4. Farnesyltransferase Inhibitors from Natural Sources and Compound Libraries

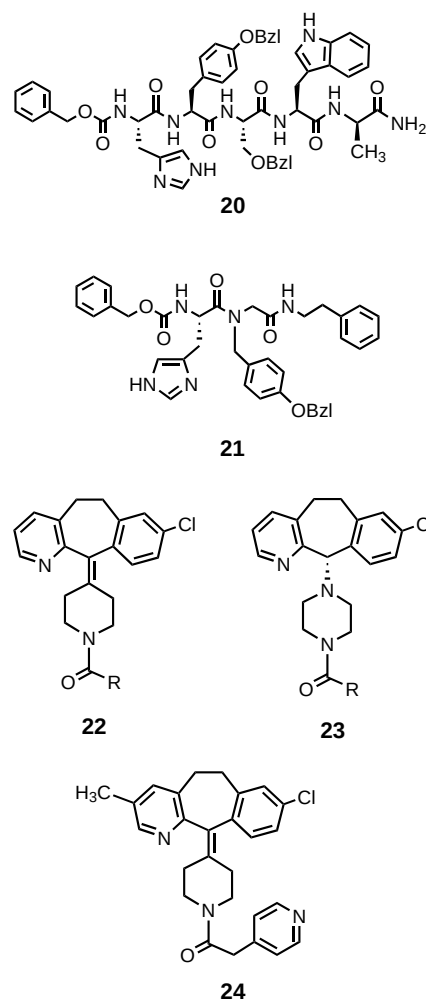
In addition to peptidinnamin E several natural products have been identified as FTase inhibitors, including for example, manumycin (**17**) and analogues,^[207] fusidienol (**18**)^[208] and the preussomerins, for example, **19**^[209] (Scheme 17; for further examples see ref. [182]). In general, these compounds have been less potent than the CAAX-based mimetics, typically displaying IC_{50} values in the low micromolar or the high nanomolar range. High-throughput



Scheme 17. FTase inhibitors from natural sources.

screening of compound libraries was used to identify further inhibitors of farnesyltransferase. Thus a highly potent pentapeptide **20** was identified (Scheme 18) that inhibited FTase with $IC_{50} = 17$ nM and that antagonized Ras in *Xenopus* oocytes.^[210] Systematic derivatization and truncation of the peptide backbone finally led to a dipeptide derivative **21** that inhibited Ras processing in cells and increased the life span of tumor-bearing nude mice by 35% at a dosage of 200 mg kg^{-1} .^[182, 211]

In particular, a series of completely nonpeptidic non-sulfhydryl FTase inhibitors **22–24** was uncovered (Scheme 18). These chlorobenzocycloheptapyridines display pronounced selectivity for FTase over GGTase I. They show improved in vivo antitumor activity and pharmacokinetic profiles in mice when administered orally and inhibit H-Ras processing in Cos monkey kidney cells.^[212, 213] Extensive variation of the substituents on the piperidine moiety and the tricyclic ring system led to the development of compound



Scheme 18. FTase inhibitors identified from compound libraries.

23, which inhibits FTase with $IC_{50} = 40$ nM and is inactive toward GGTase I.

3.1.5. Lack of Toxicity to Normal Cells

Although FTase inhibitors influence the farnesylation of Ras they are likely to interfere with the posttranslational modifications of other CAAX-containing proteins as well. Apart from the approximately 20 farnesylated proteins that are known today, farnesylation is also required for normal Ras function, which in turn is critical for normal cell viability. For these reasons farnesyltransferase inhibitors were thought to be potentially toxic. However, these drugs proved to be surprisingly nontoxic and did not display inhibitory activity against rodent fibroblast cells.^[214] Farnesyltransferase inhibitors not only inhibited the growth of transformed cells in culture far more than the growth of normal cells, but also showed surprisingly few side effects in the treatment of tumor-bearing animals, with no obvious toxicity in normal tissues following treatments of up to six weeks.

There are several possible explanations to account for this apparent lack of toxicity. Some geranylgeranylated Ras-related proteins might compensate for the loss of Ras function.^[215] Alternatively, inhibition of farnesyltransferase

may reduce Ras activity below the level required for transformation, yet still allow sufficient Ras activity to maintain normal cell viability.^[216] Alternatively, a different signaling pathway may be activated when Ras is not anchored to the plasma membrane. Toxicity and effectivity studies have often been performed in rodent fibroblast cells containing oncogenic H-Ras. However, prenylated K-Ras4B and N-Ras are not as effectively blocked by the farnesyltransferase inhibitors as H-Ras (see Section 3.1.6).^[217] Thus normal cells may be less sensitive to these drugs because they express K-Ras4B and N-Ras. In this context it should be noted that H-Ras mutations are relatively uncommon in human tumors.^[218] Rather, the *K-Ras* gene is the most frequently mutated in solid human cancers, whereas the *N-Ras* gene is prevalent in leukemias. Thus the preclinical evaluation of the farnesylation inhibitors has yet to be critically re-evaluated for trials in humans.

3.1.6. Mechanism of Action

Intensive investigations of a variety of FTase inhibitors has clearly revealed that these drugs demonstrate antiproliferative activity in cell cultures against a variety of transformed cell lines and tumors in animals. In particular, cells with a mutant H-Ras protein are sensitive against these drugs. Although the growth of K-Ras4B-transformed cells is also blocked, much higher concentrations are needed for this effect since this protein is a much better substrate for FTase than H-Ras.^[219] Furthermore, the K-Ras4B protein can more easily be geranylgeranylated by GGTase, thus compensating for its lack of farnesylation.^[220, 221] Since geranylgeranylated forms of Ras proteins can also potently transform cells, farnesyltransferase inhibitors are not expected to inhibit the growth of tumors with mutant K-Ras4B. However, growth inhibition is observed despite this alternative prenylation. Clearly blocking the farnesylation of one or more proteins other than Ras is responsible for, or at least contributes to, the observed effect. This proposal is supported by the finding that growth of fibroblast cells transformed by an N-terminal myristoylated (and thereby membrane anchored) Ras that cannot become farnesylated or geranylgeranylated is inhibited by FTase inhibitors as well.^[222]

These findings have raised the questions as to which additional proteins are the molecular targets of FTase inhibitors. One class of candidates involves members of the Rho family of proteins, in particular RhoB. RhoB is a particularly attractive target as Rho proteins have been shown to be required for Ras transformation and since RhoB is an immediate early gene response protein activated by growth factor signaling. RhoB is targeted by FTase inhibitors (see Section 3.1.2 and ref. [223]). Another novel Ras-related farnesylated protein, Rheb, may also be a target. Rheb modulates Ras function and is an antagonist of Ras transformation. Its processing and membrane localization can be inhibited by a FTase inhibitor.^[224]

Many of the biological studies with FTase inhibitors suggested that these agents are cytostatic and suppress cell growth, rather than being cytotoxic in patients. However, farnesyltransferase inhibition was also observed to induce

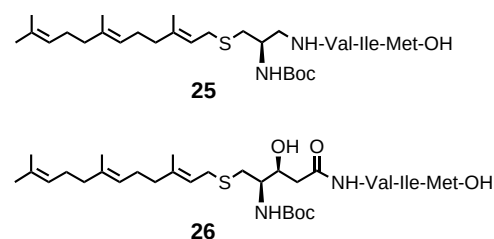
tumor regression of mammary and salivary carcinomas in a mouse tumor model.^[214] The mechanism of shrinkage was unclear, but a recent study showed that a FTase inhibitor can induce apoptosis (cellular suicide) in cell cultures, and is independent of the p53 tumor suppressor protein (a mediator of apoptosis).^[225] These observations may be clinically important because resistance to standard anticancer agents is often conferred by alterations in p53. Thus FTase inhibitors may still be efficacious against tumors containing mutant p53.

Many of the questions concerning the biological activity of FTase inhibitors have been addressed, however numerous questions remain to be answered. In particular the consequences of the K-Ras4B geranylgeranylation bypass and the uncertainty about the cellular targets of these drugs deserve intensive investigation. Nonetheless it is clear and accepted that FTase inhibitors have proven effective and shown significant antitumor activity against Ras-dependant tumors with little or no whole animal toxicity in many animal tumor models. They have now reached the stage of human clinical evaluation.^[211, 226] The clinical trials will provide answers to questions about efficacy, toxicity, development of resistance, and mechanism of action of these compounds such that their full therapeutic potential can be appreciated.

3.2. Carboxyl Terminal Protease, Methyltransferase, and Palmitoyltransferase/esterase as Targets

In addition to farnesylation, complete processing of Ras is required for the protein to have full biological activity. Thus, the protease, the methyltransferase, and the protein that introduces the palmitic acid thioester could be alternative targets for new anti-Ras drugs. The endoproteinase can be inhibited by analogues of the C-terminal CAAX tetrapeptide in which the cysteine amide group is reduced to a methyleneamine or to a hydroxymethyleneamine.^[227, 228]

Compounds **25** and **26** (Scheme 19) displayed K_i values in the 60–80 nM range. These inhibitors could serve as starting points for the design of new drug candidates. This opportunity is further strengthened by the recent isolation and molecular cloning of the Rce1 protein from *Saccharomyces cerevisiae* as the CAAX protease.^[229] It is localized at the membrane and its deletion leads to defects in Ras localization. The methyltransferase that methylates Ras has been identified in several mammalian tissues and can be inhibited by simple farnesyl-cysteine analogues such as farnesylthioacetic acid.^[230] The use of methyltransferase inhibitors for interfering with Ras function has, however, not been investigated.



Scheme 19. Inhibitors of Ras carboxyl terminal protease. Boc = *tert*-butoxycarbonyl.

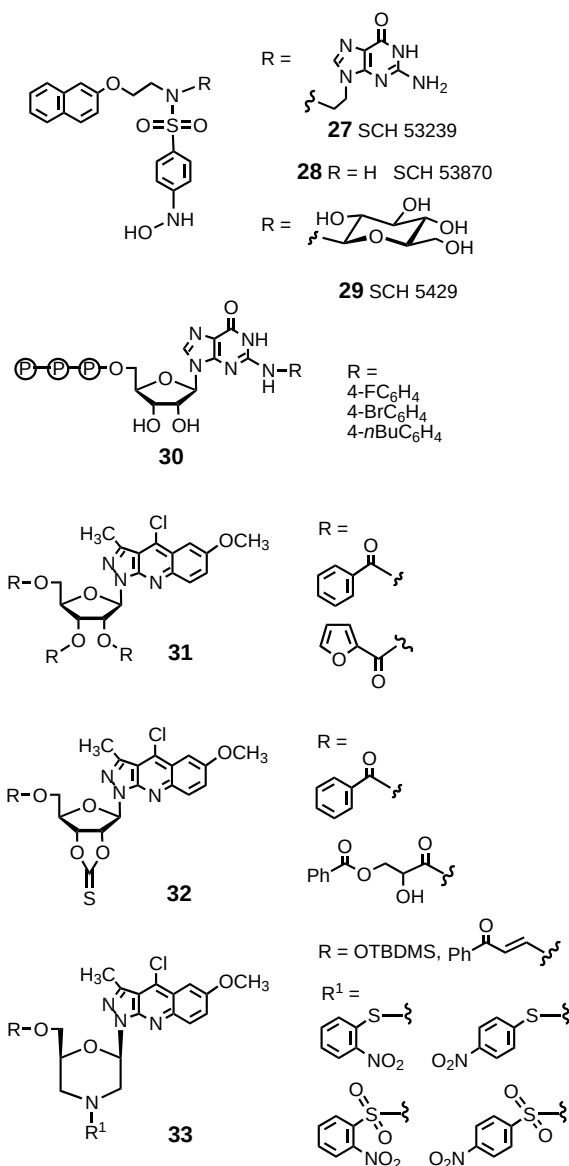
The possible involvement of a putative palmitoyltransferase in the final processing of H- and N-Ras has attracted considerable interest^[231] since these Ras-protein isoforms exert their full biological function only if they are palmitoylated. Indeed, an enzymatic activity was described that palmitoylates Ras,^[232] and this later turned out to be a thiolase. Until now the long-searched-for transferase has not been isolated. However, a protein palmitoyl thioesterase has been identified that processes Ras.^[233] The enzyme was described as “the first bona fide player” in Ras palmitoylation, and inhibiting its function may open up new alternatives for regulating the localization at the plasma membrane and thereby the biological activity of H- and N-Ras.

3.3. Inhibition of Nucleotide Exchange

The rationale to search for inhibitors of the GDP-GTP exchange reaction is not entirely clear, since oncogenic versions of Ras are mostly bound to GTP in the cell.^[146] Nevertheless, attempts to identify such compounds have been undertaken. They could potentially be useful in cases where upstream components of the Ras pathway rather than Ras itself are activated. On the basis of molecular modeling studies compounds SCH 53239 (**27**), SCH 53870 (**28**), and SCH 54292 (**29**) were designed to bind competitively with GDP in the nucleotide binding site (Scheme 20).^[234] Indeed these compounds inhibited nucleotide exchange on Ras with IC₅₀ values of 0.5–0.7 μM, but they do not compete with GDP binding. NMR analysis of SCH 54292 bound to Ras revealed that it binds to the Switch II region of Ras and not to the nucleotide binding site. The Switch II region (residues 60–70) is involved in the interaction of Ras with nucleotide exchange factors. When the PC 12 cells stimulated with nerve growth factor were treated with SCH 53870 the Ras signaling was blocked. Glycoside SCH 54292 did not show this effect, probably because of reduced membrane permeability.

A series of nucleoside triphosphates and nucleoside analogues was investigated for their ability to interfere with GDP binding to the Ras proteins. Several GTP analogues with alterations in the ribose part displayed drastically decreased binding affinity to both normal and mutated Ras.^[235] Methylation of N-7 or replacement of N-7 by a CH group were also ineffective. However, several N-2-substituted GTPs **30** (Scheme 20) showed affinities higher than that of GDP itself, and the 4-(*n*-butyl)phenyl derivative bound to the oncogenic mutant twice as strongly as to the cellular protein. Pyrazolo[3,4-*b*]quinoline ribosides **31** and **32** as well as the morpholino analogues **33** were found to be moderate in vitro inhibitors of the nucleotide exchange process of oncogenic Ras.^[236] Surprisingly, fully protected triesters **31** and the corresponding thionocarbonates **32** were the most effective ribo compounds, displaying IC₅₀ values in the low μM range. A slight improvement was recorded in the morpholines **33**.

These first examples demonstrate that one can, in principle, inhibit the exchange of GDP for GTP on Ras and thus prevent its activation. However, rather than trying to inhibit nucleotide exchange itself or inhibit the Ras-GEF interaction, it might be more reasonable to develop noncompetitive inhib-



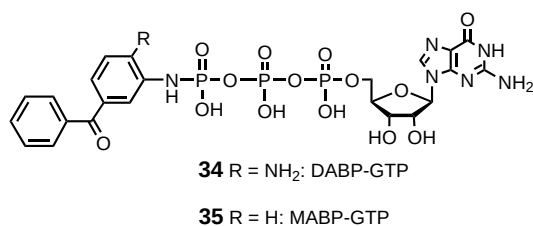
Scheme 20. Inhibitors of Ras GDP-GTP exchange. TBDMS = *tert*-butyldimethylsilyl.

itors of the GEF-catalyzed nucleotide exchange, similar to the action of Brefeldin A. The latter compound stabilizes the abortive ArfGDP-Sec7 domain complex.^[237, 238] A similar noncompetitive approach for the Ras·GEF complex would be to inhibit its interaction with effectors that form an overlapping interface.

3.4. Ras GTPase as a Target

Since Ras is an oncogene and the major problem with oncogenic Ras is its inability to hydrolyze GTP, it was always considered a possibility that stimulating the GTPase of oncogenic Ras would be a valuable target for the development of anti-Ras drugs. The discovery that the (apparently simple) introduction of an arginine residue into the active site of Ras stimulates the GTPase very efficiently has fueled this idea considerably, although GAP itself is unable to act on

oncogenic Ras. The idea of GTPase inducers has gained further support by some recent data with the GTP analogue DABP-GTP (**34**; Scheme 21).^[239, 240] Substitution of the β -phosphate by any ester or amide normally completely blocks the hydrolysis of that compound. In the case of DABP-GTP it was found, first for G_{α} subunits of heterotrimeric G proteins



Scheme 21. Structure of DABP-GTP and MABP-GTP—GTP analogues that are hydrolyzed by oncogenic Ras.

and then in a more thorough study on Ras, that this analogue is efficiently hydrolyzed on the proteins. The aromatic amino group is mostly responsible for this effect, since MABP-GTP (**35**) is only very slowly hydrolyzed. Furthermore, Gln 61 is not necessary for this effect and actually all oncogenic mutants of Ras including those of Gly 12 hydrolyze DABP-GTP more efficiently than the wild-type.^[241] This is good news for drug development since it shows that in principle oncogenic Ras can be induced to break the β, γ phosphate bond efficiently if the appropriate chemistry is supplied to the active site. Clearly such GTP analogues themselves are not good lead compounds, since the high affinity for Ras and the high concentration of GTP in the cells renders this approach unfeasible, but the search for small compounds homing into the active site of Ras and carrying reactive chemical groups as indicated by the GAP and DABP-GTP studies makes this approach a valid one. The structure of DABP-GppNHp bound to Ras^[241] should be helpful in designing the proper scaffold for a GTPase-directed lead compound.

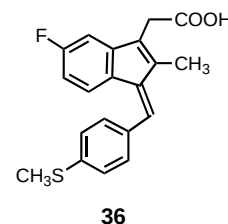
3.5. Inhibition of the Interaction of Ras with Effector Proteins

A key event in Ras-mediated signal transduction involves the interaction of Ras with downstream effector targets. A critical effector is the Raf serine/threonine kinase, but it is clear that Ras function is mediated through its interaction with various effectors. Inhibition of the interaction between Ras and one or several of its downstream effectors could open up new alternatives for interfering with and regulating Ras signaling. Although the functionally different effectors have a common fold, they lack sequence homology and use different residues for the interaction with Ras. Accordingly, it appears possible to develop selective inhibitors.

That this is indeed possible was shown by employing peptides corresponding to the Ras-effector region or to the Ras-binding domains of the downstream targets. The interaction between c-Raf-1 and Ras·GTP was successfully inhibited by pentadecapeptides^[242] as well as hepta- and octapeptides derived from the Ras binding domain of Raf-

1.^[106, 243] Some of these compounds inhibited Ras-Raf binding with IC₅₀ values as low as 7 μ M (observed for one of the heptapeptides) and blocked the Ras-mediated activation of MAP-kinase. In many cases, the reason for the inhibition is not obvious, since these peptides were not derived from the interface of the Ras·RafRBD complex determined from the three-dimensional structure.^[115, 117] A series of 13–28 amino acid peptides derived from the effector-binding region of H-Ras and bearing various amino acid substitutions were investigated as inhibitors of the association of Ras with different effectors.^[244] The peptide corresponding to the original H-Ras sequence inhibited Ras association with three different effectors at K_i values of 1–10 μ M. Introducing amino acid substitutions corresponding to Ras mutations led, however, to peptides that selectively inhibited one or two Ras-effector interactions. Thus, the specificity observed with the whole Ras protein was retained in the effector-region peptide. These results suggest that, in principle, it should be possible to develop small molecules as inhibitors of Ras-mediated protein–protein interactions. However, this process is generally considered to be difficult because of the fact that the interface usually covers a large nonsolvent-accessible surface area between 1000–5000 Å² (in the case of the Ras·RafRBD: 1300 Å²). Protein–protein interfaces are additionally rather flat and do not contain deep water-filled pockets as found in enzyme active sites. Furthermore, mutational studies have shown that residues in the interface of a protein·protein complex do not contribute equally to the binding energy and that sometimes 1–3 residues are responsible for most of the binding energy, in a way that is not at all obvious from the structure.^[245–247] It might thus be necessary to construct new types of scaffolds specifically designed to cover a large surface area of the protein in question.

A successful candidate to inhibit the Ras-Raf interaction is the nonsteroidal anti-inflammatory drug (NSAID) sulindac sulfide (Scheme 22).^[248] Sulindac itself has been used in the therapy of tumors in patients with the inherited cancer disposition Familial Adenomatous Polyposis^[249, 250] and seems to target tumors carrying a Ras oncogene.^[251] Recent biochemical and cell biology studies suggest that sulindac sulfide inhibits Ras transformation apparently by blocking the Ras-Raf interaction.^[248] Although the affinity is presently too low to be a meaningful drug, it constitutes a promising lead compound.



Scheme 22. Sulindac sulfide, an inhibitor of the Ras-Raf interaction.

3.6. Miscellaneous Approaches to Interfering with Ras Function

In addition to the strategies summarized in Sections 3.1 to 3.5 a few other approaches have been taken to interfere with Ras. For instance, RNA aptamers were selected against the farnesylated peptide Boc-Cys-Lys-Ser-Lys-Thr-Lys-Cys(Farn)-OH, which is modeled on the C terminus of K-Ras.^[252] The peptide was immobilized on a column chro-

In a further approach antisense oligonucleotides have been designed that interact with H-Ras mRNA codon 12, that is, the site at which the oncogenic mutation occurs, and repression of mutant H-Ras.^[254] After c nanoparticles these oligonucleotides growth of HBL100Ras1 in nude mice.

Since Ras is the most frequent oncogene found in human tumors it has long been considered an attractive target for

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Scheme 23. A branched peptide identified from a library that binds to H-Ras peptides and GFP-CAAX fusion proteins (GFP = green fluorescent protein) and prevents their farnesylation.

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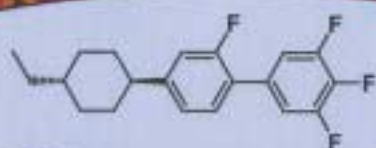
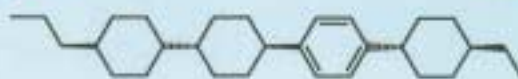
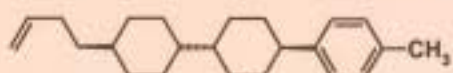
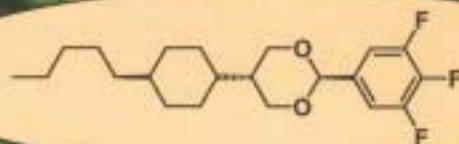
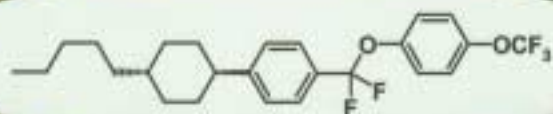
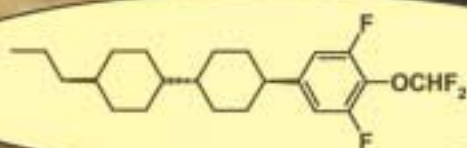
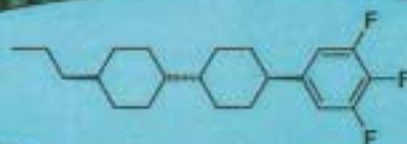
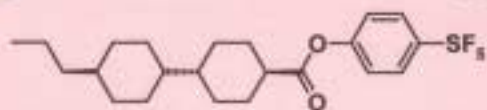


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Nematic Liquid Crystals for Active Matrix Displays: Molecular Design and Synthesis**

Peer Kirsch* and Matthias Bremer*

Substances forming calamitic mesophases have been known for more than 100 years but only the recent, rapid advance in active matrix liquid crystal display (AM-LCD) technology helped these materials to achieve the crucial position in flat panel display technology they hold today. Due to their high contrast, large viewing angle, and rapid switching times, modern AM-LCDs offer a superior picture quality even compared to conventional cathode ray tubes. Their flatness, low weight, and low energy consumption render them the technology of choice for all kinds of portable devices. Some of the future promises of AM-LCD technology are centered around the development of liquid crystalline materials for the

different subtypes of active matrix applications. This development is aimed, on the one hand, towards improved electrooptical and viscoelastic properties; on the other hand, the increasing performance of LCDs leads to extremely stringent reliability demands on the liquid crystals. Responding to these high standards of performance and quality, most liquid crystals for contemporary AM-LCD applications are multiply fluorinated compounds with very high purities, as is typical for materials used in the electronics industry. The synthesis of these superfluorinated materials (SFMs) often requires specialized methods, which, in several cases, had to be introduced for the first time into the

canon of industrial production. The immense market pressure, as well as the rapid advance of AM-LCD technology on the side of the display manufacturers, urges an increasing pace of the materials development. This demand for new materials can no longer be fulfilled by conventional trial-and-error approaches. As in the pharmaceutical industry, in the search for new, superior liquid crystals, the purely empirical methods are increasingly supported by a rational design based on computational methods.

Keywords: fluorine chemistry • liquid crystals • mesogens • metallation • molecular modeling

1. Introduction

In the past few years, liquid crystal displays (LCD) have become a nearly inseparable part of our daily life. In 1999, more than two billion LCDs were produced worldwide;^[1] half of them small monochrome displays for watches and video games. A smaller but rapidly growing fraction of this overall number are full-color displays capable of high-resolution graphics. Perhaps the most prominent example for this type of

high information content display are the desktop computer monitors (about 3.6 million units produced in 1999, most of them with a 15" (37.5 cm) diagonal screen), which have started to compete seriously with the long-established cathode ray tube (CRT) monitors. Having overcome some of the major obstacles on the way to larger panel sizes, display manufacturers are now targeting the "hang-on-the-wall" flat-screen TV as their next strategic goal, which will surely be accomplished within the first half of the next decade.

Most of these high-end displays are based on the active matrix (AM) technology,^[2, 3] where each picture element ("pixel") is controlled separately by a thin film transistor (TFT) integrated on the panel glass. The principle components of an LCD are the glass substrate, covered by an indium tin oxide (ITO) layer as transparent electrode material, polarizer and birefringent compensator films, and color filters. The "heart" of most commercial LCDs is a 5–6 μm thick film of a nematic liquid crystal. The apparently quite small world production volume of liquid crystals (about 55 t in 1999)^[4] is easily explained by the fact that most LCDs require only 0.5–

[*] Dr. P. Kirsch, Dr. M. Bremer
Merck KGaA, Liquid Crystals Division
64271 Darmstadt (Germany)
Fax: (+49) 6151-722593
E-mail: peer.kirsch@merck.de, matthias.bremer@merck.de

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0.6 mg of liquid crystal per square centimeter. Currently around 40 % of the worldwide liquid crystal production is used for AM-LCDs.

The broad application of liquid crystal displays became feasible when, in 1971, the twisted–nematic (TN) cell was invented by Schadt and Helfrich.^[5] In a liquid crystal cell based on the TN mode, a nematic liquid crystalline material, with positive dielectric anisotropy ($\Delta\epsilon$) and helically twisted by 90° , is placed in an ITO lined glass cell and arranged in an homogeneously aligned layer (that is, parallel to the glass) between crossed polarizers (Figure 1).^[6–12] The orientation of

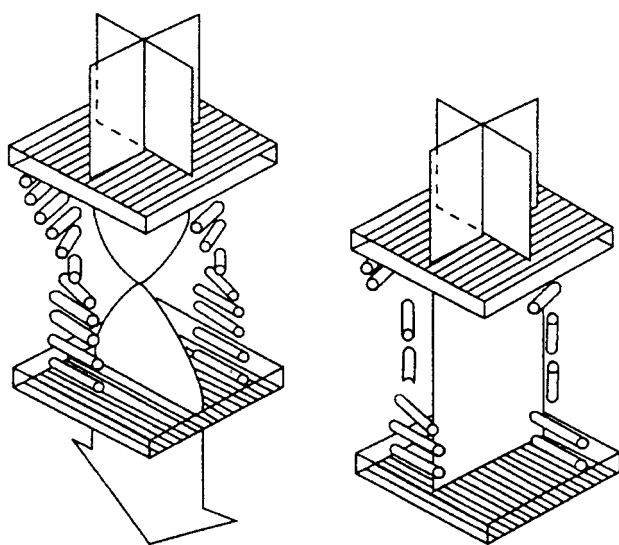
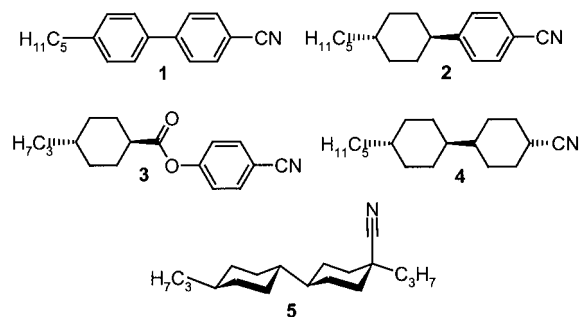


Figure 1. Schematic representation of the working principle of a TN cell in the off (left) and on (right) states. Reproduced from ref. [2] with permission.

the liquid crystal is achieved by an alignment layer of directionally rubbed polyimide within the cell. In order to ensure a homogeneous handedness of the helical structure and, thus, to avoid the formation of domains in the display, a small amount (up to 0.1 %) of a chiral dopant is added to the liquid crystal material.^[13] In the off state, the incoming light is polarized and the polarization plane of the light passing through the liquid crystal layer is rotated by 90° and is thus able to exit the second polarizer. If an electrical potential is applied, the liquid crystal helix is deformed and the incoming light cannot pass the crossed polarizers. Thus, in the off state, the cell, which is illuminated from the back, appears white; in the on state, black. A grey scale can be achieved by applying a voltage between the threshold voltage (V_{th}) and the saturation voltage.

At the early 1970s, Gray and co-workers^[14] synthesized the first chemically and photochemically stable liquid crystals that exhibited a nematic phase at room temperature (**1**, Scheme 1). Another commercially important new class of materials also



Scheme 1. Examples of the first chemically stable liquid crystals which have a nematic phase around room temperature.

Peer Kirsch was born in Herford, Germany, in 1965. He studied chemistry at Heidelberg University and received his Ph.D. early in 1993 under the supervision of Prof. Heinz A. Staab (Max Planck Institute for Medical Research) in physical organic chemistry. After a collaboration with Prof. R. Heiner Schirmer (Heidelberg University) on flavin-based enzyme inhibitors, he was from 1994 to 1995 on a Feodor Lynen fellowship of the Alexander von Humboldt Foundation and a fellowship of the Japanese Science and Technology Agency (STA) at the Institute of Physical and Chemical Research (RIKEN) in Wako, Saitama. There he worked with Prof. Tomoya Ogawa in the field of carbohydrate chemistry. He joined the Liquid Crystal Division of Merck KGaA in November 1995. His main research interest is the application of fluorine chemistry for the design and synthesis of liquid crystals.



M. Bremer

P. Kirsch

Matthias Bremer received his “Diplom-Chemiker” from the Erlangen University in 1986 and a Ph.D. degree from the same institution in 1989. In Erlangen, his work was on physical organic chemistry under the supervision of Prof. Paul von Rague Schleyer. From late 1989 until December 1990 he was a Feodor Lynen fellow of the Alexander von Humboldt Foundation at the University of California in Berkeley, where he studied chiral organometallic compounds with Prof. Andrew Streitwieser. He joined Merck KGaA in early 1991 and has worked in the Liquid Crystal research department since. In addition to a number of patents, he has published some 30 research papers on computational chemistry, synthetic and physical organic chemistry, X-ray crystallography, and liquid crystals.

with a nematic phase around ambient temperature, the cyanophenylcyclohexanes (**2**), were introduced by Eiden-schink^[15] in 1977. By systematically utilizing the melting point depression of mixtures of these and similar materials,^[16–19] it was possible to produce TN-LCDs with a reasonable working temperature range and, in principle, an unlimited lifetime.^[20] Soon, the range of commercially applicable liquid crystalline materials was further broadened by other cyano based compounds.

The first TN-LCDs were simple, directly addressed segment displays, as still used today in wrist watches, for example. In attempting to increase the information content of the displays, by time-sequential addressing in rows and lines, the limits of the TN cell were soon met: At higher multiplex ratios,^[21] a severe loss in contrast occurred due to the ever shorter addressing times. The development of the super-twisted nematic (STN) cell in 1984^[22] pushed the practicable limit to higher multiplex ratios but did not lead to a general solution to the problem.

Already at the end of the 1960s, the first commercial LCDs still based on the dynamic scattering mode (DSM)^[23, 24] had faced the same principal problem of addressability at higher multiplex ratios. As a solution, the use of an “active matrix” of one thin film transistor (TFT), in combination with a voltage-holding capacitor for each pixel, was proposed.^[25–27] In the long term, the DSM mode itself did not prove feasible but the general idea of active matrix addressing was intensively re-evaluated during the 1980s for TN displays,^[2] in order to precisely control the applied voltage and thus the optical transmission for each pixel separately. These efforts resulted in the first prototype of a 3” (7.5 cm diagonal) TFT display presented by Sharp in 1986. Since the manufacturing process of the TFT arrays is highly complex and expensive in terms of financial investment and human resources, the larger scale production of AM-LCDs started only 1989 for use in notebook computers.^[28]

The major drawback of the first AM-LCDs was the strong dependance of the contrast on the viewing angle, which resulted in grey-scale inversion and color shifts in the display when viewed from any than an approximately perpendicular direction. This problem was solved for the “classic” TN-TFT design by using birefringent compensator films, sometimes in combination with multidomain technology.^[29] On the other hand, the last five years showed a technological diversification targeted to further improve the performance of active matrix addressed LCDs: The in-plane switching (IPS) mode^[30] and the multidomain vertical alignment (MVA) LCD mode^[31] increased the viewing angle to 160° and resulted in a dramatically improved contrast ratio (up to 1:300 for the MVA-LCD). The plasma-addressed LCD (PALC)^[32] uses plasma discharges instead of solid state TFTs to address the pixels and allows the design of flat TV screens up to 42” (105 cm) diagonal.

2. Physical Properties of Nematic Liquid Crystals

In order to obtain an optimal performance of any LCD mode, a certain set of physicochemical requirements has to be

met by the liquid crystal material. These specifications include, among others, the nematic phase range, dielectric anisotropy ($\Delta\epsilon$), birefringence (Δn), rotational viscosity (γ_1), and elastic constants (K_1 , K_2 , K_3).^[8, 33–36]

The most basic prerequisite for all types of commercially significant LCDs is surely a broad nematic mesophase range of the liquid crystal. Especially for the rapidly spreading automotive uses, such as car navigation displays, the material must remain stably nematic in a wide temperature range, in many cases from -40 to 110°C .

In order to respond to an applied switching voltage, a liquid crystal has to exhibit a dielectric anisotropy ($\Delta\epsilon = \epsilon_{\parallel} - \epsilon_{\perp}$),^[8, 21, 37] defined as the difference between the dielectric constants parallel and perpendicular to the director of the nematic phase. At the molecular level, $\Delta\epsilon$ is correlated to the square of the dipole moment μ and to the angle β between the dipole moment vector and the effective orientation axis of the liquid crystal molecule.^[38] In order to make Equation (1) useful for the design of materials on an atomistic molecular level and for the prediction of $\Delta\epsilon$ by computational methods, the orientation axis is usually approximated by the “long molecular axis” (here defined as the rotation axis with the smallest moment of inertia) of the rodlike liquid crystals, even if this leads, in exceptional cases, to inaccurate results.

$$\Delta\epsilon = \frac{N h F}{\epsilon_0} \left\{ \Delta\alpha - F \frac{\mu^2}{2 K_B T} (1 - 3 \cos^2 \beta) \right\} S \quad (1)$$

For the design of liquid crystals, two implications of the Maier–Meier Equation (1)^[38] have to be kept in mind: Firstly, independent from the absolute value of the dipole moment μ , at the “magic” angle $\beta = 54.7^\circ$ the contribution from the molecular dipole to $\Delta\epsilon$ always vanishes, to leave only the very small contribution from $\Delta\alpha$. Secondly, if a local dipole moment is oriented parallel to the orientation axis, it is by a factor of $\sqrt{2}$ as effective in “generating” absolute dielectric anisotropy ($|\Delta\epsilon|$) than in the case of perpendicular orientation.

The threshold voltage (V_{th}) of the electrooptical response of a dielectrically positive liquid crystal mixture (Figure 2)—and therefore also the driving voltage of a TN display—depends

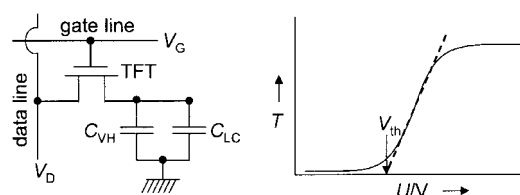


Figure 2. Function of an active matrix liquid crystal display (AM-LCD). Left: the electronic scheme; V_D denotes voltage on the data line, V_G voltage on the gate line, C_{VH} voltage-holding capacitance, C_{LC} capacitance of the liquid crystal layer. Right: the electrooptical response curve of a “normally black” TN cell (parallel polarizers) showing optical transmission T against the applied voltage U ; V_{th} denotes the threshold voltage of the electro-optical response.

[Eq. (2)], besides the dielectric anisotropy ($\Delta\epsilon$), on a set of elastic constants (K_1 , K_2 , and K_3) also.^[21, 37] They describe the

$$V_{\text{th}} = \pi \sqrt{\frac{K_1 + \frac{(K_3 - 2K_2)}{4}}{\epsilon_0 \Delta\epsilon}} \cong \pi \sqrt{\frac{K_1}{\epsilon_0 \Delta\epsilon}} \quad (2)$$

different types of elastic deformation of the helical liquid crystal alignment under the influence of an applied electric or magnetic field. Most decisive for TN type cells is the constant K_1 , the so-called “splay” deformation.^[37]

In order to obtain a good contrast ratio in a display based on the normal TN mode (90° twist, “normally black” mode with parallel polarizers), the relation between the birefringence Δn of the liquid crystal^[37] and the cell gap d has to be chosen in a way that in the off state the transmission T , according to the Gooch–Tarry Equation (3), is minimized.^[21, 39, 40]

$$T(u) = \frac{1}{2} \frac{\sin^2\left(\frac{\pi}{2} \sqrt{1+u^2}\right)}{1+u^2}; u = 2d \frac{\Delta n}{\lambda} \quad (3)$$

For active matrix displays, the first minimum is usually used, to lead to the best viewing angle independence of the picture quality.^[41] This arrangement corresponds to a typical birefringence Δn value of 0.0837 for a cell gap of 6 μm . Recently, many display manufacturers are using birefringent compensation films to improve further the viewing angle of the panel. The optical properties of these films have also to be taken into account for the specification of the birefringence of the liquid crystal material. On the molecular level, the birefringence of a nematic liquid crystal mostly depends on the anisotropy of the polarizability ($\Delta\alpha = \alpha_{\parallel} - \alpha_{\perp} = \alpha_{xx} - (\alpha_{yy} + \alpha_{zz})/2$, [Eq. (4)]; the xx axis is the molecular rotation axis with the smallest moment of inertia).^[8b, 42]

$$\frac{n_e^2 - 1}{n^2 + 2} = \frac{N}{3\epsilon_0} \left(\alpha + \frac{2\Delta\alpha S}{3} \right); \frac{n_o^2 - 1}{n^2 + 2} = \frac{N}{3\epsilon_0} \left(\alpha - \frac{\Delta\alpha S}{3} \right); n^2 = \frac{n_e^2 + 2n_o^2}{3} \quad (4)$$

For most AMD applications, the switching time has become a decisive factor. The time interval between two video frames at 60 Hz is 16.67 ms. Therefore, multimedia and video applications usually require a fast switching time ($\tau = \tau_{\text{on}} + \tau_{\text{off}}$; [Eq. (5)]^[43] of, at least, less than 20–25 ms.

$$\tau_{\text{on}} = \frac{\gamma_1 d^2}{\pi^2 K_1 \left(\frac{V_{\text{on}}^2}{V_{\text{th}}^2} - 1 \right)}; \tau_{\text{off}} = \frac{\gamma_1 d^2}{\pi^2 K_1} \quad (5)$$

On the materials side, the switching time predominantly depends on the rotational viscosity γ_1 ,^[43] which can vary over a wide range for differently structured liquid crystals. The molecular parameter predominantly influencing γ_1 is the length of the liquid crystal molecule. The “splay” elastic constant K_1 is a far less variable parameter. A theoretical model quantitatively correlating molecular parameters with the viscoelastic parameters of the nematic phase, such as γ_1 or K_1 , would be of value but is not available so far. On the side of display design, the switching time can be reduced by a smaller cell gap d . A consequence of this would be the requirement to increase the birefringence Δn of the liquid crystal, according to Equation (3), in order to obtain an optimized viewing angle independence of the contrast. So far, for the larger panel sizes used in notebook, monitor, or TV applications, the reduction of the cell gap, with sufficient accuracy, is technically feasible to about 4.5 μm .

Table 1 shows the most important types of active matrix displays currently in use and their requirements for the physicochemical properties of the liquid crystalline material.

The complex property profile required for the optimum performance of each display type cannot be met by currently existing *single* nematic liquid crystals. The initial task, just to produce a liquid crystal mixture which is nematic around room temperature,^[20b] has thus evolved into a multidimensional optimization problem, which is usually approached by mixing 10 to 15 and, sometimes, even more single substances.

In general, the development of nematic liquid crystal mixtures is still based on eutectic “blocks”^[20b] of different alkyl homologues of the same classes of materials. Not all components of a liquid crystal mixture have to exhibit a thermodynamically stable nematic phase. Some commonly used materials (for example, the dialkylbicyclohexanes) show only a smectic B (S_B) phase or even no mesophase at all. Nevertheless, if applied in moderate concentrations, they do not induce a smectic phase in the mixture and thus allow

Table 1. Overview over the material requirements for the most important active matrix addressed LCD types.

Technology	Applications	Material Requirements	Characteristics
Standard AM-LCD (5V/4V driver)	PC monitors, notebook computers	$\Delta\epsilon$ 4–6, Δn 0.085–0.10, T_{NI} 80–120 °C	Well established technology; use of compensation films for improvement of viewing angle independence of contrast
Low V_{th} AM-LCD (3.3V/2.5V driver)	Notebook computers, Personal Digital Assistant (PDA), camera view finders	$\Delta\epsilon$ 10–12, Δn 0.085–0.10, T_{NI} $\sim$ 70 °C	Allows cheaper and more compact driving electronics than for standard AM-LCD; lower power consumption; very sensitive towards impurities of the liquid crystal material
Reflective TFT	Video games, small notebook computers, PDA	$\Delta\epsilon$ 4–8, Δn 0.06–0.07, T_{NI} $\sim$ 80 °C	No backlight required, therefore reduction of power consumption by up to $\sim$ 90 %; relatively low brightness and contrast
In-Plane Switching (IPS) ^[a]	PC monitors	$\Delta\epsilon$ 12–16, Δn $\sim$ 0.075, T_{NI} 70–85 °C	Very wide viewing angle and superior picture quality; nitrile based materials can also be used
VA-TFT	PC monitors	$\Delta\epsilon$ $\sim$ 4.5, Δn $\sim$ 0.08, T_{NI} $\sim$ 70 °C	Very wide viewing angle and superior picture quality; very high contrast and fast switching time
Plasma Addressed LCD (PALC)	TV, advertising	$\Delta\epsilon$ < 0, Δn $\sim$ 0.08, T_{NI} $\sim$ 70 °C	Large size (1 m diagonal); very good picture quality and fast switching time

[a] The IPS mode is not based on a twisted nematic (TN) cell but on an interdigital electrode arrangement.^[30]

utilization of their otherwise quite advantageous properties, such as low viscosity or low birefringence. Even for materials with a stable nematic phase, the comparative evaluation of their application potential is severely complicated by the strong dependence of all anisotropic parameters on the temperature and on the nematic–isotropic transition point of each individual compound.^[8]

One method to obtain a set of comparable characteristics for any kind of material, independent of its phase sequence, is the introduction of so-called “virtual” parameters: nematic–isotropic transition points ($T_{\text{NI,extr}}$), electrooptic ($\Delta\epsilon$, Δn), and viscoelastic parameters (γ_1) are measured in a defined solution of the respective single compound in a standardized nematic host mixture. The values thus obtained are empirically corrected for the change in the order parameter induced by the addition of the single compound and extrapolated to 100% pure, single compound. Even if, from the theoretical point of view, this method is based on some simplifying assumptions, in most cases the “virtual” parameters are linearly additive to a degree, sufficient for the calculation and prediction of mixture properties from these standardized single-compound properties. The practical development of commercial liquid crystal mixtures is based strongly on this method.

3. Material Requirements for Active Matrix Displays

In addition to the above-mentioned physicochemical requirements, liquid crystals for display applications also have to meet a different set of specifications, which are usually collected under the term “reliability”. The exact definition of “reliability” varies for each application and also for each display manufacturer but every liquid crystal mixture, prior to commercialization, usually has to pass tests focusing on the purity, long term chemical and photochemical stability, and specific dielectric properties.

In desktop computer monitors, most components of the display are permanently heated by the backlight to sometimes more than 60 °C. Here, the long term stability of the liquid crystal mixture against thermal or photochemical degradation is a decisive point for the material development. Whole classes of materials with otherwise excellent properties, such as tolane (diphenylethyne) or (*E*)-stilbene ((*E*)-diphenylethene)-based liquid crystals, are generally not used for AM-LCDs due to their limited photochemical stability. High stability towards UV irradiation is especially important for materials used in projection displays with high pressure xenon or mercury lamps as the light source.

In an AM-LCD, the driving voltage is applied to each pixel once per refresh cycle. Until the next cycle, the voltage has to be kept constant by the capacitance of the liquid crystal layer and by the voltage-holding capacitor integrated into each pixel (Figure 2).

If there is a voltage drop, such as one due to an electric current leaking through the liquid crystal, the contrast of the display is reduced or flicker phenomena are observed.^[44, 45] Reliability tests include measurements of the voltage-holding

ratio (VHR, the ratio of the voltages applied to a pixel at the end and beginning of a defined timespan), the specific resistivity (SR),^[46] and the ion density.^[47] Additional reliability problems, such as image sticking, are caused by the residual direct current (DC),^[48] which is linked to an inhomogeneous distribution of charge carriers within the liquid crystal cell after application of a voltage for a prolonged period.^[49, 50] Changes in the ion density, VHR, SR, or of the clearing point under the influence of elevated temperatures or UV irradiation usually indicates chemical degradation of the liquid crystal or of another peripheral material, such as the alignment layer.

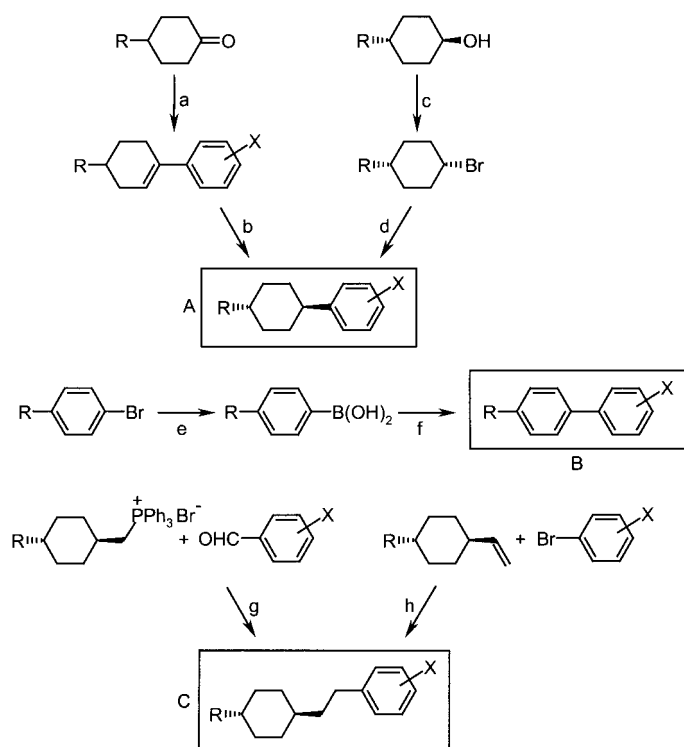
Insufficient reliability of a liquid crystalline material, especially an insufficiently low VHR, strongly affects the observable picture quality—and thereby also the production yield—of a display panel. Therefore, reliability has become one of the most decisive factors for the development of new materials designed for active matrix displays.

Already during the initial development phase of the AM-LCD technology, a body of evidence developed that indicated the cyano-based materials shown in Scheme 1 could not meet the stringent requirements on the specific resistivity and the VHR for active matrix displays. Even after extensive purification and removal of potential ionic contaminants, the VHR of most of these materials could not be raised to a sufficient level.^[51]

Since the early 1980s, a large number of liquid crystals carrying fluorine substituents were synthesized. The initial interest in such materials was aroused by the greater nematic phase range, often dramatically increased, of laterally fluorinated liquid crystals compared to their nonfluorinated analogues. Later, it was found that liquid crystals that derive their molecular dipole moment not from a terminal cyano group but from one or more carbon–fluorine bonds—the so-called superfluorinated materials (SFMs)^[52–54]—fulfilled the reliability requirements for materials used in AM-LCD.

4. Chemistry of Highly Fluorinated Liquid Crystals

Due to the high fluorination degree of most liquid crystals used in active matrix applications, a specialized methodology for the synthesis of these materials was developed. Usually the syntheses commence from aromatic building blocks already carrying the required fluorination pattern. These building blocks are either functionalized via their commercially available halogen derivatives or directly by *ortho*-metallation.^[55] In 1989, when the first liquid crystals with an *ortho*-metallation step in their synthetic sequence were introduced to the market, it was also the first time that the necessary low temperature reactions ran on an industrial scale. The activated intermediates can be subsequently treated with a variety of organometallic C–C coupling reactions^[56–58] to carbonyl compounds, (cyclo)alkyl bromides,^[59] aromatic halides,^[60] or boronic acids.^[61] Other, often used, types of C–C coupling reactions are the Wittig reaction^[62] and the Heck coupling (Scheme 2).^[63]



Scheme 2. General synthetic procedures with typical reaction conditions and yields for the important substructures of nematic liquid crystals: a) 1. ArMgBr, THF; RT, 2 h; 2. Cat. TsOH, toluene; azeotropic removal of water (50–60%). b) H₂, THF, 5% Pd/C (25–30% pure *trans* product). c) Ph₃PBr₂, CH₃CN; 0→50 °C, 5 h (80–85%). d) 1. Li, ZnBr₂, THF, toluene; sonication at 5–15 °C; 2. ArBr, 0.05 equiv [Pd(dppf)Cl₂]; RT, 48 h (50–60% pure *trans* product). e) 1. *n*BuLi, THF; –78 °C, 30 min; 2. B(OMe)₃; –78 °C→RT; 3. 2N HCl (75–80%). f) ArBr, aq. Na₂CO₃, toluene, 0.05 equiv [Pd(PPh₃)₄]; 50 °C, 18 h (70–80%). g) 1. KOtBu, THF; –10 °C→RT; 2. H₂, 5% Pd/C, THF (80–90%). h) 1. ArBr, 0.05 equiv (*o*-tolyl)₃P, NEt₃, CH₃CN; reflux, 96 h; 2. H₂, 5% Pd/C, THF (85–90%). RT=room temperature, Ts=4-toluene sulfonyl, dppf=1,1'-bis(diphenylphosphinyl)ferrocene.

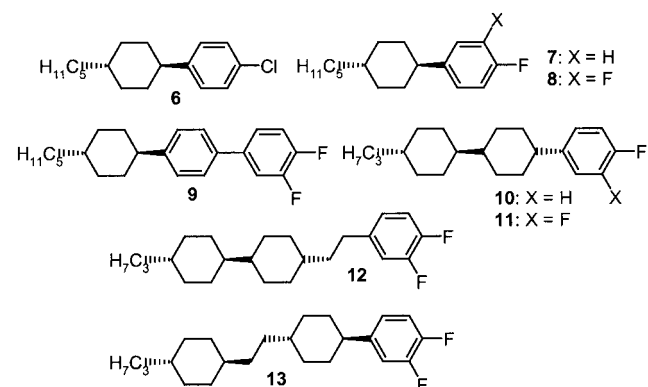
4.1. Materials for Standard AMD Applications

Currently, most AM-LCD in use as, for example, desktop computer monitors are based on 5 or 4 V driver circuits, that require a threshold voltage V_{th} of around 2 V for the liquid crystal mixture. The mixtures used for this applications usually have a dielectric anisotropy $\Delta\epsilon$ value between four and six and a birefringence Δn in the range of 0.085 to 0.10, depending on the cell gap of the display and the choice of compensation films for the improvement of brightness or viewing angle independence of the contrast.

Cyano materials such as **1**, **2**, or **3** are not used due to their low voltage-holding ratio. Therefore, the required $\Delta\epsilon$ value has to be achieved by other means, such as the dipole moment of terminal carbon–halogen bonds. The electronegativity differences ΔEN are 0.33 and 1.60 for the carbon–chlorine and the carbon–fluorine bond, respectively, while the lengths of the aromatic carbon–halogen bonds are 170 and 136 pm for chlorine and fluorine, respectively. A phenylcyclohexane-based liquid crystal with one terminal C_{ar}–F dipole^[64] has a $\Delta\epsilon$ value of only around four. One often applied method to increase the dielectric anisotropy is the introduction of

additional lateral fluorine atoms in order to augment the molecular dipole moment.^[52] As the comparison between **2**, **6**, and **7** illustrates (Table 2), it is generally much more difficult to achieve a high dielectric anisotropy with materials in which their polarity is based solely on carbon–halogen bonds than for cyano-based materials.

Table 2. Examples for the first generation of commercially used materials used in TN and AM-LCDs.^[65]



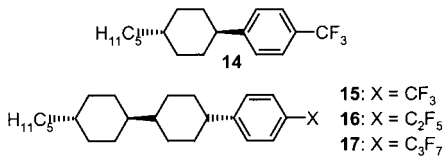
No.	Mesophases	$T_{NI,extr}$	$\Delta\epsilon$	Δn	γ_1
1	C 23 N 35.1 I	4.2	21.6	0.237	112
2	C 31 N 54.6 I	25.7	18.0	0.125	199
3	C 56 N 72 I	49.8	16.8	0.128	–
4	C 65 S _B (52) N 86.2 I	34.3	8.3	0.067	408
5	C 34 N (19) I	14.1	–8.2 ^[a]	0.027	–
6	C 32 I	–12.4	4.2	0.108	62
7	C 34 I	–56.9	4.0	0.075	27
8	C –6.1 I ^[b]	–	–	–	–
9	C 55 N 105.4 I	107.8	6.3	0.144	210
10	C 90 N 158.3 I	161.3	3.0	0.079	156
11	C 46 N 124.3 I	116.0	6.4	0.079	160
12	C 25 S _B 53 N 119.1 I	111.7	5.1	0.082	229
13	C 39 N 104.3 I	105.7	5.5	0.067	247

[a] Extrapolated from the Merck mixture ZLI-2857. [b] Data cited from ref. [52].

From the data shown in Table 2, a principal disadvantage of the superfluorinated materials (SFMs) becomes obvious immediately: The clearing points (real nematic–isotropic transitions as well as “virtual” extrapolated values) are much lower than for the cyano materials (**2**→**6**→**7**). On the other hand, the rotational viscosities γ_1 are also far lower, so that especially the fluorinated two-ring materials (**7**, **8**) become interesting as mixture components in order to reduce the switching time of the display. Comparison of the pair **10** and **11**, for example, shows that the lateral fluorination is effective for increasing the value of $\Delta\epsilon$ by three to four units. On the other hand, the major drawback of the lateral fluorination strategy is a significant decrease of the clearing temperatures by 30 to 40 K for each lateral fluorine substituent.

In order to increase $\Delta\epsilon$ values and avoid the concomitant decrease of the clearing points, the next logical step was to make use of the cumulated carbon–fluorine dipole moments of highly fluorinated terminal alkyl groups.^[66]

As shown in Table 3, terminal perfluoroalkyl chains are well suited to achieve a strong dielectric anisotropy compared to the terminally fluorinated materials. On the other hand, use

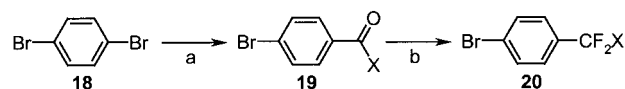
Table 3. Examples for liquid crystals deriving their polarity from highly fluorinated terminal alkyl chains.^[65]


No.	Mesophases	$T_{NI,extr}$	$\Delta\epsilon$	Δn
14	C 23 I	−71.0	9.2	0.086
15	C 43 S ₇ 109 N 122.9 I	100.0	9.1 ^[a]	0.103 ^[a]
16	C 89 N (88.6) I	116.1	6.3 ^[a]	0.083 ^[a]
17	C 127 N (126) I	110.8	7.5	0.084

[a] Extrapolated from the Merck mixture ZLI-1132.

of longer fluorinated side chains does not increase $\Delta\epsilon$ but often results in higher melting temperatures and occasionally also in solubility problems. Despite their reasonably high virtual clearing temperatures, many of the highly fluorinated materials (such as **15**, **16**, or **17**) show only a monotropic nematic phase.

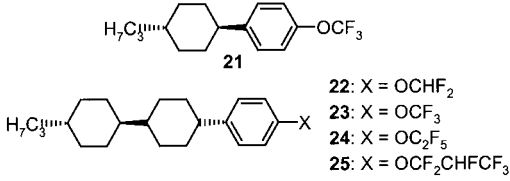
The synthesis of the (per)fluoroalkyl-substituted liquid crystals is based on the bromoaromatic building blocks of the type **20** (Scheme 3). They are attached to the basic mesogenic core structures by the general methods depicted in Scheme 2.^[67]



Scheme 3. Syntheses for highly fluorinated 4-alkyl-bromobenzenes (**20**), used as starting materials for the liquid crystals shown in Table 3: a) 1. *n*BuLi, THF; −78 °C, 30 min; 2. EtOOC-R_f (R_f = CF₃, C₂F₅); −78 °C, 2 h (70–85 %). b) Diethylaminosulfurtrifluoride (DAST), neat; 0→50 °C (**Caution**: sometimes decomposes explosively), 5 h (65–75 %).

As an alternative concept, which makes use of the advantages of highly fluorinated alkyl chains (namely, their strong dipole moment) while improving the mesogenic properties, fluorinated alkoxy functions were introduced to the basic mesogenic core structures (see Table 4).^[68, 69]

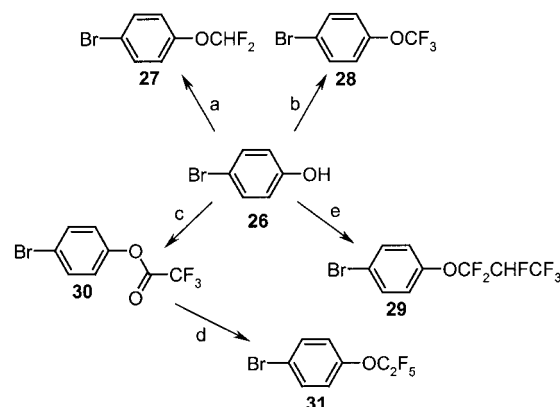
In spite of their tendency to form smectic mesophases in the pure state, some of the materials based on a terminal trifluoromethoxy group (such as **23**) are playing a central role in commercially used standard AMD liquid crystal

Table 4. Liquid crystals with fluorinated alkoxy groups.^[65]


No.	Mesophases	$T_{NI,extr}$	$\Delta\epsilon$	Δn	γ_1
21	C −3 I	−68.0	7.1	0.083	25
22	C 52 S _B 69 N 173.6 I	163.2	5.2	0.086	–
23	C 39 S _B 70 N 154.7 I	146.2	6.9	0.087	142
24	C 119 S _B 152 N 168.6 I	152.1	6.5	0.088	–
25	C 178 I	157.3	3.9	0.074	–

mixtures, together with the 3,4-difluorobenzene-derived materials (**11–13**; Table 2).^[28] Compared to their structurally analogous 3,4-difluorobenzene derivatives, materials with a terminal trifluoromethoxy group possess higher clearing temperatures, which allows the operating temperature range for LCDs to be extended to higher temperatures.

The synthesis of the building blocks (Scheme 4) used for this type of materials starts from 4-bromophenol **26** and involves three different types of reactions: Simple nucleophilic substitution (→**27**; probably via a difluorocarbene



Scheme 4. Syntheses for highly fluorinated 4-alkoxy-bromobenzenes used as starting materials for the liquid crystals shown in Table 4: a) KOH, CHClF₂, dioxane/H₂O (1/1); 40 °C, 1–3 bar, 4 h (55 %). b) CCl₄, HF; 150 °C, 18 h (61 %). c) (CF₃CO)₂O, neat; reflux, 5 h. d) SF₄, HF; 100→175 °C over 10 h (70–75 %). e) NEt₃, F₂C=CF₃; 75 °C, 45 min (53 %).

intermediate),^[70] nucleophilic substitution followed by a Lewis acid catalyzed fluorination (→**28**; Swarts-type reaction),^[71] nucleophilic addition to perfluoroolefins (→**29**),^[72] or fluorination of an intermediate trifluoroacetate (→**30**) with sulfur tetrafluoride to the pentafluoroethoxy function (→**31**).^[73]

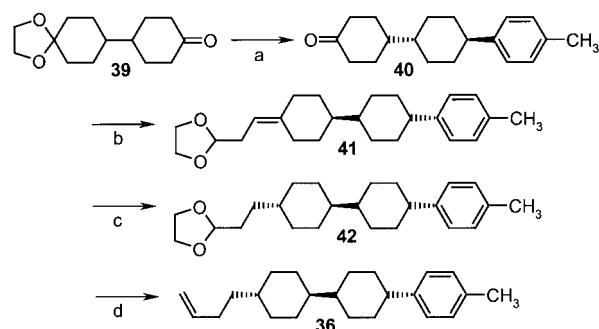
The main characteristic of the materials listed in Tables 2–4 is their strong dielectric anisotropy $\Delta\epsilon$. This basic property is sometimes accompanied by relatively low clearing temperatures T_{NI} , high rotational viscosities γ_1 , or a narrow nematic phase range. In order to improve the operating temperature range of the display and to adjust the birefringence Δn and dielectric anisotropy to the exact specifications dictated by the display design, often less polar compounds are used to fine tune the liquid crystal mixture.^[28] Some commonly used examples of such materials are listed in Table 5.

Compounds **35** and **36** have an olefinic side chain. Such materials often have lower rotational viscosities γ_1 and a more pronounced tendency to form a nematic mesophase than their saturated analogues.^[74, 75] The synthetic strategy leading to alkenyl compounds, such as **36**, is based on Wittig reactions, either for the homologation of carbonyl compounds by one to two carbon atoms or for the generation of the double bonds in the side chain (Scheme 5).^[76]

The comparison between the four-ring materials **37**^[77] and **38**^[78] demonstrates the aforementioned expansion of the nematic phase range upon lateral fluorination.^[79]

Table 5. "Neutral" materials used for miscellaneous purposes, such as the exact adjustment of electrooptic properties, reduction of the rotational viscosity, or increasing the clearing temperature of mixtures for LCDs.^[65]

No.	Mesophases	$T_{NI,extr}$	$\Delta\epsilon$	Δn	γ_1
32	C 42 N (37.6) I	28.3	-0.1	0.098	44
33	C 22 S _B 98 I	56.2	-0.5	0.049	31
34	C 49 N 49.6	27.2	-0.4	0.048	46
35	C -9 S _B 52 N 63.1 I	51.8	0.3	0.054	39
36	C 54 S _B 104 N 176.6 I	186.5	-1.0	0.097	159
37	C 158 S _B 212 S _A 223 N 327	330	-0.1	0.137	491
38	C 133 N 302 I	299.5	0.0	0.126	651


 Scheme 5. Example synthesis of the alkenyl compound **36**: a) 1. 4-MePhMgBr, THF; 2. cat. TsOH, toluene; azeotropic removal of water (70%); 3. H₂, 5% Pd/C, THF; 4. 98% HCOOH, toluene; 5. selective crystallization of the *trans* isomer from *n*-heptane (40–60%). b) 2-(1,3-dioxolan-2-yl)ethylphosphonium bromide, KOtBu, THF; -10 °C → RT. c) H₂, 5% Pd/C, THF (64%). d) 1. 98% HCOOH, toluene; 2. MePPh₃⁺Br⁻, KOtBu, THF; -10 °C → RT (66%).

4.2. Materials for Displays with Low Driving Voltages

Recently, on the side of LCD manufacturers there is a trend to reduce the driving voltage, especially for notebook computer displays. A reduction of the driving voltage from 4–5 V to 3.3 V or even 2.5 V allows a denser integration of the electronic components, which is, of course, an advantage for portable devices. The power consumption of the electronic components of the display is also lower, to result in a longer battery lifetime. For the liquid crystal material, a reduced driving voltage requires a lower threshold voltage V_{th} for the electrooptical response, which is most effectively met by increasing the dielectric anisotropy $\Delta\epsilon$.

The consequent application of this lateral fluorination concept in order to increase $\Delta\epsilon$ leads from the 3,4-difluorobenzene derivatives (Table 2) to the 3,4,5-trifluorobenzene-based liquid crystals listed in Table 6.^[68, 80]

 Table 6. Materials deriving their polarity from a 3,4,5-trifluorophenyl group.^{[65][a]}

No.	Mesophases	$T_{NI,extr}$	$\Delta\epsilon$	Δn	γ_1
43	C 25.6 I	-103.0	6.8	0.034	49
44	C 40.7 N (33.2) I	57.8	12.8	0.137	151
45	C 64.7 N 93.7 I	74.0	8.3	0.073	171

[a] Except γ_1 values, all data are cited from ref. [80].

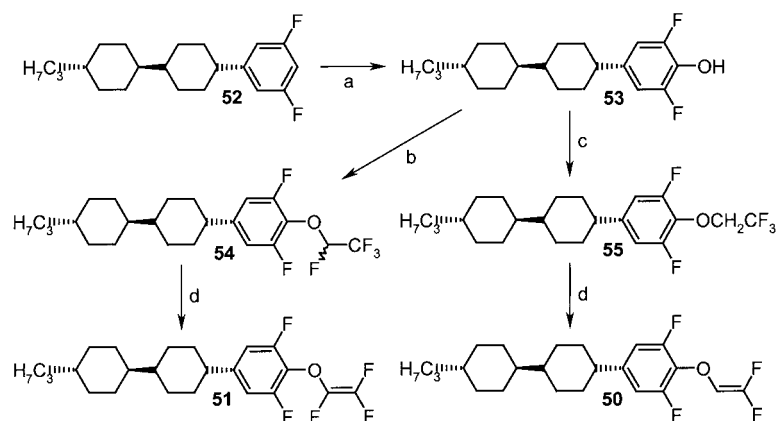
The values of the dielectric anisotropies $\Delta\epsilon$ of this class of substance range between 7 and 13, depending on the mesogenic core structure. The rotational viscosities γ_1 are also reasonably low, allowing for displays with switching times compatible with video demands. Nevertheless, the lateral difluorination results in a severe drop of real as well as of virtual clearing temperatures of the two and three-ring materials. In order to address this problem, the lateral fluorination concept was combined with polar terminal groups, such as trifluoromethoxy and difluoromethoxy moieties, which are known to induce higher clearing temperatures.^[53, 54]

In the first generation of liquid crystal mixtures for use in AM-LCD at low driving voltages, compound **47** and its alkyl homologues played an especially dominant role. While the laterally nonfluorinated materials in Table 4 have a tendency to form smectic phases, the laterally fluorinated liquid crystals in Table 7 are all purely nematic.

The syntheses of laterally fluorinated (per)fluoroalkoxy compounds are based on *ortho*-metallation^[55, 81] of precursors such as **52**.^[13] The lithiated species is oxidized to the phenol via a boronic ester intermediate and, subsequently, converted to the desired liquid crystals by the general methods shown in

 Table 7. Materials with increased dielectric anisotropy by lateral fluorination or difluorination.^[65]

No.	Mesophases	$T_{NI,extr}$	$\Delta\epsilon$	Δn	γ_1
46	C 33 N 144.6 I	123.8	7.4	0.087	242
47	C 62 N 127.5 I	86.9	8.8	0.083	313
48	C 46 N 129.8 I	107.9	9.0	0.089	200
49	C 66 N 118.3 I	86.2	10.5	0.083	279
50	C 49 N 135.9 I	105.3	9.8	0.099	132
51	C 64 N 80.9 I	53.6	10.7	0.088	158

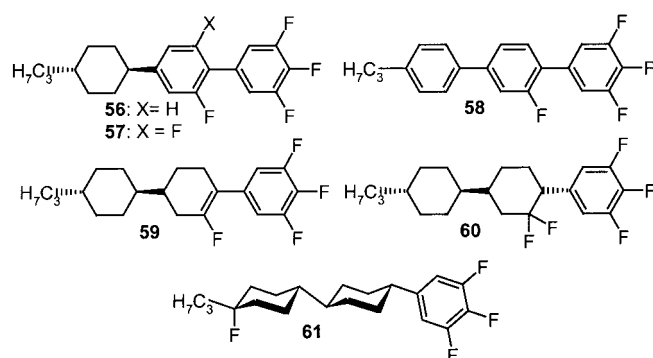


Scheme 6. Syntheses for liquid crystals **50** and **51**: a) 1. *n*BuLi, THF; -70°C , 30 min. 2. $\text{B}(\text{OMe})_3$; $-70^{\circ}\text{C} \rightarrow \text{RT}$. 3. 30% H_2O_2 , HOAc; $30 \rightarrow 50^{\circ}\text{C}$ (50–60%). b) 1. CF_3CHO , THF; 5°C , 30 min; 2. DAST, CH_2Cl_2 ; $10^{\circ}\text{C} \rightarrow \text{RT}$, 18 h (35%). c) 1. NaH, THF; 2. $\text{TsOCH}_2\text{CF}_3$; reflux, 18 h (45%). d) LDA, THF; $-70^{\circ}\text{C} \rightarrow \text{RT}$ (70–90%). LDA = lithium diisopropylamide.

Scheme 2. The fluoroalkenyloxy derivatives **50** and **51** are synthesized as depicted in Scheme 6.^[82, 83]

If the lateral fluorination concept is applied to additional aromatic rings in the mesogenic core structure, materials with dielectric anisotropies $\Delta\epsilon$ up to about 20 (**57** in Table 8) can be obtained.^[84] On the other hand, the same example also illustrates the extreme effect multiple lateral fluorination has on the (virtual) clearing temperature, which has dropped below 0°C .

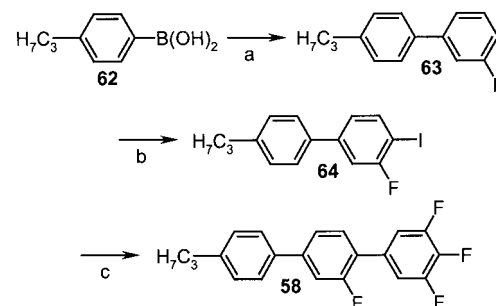
Table 8. Materials with increased dielectric anisotropy by lateral fluorination or difluorination on a second ring structure.^[65]



No.	Mesophases	$T_{\text{Nl,extr}}$	$\Delta\epsilon$	Δn	γ_1
56	C 64 I	25.0	15.2	0.135	173
57	C 123 I	-9.6	20.5	0.117	–
58	C 54 N (35.7) I	39.7	17.6	0.219	168
59	C 46 N 69.6 I	60.8	15.1	0.093	210
60	C 65 I	25.8	13.9	0.075	508
61	C 73 N 115 I	89.4	9.8	0.082	301

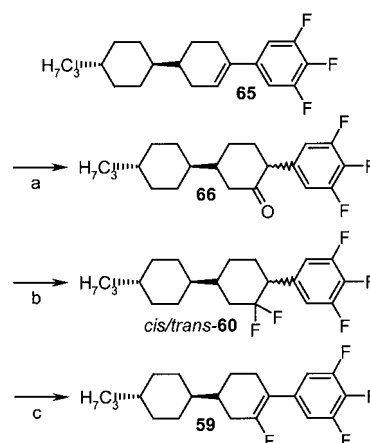
Materials such as **56**, **57**, or **58** are usually synthesized by repetitive *ortho*-metallation, iodination, and subsequent palladium-catalyzed addition of the ring increments as boronic acids (Scheme 7).

The introduction of a second aromatic substructure in order to attach the additional lateral fluorine atoms leads to a concomitant increase in birefringence (for example **45** $\rightarrow$ **44**). If a lower birefringence is required, it is possible to (on paper)



Scheme 7. Example of the sequential synthesis of a terphenyl-based liquid crystal (**58**): a) 3-Fluorobromobenzene, Na_2CO_3 , EtOH, toluene, 0.03 equiv $[\text{Pd}(\text{PPh}_3)_4]$; 50°C , 18 h (92%). b) 1. KOtBu, BuLi, THF; -100°C ; 2. I_2 ; $-100^{\circ}\text{C} \rightarrow \text{RT}$ (51%). c) 3,4,5-Trifluorophenylboronic acid, 0.03 equiv $[\text{Pd}(\text{PPh}_3)_4]$, Na_2CO_3 , EtOH, toluene; 50°C , 18 h (98%).

“reduce” the second aromatic moiety to a cyclohexene structure (**59**), which can also serve as a scaffold to attach the fluorine atom but does not contribute so much to the anisotropy of the molecular polarizability (Scheme 8).^[85]



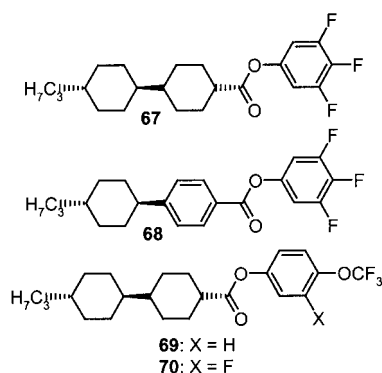
Scheme 8. Synthesis of the fluorocyclohexene-based liquid crystal **59**: a) 1. $\text{BH}_3 \cdot \text{THF}$; $2^{\circ}\text{C} \rightarrow \text{RT}$; 2. 30% H_2O_2 , HOAc; $\text{RT} \rightarrow 35^{\circ}\text{C}$ (66% isomer mixture); 3. PCC, CH_2Cl_2 ; RT, 18 h (85–90%). b) DAST, CH_2Cl_2 ; reflux (86% crude product). c) KOtBu, THF; 70°C , 5 h (76%). PCC = pyridiniumchlorochromate.

A recent approach to compensate the unacceptably low clearing temperatures of highly fluorinated liquid crystals is the exchange of axial hydrogen atoms of cyclohexane substructures by fluorine. Materials such as **61** ($T_{\text{NI}} = 115^{\circ}\text{C}$) generally have virtual clearing points 10–15 K higher than their axially nonfluorinated analogues (**45**; $T_{\text{NI}} = 93.7^{\circ}\text{C}$).^[86, 87]

The reduction of the threshold voltage V_{th} of a liquid crystal mixture by exclusively increasing the dielectric anisotropy also increases the average dielectric constant ($\epsilon_{\text{mean}} = (\epsilon_{\parallel} + 2\epsilon_{\perp})/3$). Materials with a high ϵ_{mean} value promote the dissociation of ionic trace impurities, to result in a decrease in the VHR and specific resistivity.^[44, 80] Therefore, a more differentiated approach is the utilization of mixture components with a small elastic constant K_1 for the “splay” deformation, according to Equation (2).^[53] A substance class generally suitable for this strategy are fluorinated phenol

esters, such as **67**–**70** (Table 9). Even if an ester-based mixture has a lower overall dielectric anisotropy, it can still have a lower threshold voltage for the electrooptical response than a conventional ester-free material.

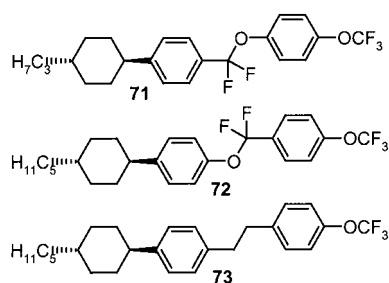
Table 9. Strongly polar, phenol ester structures.^[65]



No.	Mesophases	$T_{\text{NLe},\text{extr}}$	$\Delta\epsilon$	Δn	γ_1
67	C 56 N 117.2 I	110.6	11.1	0.067	175
68	C 101 N (90) I	82.8	21.4	0.129	216
69	C 52 S _B 126 N 187.4 I	172.3	6.2	0.078	199
70	C 50 N 159.3 I	142.1	8.9	0.071	235

Of course, the ester structure is potentially always sensitive towards hydrolysis, thus limiting the chemical stability of the liquid crystal material. For this reason, the more inert difluorooxymethylene bridge was intensively investigated as an alternative, inherently polar linking group for liquid crystals (Table 10).^[88]

Table 10. Structures deriving additional polarity from a polar difluorooxymethylene link within the mesogenic core.^[65]

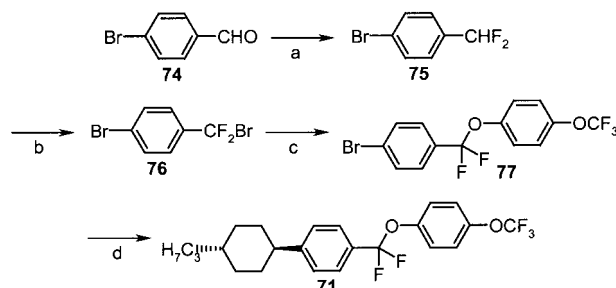


No.	Mesophases	$T_{\text{NLe},\text{extr}}$	$\Delta\epsilon$	Δn	γ_1
71	C 57 S _B 70 N 82.9 I	69.7	9.1	0.115	93
72	C 43 S _B 116 I	100.2	4.4	0.105	–
73	C 47 S _B 68 N 73.7 I	62.3	6.5	0.120	204

Use of this group, as exemplified by **71**, results in a significant increase of $\Delta\epsilon$ compared to the ethylene-linked reference compound **73**, since the dipole moments of the polar terminal trifluoromethoxy group and of the polar difluorooxymethylene bridge are added. If the direction of the linking group is reversed, the dielectric anisotropy is decreased (**72**).

Difluorooxymethylene-based liquid crystals can be synthesized from 4-bromobenzaldehyde **74**, which is fluorinated with diethylaminosulfurtrifluoride (DAST), photobrominated, and

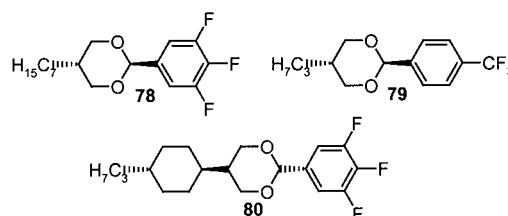
treated with the corresponding phenolate (Scheme 9). The resulting intermediate **77** is coupled to the zinc derivative of a suitable *cis*-4-alkylbromocyclohexane.^[89]



Scheme 9. Synthesis of the difluorooxymethylene-linked liquid crystal **71**: a) DAST, CH₂Cl₂; reflux, 18 h (80 %). b) Br₂, CCl₄; *hν*, 14 d (70–80 %). c) 3,4,5-trifluorophenol, NaH, DMF; 50–60 °C, 15 h (51 %). d) 1. *cis*-4-propylbromocyclohexane, Li, ZnBr₂, THF, toluene; sonication, RT, 4 h; 2. **77**, cat. [Pd(dppf)Cl₂], THF, toluene; RT, 18 h (46 %).

An alternative method to augment the dipole moment of a polar terminal group is the introduction of a polar ring increment into the mesogenic core structure.^[90, 91] Replacement of a cyclohexane substructure by a 1,3-dioxane unit leads to a dramatic increase in the value of $\Delta\epsilon$ without the pronounced drop of the clearing temperatures, which is observed for lateral fluorination (Table 11). The dioxane-based materials **78**–**80** are synthesized by acid catalyzed reaction of an aldehyde with a suitable 1,3-diol.^[90]

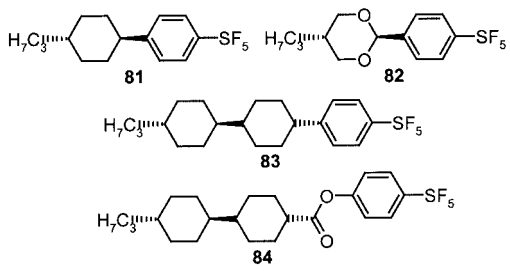
Table 11. Structures that derive additional polarity from a polar 1,3-dioxane subunit within the mesogenic core.^[57]



No.	Mesophases	$T_{\text{NLe},\text{extr}}$	$\Delta\epsilon$	Δn	γ_1
78	C 20 I	– 97.6	15.2	0.080	–
79	C 57 I	– 88.3	16.1	0.093	–
80	C 74 N (51.2) I	69.7	17.0	0.068	201

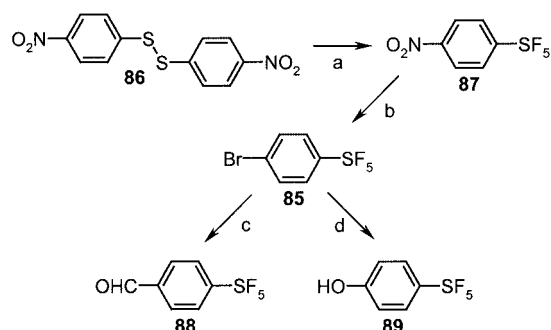
The most severe drawback of liquid crystals carrying lateral fluorine atoms at aromatic substructures still are their low clearing temperatures compared to, for example, the cyano derivatives. Therefore, it is a most important target for application-oriented liquid crystal research to identify new, highly polar terminal groups, which induce reasonably high clearing points and have a minimized interaction with ionic trace impurities. The most polar liquid crystals resulting so far from this line of research are based on sulfur in its highest oxidation state (Table 12).^[82, 92]

A terminal pentafluorosulfuranyl group^[92] has a chemical stability comparable to the trifluoromethyl group. The resulting materials are more polar than the corresponding trifluoromethyl analogues.^[93] Judging from its influence on the

Table 12. Highly polar liquid crystals based on sulfur-containing terminal groups.^[65]


No.	Mesophases	$T_{N\text{Lextr}}$	$\Delta\epsilon$	Δn	γ_1
81	C 11 I	−96.8	12.0	0.087	133
82	C 69 I	−79.9	20.2	0.091	145
83	C 121 I	97.8	11.6	0.094	612
84	C 90 N 152.8 I	132.9	12.3	0.087	–

electrooptic properties of liquid crystals, the pentafluorosulfuranyl group can be considered as a kind of “super-trifluoromethyl” group. The central building block **85**, which carries the pentafluorosulfuranyl function, is most conveniently prepared by direct fluorination of the disulfide **86**,^[94] followed by hydrogenation and the Sandmeyer reaction. The bromide **85** can be converted to other building blocks, such as **88** or **89**, via its lithiated derivative (Scheme 10).

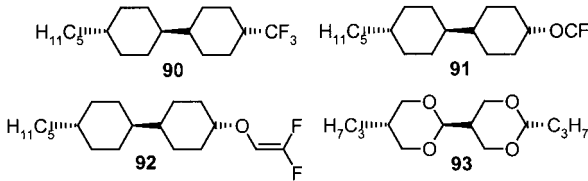


Scheme 10. Synthesis of the central building blocks **85**, **88**, and **89** for pentafluorosulfuranyl-based liquid crystals: a) 10% F_2 in N_2 , CH_3CN ; $-10^\circ C$ (80%). b) 1. H_2 , 5% Pd/C , THF; 2. HBr , $NaNO_2$; $-5^\circ C$; 3. $CuBr$; $RT \rightarrow 80^\circ C$ (46%). c) 1. $tBuLi$, Et_2O , $-78^\circ C$; 2. N -formylpiperidine; $-40^\circ C \rightarrow RT$ (76%). d) 1. $tBuLi$, Et_2O , $-78^\circ C$; 2. $B(OMe)_3$; $-70 \rightarrow -20^\circ C$; 3. 30% H_2O_2 , $HOAc$; $-20 \rightarrow 35^\circ C$, 1 h (58%).

4.3. Materials with Very Low Birefringence

The largest contribution to the overall power consumption of a LCD (about 70–90%) is due to the backlight used to illuminate the display. Recently, many portable devices, such as small notebook computers, video games, or personal digital assistants (PDAs, such as electronic notebooks and calendars), have therefore been equipped with reflective TFT displays^[95] in order to increase the battery lifetime. Since the optical path of the reflected light through a reflective display is different from a usual TN-LCD, there are other requirements for the birefringence of the liquid crystal materials. While a standard TFT display requires a Δn value of roughly around 0.1, an optimal picture quality reflective with TFT display materials need Δn values around 0.06.

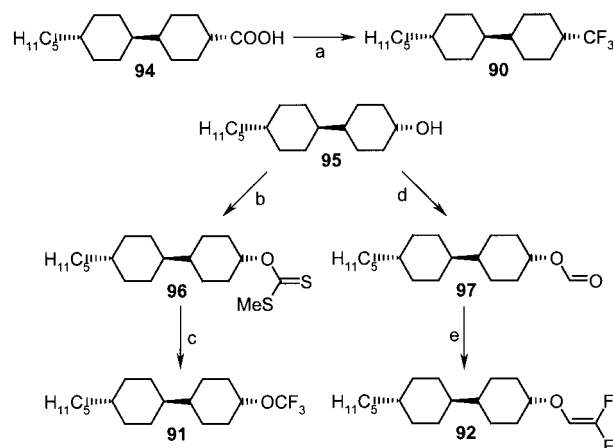
One possible empirical approach to materials with very low birefringence is the minimization of the number of polarizable structure elements, such as double or triple bonds within the molecule. On the other hand, the indirect contribution of the polarizability to the molecular dipole moment via induced dipole components makes it relatively difficult to obtain a large dielectric anisotropy $\Delta\epsilon$ with purely cycloaliphatic materials. This effect is illustrated by the trifluoromethyl bicyclohexane **90**,^[96] which has a significantly lower virtual $\Delta\epsilon$ than its structurally similar phenylcyclohexane analogue **14**,^[66] that carries the same polar terminal group (Table 13).

Table 13. Polar two-ring structures with very low birefringence based solely on alicyclic substructures in the mesogenic core.^[65]


No.	Mesophases	$T_{N\text{Lextr}}$	$\Delta\epsilon$	Δn	γ_1
90	C 35 S_2 (33) I	−20.5	5.3	0.051	99
91	C 33 N (18.2) I	−13.2	6.9	0.059	89
92	C 34 N (31.0) I	15.1	5.6	0.065	65
93	C 82 S_B 125 I	−0.7	8.3	0.048	98

This difficulty was also addressed by combination of two 1,3-dioxanes within the mesogenic core structure (**93**).^[97] Unfortunately, many of the more polar structures with low birefringence suffer from broad smectic mesophases or low solubility (**93**) in liquid crystal mixtures.

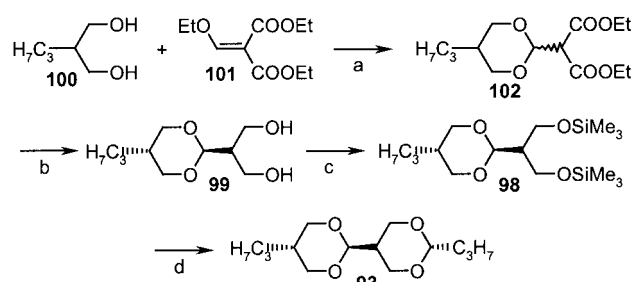
Due to the sensitivity of many cyclohexane-derived substances (such as **91**) towards base- or acid-catalyzed elimination, the introduction of the polar terminal groups must rely on a completely different methodology than for the aromatic derivatives. Thus, the trifluoromethyl function is most conveniently introduced by reaction of the corresponding *trans*-carboxylic acid with sulfur tetrafluoride (Scheme 11).^[96, 98] The aliphatic trifluoromethoxy group is available by oxidative



Scheme 11. Synthesis of the bicyclohexane derivatives **90**–**92**: a) SF_4 , HF , CH_2Cl_2 ; $120^\circ C$, 10 h (80%). b) 1. NaH , THF; $40^\circ C$, 2 h; 2. CS_2 ; RT , 30 min; 3. MeI ; RT , 18 h (93%). c) NBS , 50% HF /pyridine (20–30%). d) $HCOOH$, DCC , CH_2Cl_2 ; $0^\circ C \rightarrow RT$ (74%). e) $P(NMe_2)_3$, CF_2Br_2 , THF/dioxane (10/1); $0^\circ C \rightarrow RT$ (42%). NBS = *N*-bromosuccinimide.

desulfurization of a methyl xanthogenate **96** in the presence of a fluoride ion source.^[71c, 99] The difluorovinylxy function is introduced by a modified Wittig-type reaction with a formate ester **97**.^[97, 100]

The key step for the synthesis of the bis(1,3-dioxane) derivative **93** is the trimethylsilyltriflate-catalyzed ketalization^[101] of the bis(trimethylsilyloxy) derivative **98** with butyraldehyde in order to avoid oligocondensation of the “ambivalent” (protected aldehyde and diol) intermediate **99** (Scheme 12).



Scheme 12. Synthesis of the bis(1,3-dioxane) derivative **93**: a) Xylene, cat. TsOH; removal of EtOH by distillation (53%). b) 1. LiAlH₄, THF; 2. Isomer separation by crystallization from CH₃CN (29%). c) Me₃SiCl, NEt₃, DMF; 0 °C → RT (90%). d) H₇C₃CHO, cat. Me₃SiOTf, CH₂Cl₂; –78 °C, 30 min (21%). OTf = trifluoromethane sulfonate.

Since the clearing points of the two-ring materials listed in Table 13 are relatively low with regard to display applications, some additional mixture components with low birefringence Δn , high clearing temperatures, and a broader nematic phase range are required.^[102]

From the data in Tables 13 and 14, a common problem for the development of liquid crystal mixtures with very low birefringence becomes obvious: Nearly all materials with mesogenic core structures based only on cyclohexane have, in the best case, only very small nematic phase ranges. For this reason, in the future it will remain a very challenging task to develop mixtures for reflective TFT displays with a broad operating temperature range and low driving voltage.

Table 14. Materials with very low birefringence and high clearing points.^[65]

No.	Mesophases	$T_{NI,extr}$	$\Delta\epsilon$	Δn	
103	C 21 S _B 265 I	251.7	–0.1	0.057	
104	C 59 S _B 154 N 190.2 I	196.9	–1.3	0.041	
105	C 110 S _B 212 N 325 I	338.1	–0.6	0.072	
106	C 36 S _B 313 N 322.5 I	315.1	–0.3	0.077	

4.4. Dielectrically Negative Materials

Recently, AM-LCDs based on dielectrically negative liquid crystals were introduced to the market. These devices are mostly used for desktop computer monitors and, compared to standard AM-LCD, they offer a superior picture quality due to their high contrast ratio, wide viewing angle, and fast switching time.^[28, 103]

In dielectrically negative materials, the dipole moment is ideally oriented perpendicular to the orientational axis ($\beta = 90^\circ$).^[38] In contrast to a “standard” TN cell with a positive $\Delta\epsilon$ material, the dielectrically negative liquid crystals are oriented homeotropically (namely, perpendicularly) to the alignment layer in the cell. If a voltage is applied, the molecules experience a torque towards an orientation perpendicular to the applied electric field.

In principle, there are two different ways to attach a perpendicular dipole to a typical liquid crystal structure.^[19] In materials containing a cyclohexane substructure, the axial hydrogen atoms—directed in an exactly perpendicular direction to the molecular long axis—can be replaced by polar moieties, such as cyano groups^[18] (such as in **5**) or fluorine.^[86, 104, 105] In aromatic substructures, the unilateral 2,3-positions can be substituted pairwise by fluorine, in a way that the dipole vector components in the direction of the long molecular axis cancel.

Materials of the latter type (Table 15)^[106] are typical components of the dielectrically negative liquid crystal mixtures used, for example, in multidomain vertical alignment thin-film transistor (MVA-TFT) displays.^[103]

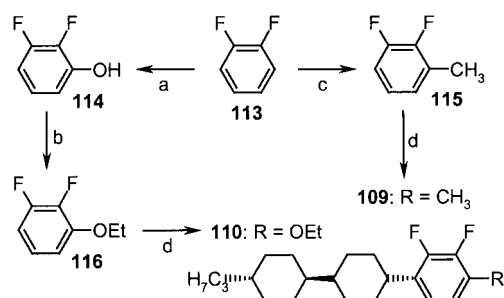
Table 15. Examples for the first generation of dielectrically negative liquid crystals suitable for AM-LCD.^[65]

No.	Mesophases	$T_{NI,extr}$	$\Delta\epsilon^{[a]}$	Δn	γ_1
107	C 14 I	–49.2	–1.8	0.086	62
108	C 49 N (12.9) I	16.5	–6.2	0.099	110
109	C 67 N 145.3 I	139.0	–2.7	0.095	218
110	C 79 S _B (78) N 184.5 I	175.4	–5.9	0.096	413
111	C 74 S _A 86 N 170.7 I	190.6	–5.3	0.146	344
112	C 80 I	44	–7.3	0.133	637

[a] Extrapolated from the Merck mixture ZLI-2857.

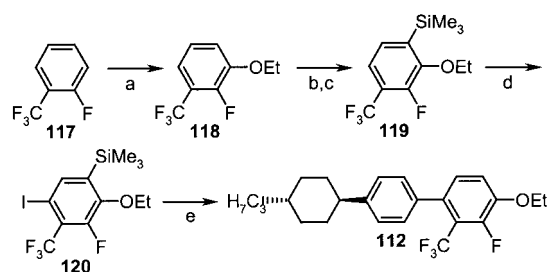
The synthesis of this type of materials starts with 1,2-difluorobenzene **113**, which is derivatized in successive *ortho*-metallation steps (Scheme 13).^[55]

The replacement of one fluorine atom by the more polar trifluoromethyl group^[19] results in a further increase of the (negative) dielectric anisotropy but also in a severe drop of the virtual clearing temperature. This very pronounced effect



Scheme 13. Synthesis of the dielectrically negative materials **109** and **110**: a) 1. *n*BuLi, THF, -70°C ; 2. $\text{B}(\text{OMe})_3$; $-70^{\circ}\text{C} \rightarrow \text{RT}$; 3. 30% H_2O_2 , HOAc; $\text{RT} \rightarrow 50^{\circ}\text{C}$ (90%). b) EtBr, K_2CO_3 , acetone; reflux, 18 h (94%). c) 1. *n*BuLi, THF, -70°C ; 2. MeI; $-70^{\circ}\text{C} \rightarrow \text{RT}$ (77%). d) 1. *n*BuLi, THF, -70°C ; 2. 4-propylbicyclohexan-4'-one; 3. Cat. TsOH, toluene; azeotropic removal of water; 4. H_2 , 5% Pd/C, THF (**109**: 35%, **110**: 38%).

is presumably due to a decrease of the length-to-breadth ratio of the molecule **112** compared to its analogue **111** (see Scheme 14 for synthesis).



Scheme 14. Synthesis of the fluorotrifluoromethylbiphenylcyclohexane derivatives **112**: a) 1. *n*BuLi, THF; -70°C ; 2. $\text{B}(\text{OMe})_3$; $-70^{\circ}\text{C} \rightarrow \text{RT}$; 3. 30% H_2O_2 , HOAc; $\text{RT} \rightarrow 50^{\circ}\text{C}$; 4. EtBr, K_2CO_3 , ethylmethylketone; reflux, 18 h (58%). b) 1. *n*BuLi, THF; -70°C ; 2. I_2 , THF; $-70^{\circ}\text{C} \rightarrow \text{RT}$ (76%). c) 1. *n*BuLi, Et_2O ; -70°C ; 2. Me_3SiCl , Et_2O ; $-70^{\circ}\text{C} \rightarrow \text{RT}$ (70%). d) 1. *n*BuLi, KOtBu, THF; -78°C ; 2. I_2 , THF; $-78^{\circ}\text{C} \rightarrow \text{RT}$ (59%). e) 1. 4-propylcyclohexylphenylboronic acid, 0.05 equiv $[\text{Pd}(\text{PPh}_3)_4]$, 2N aq. Na_2CO_3 , toluene; 50°C , 18 h (60%); 2. DMF, CsF; 80°C , 1 h (quant.).

A less favourable property of the materials listed in Table 15 is their relatively high rotational viscosity γ_1 , which limits the switching time. Therefore, as an alternative approach, liquid crystals based on axially fluorinated cyclohexane structures were extensively studied (Table 16).^[86, 104]

The first objective, the reduction of γ_1 , was clearly achieved. Further, as already mentioned before, the (virtual) clearing points are increased significantly compared to their axially nonfluorinated analogues—in the case of the bicyclohexane derivatives (such as **121**) by 50 to 70 K. Repetition of ethylene linked *ax*-fluorocyclohexane units (**121** $\rightarrow$ **123** $\rightarrow$ **125**) results in a further increase of the (negative) $\Delta\epsilon$ values. With three repetitive units, the concept meets its limitations, since **125** is not sufficiently soluble in typical liquid crystal mixtures in order to measure properties for extrapolation.^[65]

The doubly alkyl-substituted bicyclohexanes **121** have a strong tendency to form smectic B phases and, in higher concentrations, cause liquid crystal mixtures to become smectic on extended storage at low temperatures. Introduc-

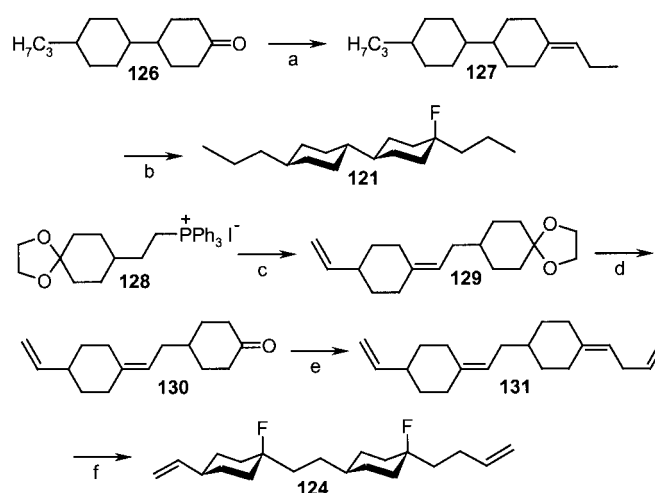
Table 16. Dielectrically negative liquid crystals based on axially fluorinated cyclohexane subunits.^[65]

No.	Mesophases	$T_{\text{NI,extr}}$	$\Delta\epsilon^{[a]}$	Δn	γ_1
121	C 75 S _B 94 I	79.6	-2.2	0.054	—
122	C 13 S _B 37 N 79.8 I	56.5	-1.9	0.057	41
123	C 78 S _B 105 I	76.5	-4.6	0.051	198
124	C 74 S _B (70) N 83.0 I	37.3	-3.8	0.061	156
125 ^[b]	C > 120, dec.	—	—	—	—

[a] Extrapolated from the Merck mixture ZLI-2857. [b] Compound **125** could not be fully characterized due to its insufficient solubility.

tion of double bonds into the side chains suppresses the smectic phases and a nematic phase emerges, as demonstrated by **122** and **124**.^[75, 105]

The synthesis of axially fluorinated cyclohexane derivatives is generally based on Wittig olefinations and a subsequent hydrofluorination step (Scheme 15). Surprisingly, the selec-



Scheme 15. Synthesis of the liquid crystals **121** and **124**: a) $\text{H}_7\text{C}_3\text{PPh}_3^+\text{Br}^-$, THF; $-10^{\circ}\text{C} \rightarrow \text{RT}$, 2 h (80–90%). b) 20 equiv 70% HF/pyridine, CH_2Cl_2 ; RT, 18 h (35%). c) 4-Vinylcyclohexanone, KOtBu, THF; $-10^{\circ}\text{C} \rightarrow \text{RT}$, 4 h (95%). d) HCOOH , toluene; RT, 18 h (73%). e) $\text{H}_2\text{C}=\text{CHCH}_2\text{CH}_2\text{PPh}_3^+\text{Br}^-$, THF; $-10^{\circ}\text{C} \rightarrow \text{RT}$, 2 h (55%). f) 1. 4 equiv 70% HF/pyridine, CH_2Cl_2 ; $-15^{\circ}\text{C} \rightarrow 10^{\circ}\text{C}$, 1 h; 2. Recrystallized three times from *n*-pentane (6%).

tivity of 70% HF/pyridine (Olah's reagent)^[107] is so high, that even tetraenes such as **131** are smoothly and selectively converted into the difluorinated bis(alkenyl) compound **124**.

5. Tools for the Rational Design of Liquid Crystals

Much of the materials research in the liquid crystal field is characterized by empirical structure–property relationships. Some of those are straightforward, such as the increase in $\Delta\epsilon$ upon the introduction of stronger polar terminal substituents or the lowering of Δn by minimizing the number of polarizable groups. Others are perhaps less obvious, such as the suppression of smectic phases by lateral fluorination or the enhancement of the nematic phase range after the replacement of fluorinated aromatics by heterocyclic analogues.^[108] Other properties, such as rotational viscosities, elastic constants, or the nature and range of mesophases, are only related in an indistinct and unpredictable manner to molecular structures.

The liquid crystalline state is governed by weak intermolecular forces, in the order of a few kcal mol^{−1}, and the prediction of bulk properties for a liquid crystal could rely only on a highly accurate description of these interactions. For the simulation of molecular ensembles, Monte Carlo or molecular dynamics calculations have been used, and simulations for simple model liquid crystals have been carried out successfully.^[109] Unfortunately, substantial simplifications must be made at the level of the individual molecule to treat the interactions of the ensemble at all. Even with these restrictions, computer times are enormous and the methods are still far from being routine modeling tools. In addition, careful parameterization of the force-field and/or the anisotropic potentials used in the simulation is crucial for reliable results.

On the other hand, classical computational chemistry^[110] deals with isolated molecules in the gas phase but allows a very detailed and accurate description of their electronic and geometric structures. The methods range from simple molecular mechanics^[111] and semiempirical methods^[110] to *ab initio*^[112] and density functional (DFT)^[113] theory.

5.1. Shape Analysis

Nematic liquid crystals, as they are used in most LCDs, are rodlike molecules. Deviations from a linear structure towards bent arrangements qualitatively result in a decrease of the clearing temperatures. It is therefore of interest to rapidly form an impression of the three-dimensional shape of novel materials that are candidates for chemical synthesis. In addition, in most cases, there will be conformational flexibility instead of complete rigidity; that is, in the condensed phase at room temperature, the thermal energy available will be sufficient to populate conformers higher in energy than the global minimum. Modeling at the force-field level of theory gives a quick overview of such conformers. For example, four low energy conformers of the bis(1,3-dioxane) **93** (Table 13) are shown in Figure 3.

In addition to these mostly qualitative aspects, it is possible to compute geometrical and electronic structure parameters. From these molecular descriptors, quantitative structure–activity relationships (QSAR)^[114] can be derived. This has

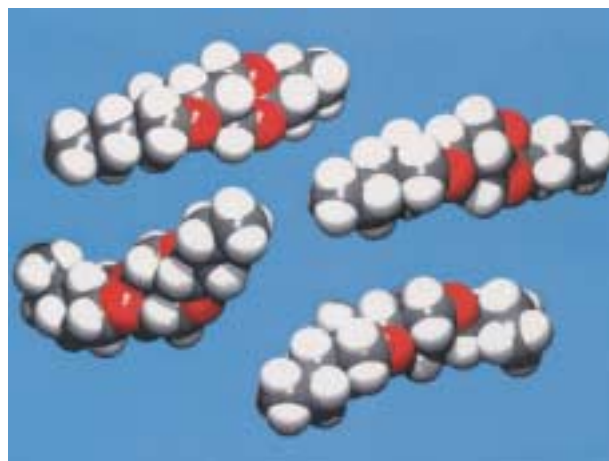


Figure 3. The four lowest energy conformers (within 0.6 kcal mol^{−1}) of the bis(1,3-dioxane) derivative **93**. The global minimum is shown in the upper left corner (MMFF94 force-field calculations).

recently been applied for the prediction of clearing temperatures for a number of liquid crystals.^[115] However, the variation in chemical structure must not be too large and the properties of structures not radically different from those used in the training set, otherwise the properties will probably not be predicted correctly.

5.2. Molecular Modeling of Electrooptic Properties

Some of the key parameters for a liquid crystal mixture are the dielectric and optical anisotropies $\Delta\epsilon$ and Δn . The dielectric anisotropy $\Delta\epsilon$ is related to molecular properties via the Maier–Meier equation [Eq. (1)]^[38] and Δn can be obtained from the molecular polarizability α from the Vuks equation [Eq. (4)].^[42] The molecular dipole moment μ and the polarizability α can be obtained from a semiempirical, *ab initio*, or DFT calculation (Figure 4).^[116–120]



Figure 4. A three-dimensional representation of the liquid crystal CCP-30CF3 **23**. The calculated (AM1) anisotropic polarizabilities are $\alpha_{xx} = 255.7$, $\alpha_{yy} = 153.2$, and $\alpha_{zz} = 159.6$ a.u.; the dipole moments are $\mu_x = 2.95$, $\mu_y = 0.08$, and $\mu_z = 0.52$ D; the angle β towards the orientational axis, approximated by the x axis of the molecule, is 10.0°. The electrooptic anisotropies derived from these values are $\Delta\epsilon_{AM1} = 5.8$ and $\Delta n_{AM1} = 0.099$. The experimentally determined virtual parameters are 6.9 and 0.087 for $\Delta\epsilon$ and Δn , respectively. (1 a.u. = 0.14818 Å³.)

For conformationally flexible molecules, the prediction, especially that of $\Delta\epsilon$, is somewhat more complicated, since different conformers may possess very different dipole moments even though they are similar in energy. It is then necessary to systematically screen the conformational space and perform a Boltzmann-type weighting of the calculated quantities.

An extreme case in point is the polar terminal group difluoromethoxybenzene. Figure 5 shows three conformers located at the B3LYP/6-311 + G**//B3LYP/6-311 + G** level of theory together with their relative energies and dipole moments. Although these calculations are at an adequate level, their predictive power is rather low. After all, we are dealing with the condensed phase where energy differences of less than 1 kcal mol⁻¹ in the gas phase are certainly insignificant. Fortunately, in most cases, there is only one minimum within a few kcal mol⁻¹ and the conformer problem disappears.

In order to assess the value of the method, calculations were carried out on a representative test set of liquid crystals that provides a wide range of electrooptical anisotropies. The predicted and experimental electrooptical anisotropies for this set are shown in Figure 6. Only single conformers were used for the calculations.

The influence of the quantum chemical method on the quality of the prediction can easily be checked by moving to higher levels of theory (Figure 7). Hartree–Fock ab initio theory with the split valence 6-31G* basis set is currently the practical limit for molecules the size of liquid crystals. Calculation times for ab initio geometry optimization and polarizability calculations are two to three orders of magnitude longer than those of the AM1 semiempirical method.

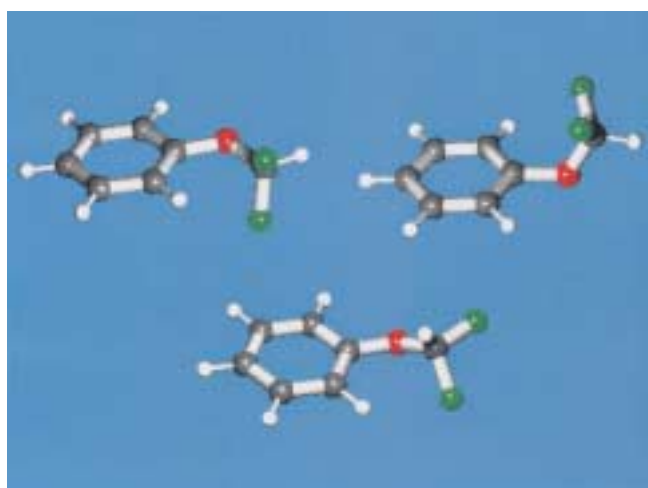


Figure 5. Three low energy conformers of difluoromethoxybenzene at the B3LYP/6-311 + G**//B3LYP/6-311 + G** level of theory: The relative energies and corresponding dipole moments are 0.4 kcal mol⁻¹ and 0.98 D (upper left), 0.0 kcal mol⁻¹ and 1.12 D (upper right), and 0.1 kcal mol⁻¹ and 2.93 D (below).

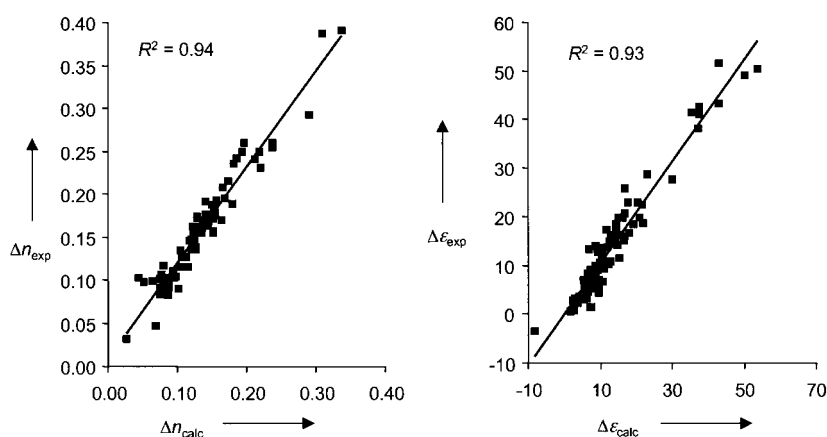


Figure 6. The correlation between semiempirically calculated (AM1) versus extrapolated experimental electrooptical anisotropies (84 samples).

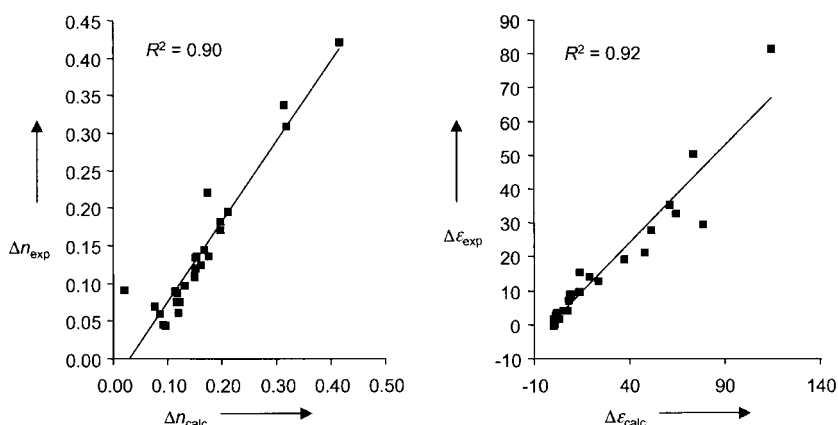


Figure 7. The correlation between ab initio (HF/6-31G**//HF/6-31G*) calculated electrooptical anisotropies versus extrapolated experimental data (26 samples).

Although the ab initio approach is more rigorous theoretically, there is a large systematic overestimation of $\Delta\epsilon$ and a (smaller) overestimation of Δn . The most likely explanation for this poor performance is the neglect of electron correlation and the use of a too small basis set. It is well known that Hartree–Fock ab initio theory systematically overestimates molecular dipole moments, while DFT and MP2 ab initio and even AM1 reproduce experimentally determined values quite well.^[121] Unfortunately, the computational problem cannot be tackled adequately at present. However, the performance of the semiempirical method (which includes some electron correlation effects via the parameterization) is quite satisfactory for standard organic materials, such as most liquid crystals. However, semiempirical theory understandably fails with molecules it was not parameterized for, which became evident with hypervalent sulfur fluorides.^[92]

5.3. Modeling-Assisted Prediction of Reliability Parameters

One of the characteristic parameters for the reliability of a liquid crystal mixture is a high VHR value. The VHR of liquid crystal mixtures has been much improved after the introduc-

tion of “superfluorinated materials” (SFM)s^[52–54] but there still remains an observable leakage current, which suggests the presence of ions or contamination in the cell.

Modeling the solvation of ions by liquid crystal molecules was supposed to provide a better understanding of the interactions involved and possibly allow predictions regarding the solvation potential of structures not yet synthesized. Many liquid crystals are polar organic materials that possess large dipole moments and lone electron pairs which can interact with ions. In principle, the solvents’ macroscopic dielectric constants, dipole moments, and polarizabilities can affect solvation but only a weak correlation with experimental data was found.^[122]

For our modeling approach, the semi-empirical AM1 method was used because of the relatively large size of liquid crystal molecules. The MOPAC program^[120] offers positively charged “artificial elements”, the “sparkles”, which can be conveniently used to model, for example, alkali metal ions. Sparkles may be regarded as unpolarizable ions of a fixed 1.4 Å diameter. They do not contribute to the orbital count and cannot accept or donate electrons. Since the strongest interactions are expected for positive ions (negative ions are generally larger and their charge is better delocalized) only singly positively charged sparkles were considered. As a suitable measure for the “ion solvation power” of a material, we chose the calculated (AM1) heat of interaction of a liquid crystal molecule with a positive sparkle.

Figure 8 shows the energy of a system consisting of benzonitrile as a simple model liquid crystal and a sparkle as a function of distance.

The calculation shows that there is a steep energy minimum at a nitrogen–sparkle distance of about 3.1 Å and an interaction energy of almost $-11 \text{ kcal mol}^{-1}$. The steepness of this energy curve leads to smooth convergence of the geometry optimization: Placing the sparkle in the initial configuration directly over the aromatic ring instead of in the ring plane leads to the same structure after energy minimization.

The geometry of the organic molecule is altered little by the presence of the sparkle; bond lengths change by less than 0.005 Å and bond angles by less than 0.3°. The charge distribution, however, is significantly perturbed by the presence of the charged particle, although the total charge in the organic part of the complex remains zero. A simple valence-bond resonance structure for benzonitrile (Figure 8) illustrates these changes qualitatively but in a strikingly simple manner.

Electron density is easily shifted in the delocalized π -system and this induces an extra dipole moment in addition to the one already present. Thus, the overall effect may best be described as a combination of ion–dipole and ion–induced dipole interactions.

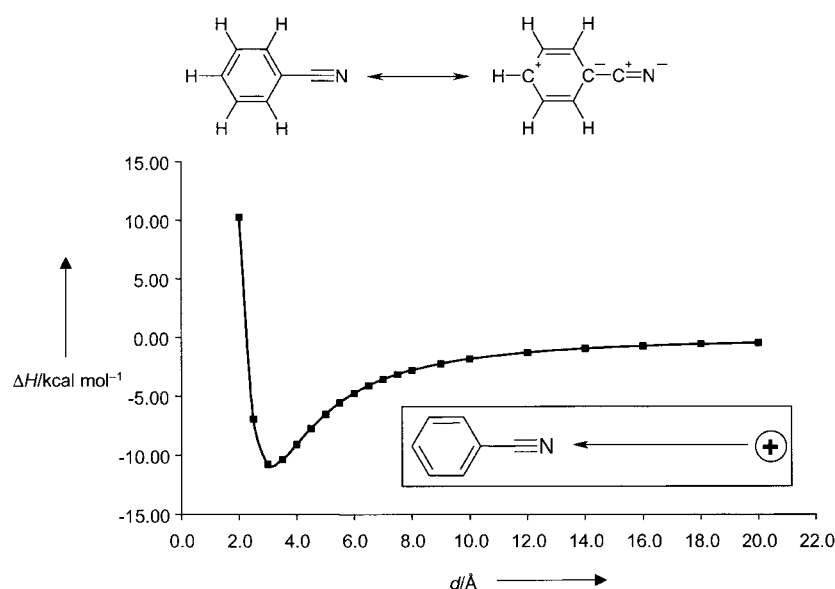


Figure 8. Energy profile for the interaction of a positively charged sparkle with benzonitrile. ΔH denotes the calculated heat of interaction (AM1) and d the distance of the sparkle from the cyano nitrogen atom.

Another case in point, typical for SFM-based materials, is 1,2,3-trifluorobenzene. As for benzonitrile, very little change in molecular geometry is observed but the change in atomic charges is now reduced. Consequently, the heat of interaction is only $-4.4 \text{ kcal mol}^{-1}$, which implies a smaller solvation power. It is well known, that for active matrix applications, nitriles are not suitable because of poor voltage-holding behavior. The empirical and perhaps initially puzzling finding, that only the recently introduced SFMs are suitable for active matrix displays, is nicely corroborated by this simple model of ion solvation.

Finally, instead of the model systems discussed so far, a set of complete liquid crystals for TN, as well as AMD, applications was used. Immediately, the problem of more than one polar group in a given molecule arises. For example, in esters of the type shown in Figure 9, the sparkle may interact with the ester group (a) or with the terminal nitrile (b).

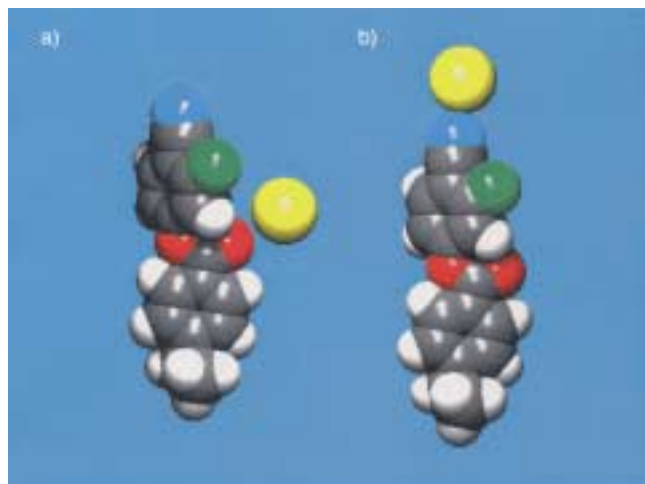


Figure 9. Two local minima for the interaction of a positive sparkle (yellow) with the liquid crystal ME2N.F (132).

Both structures are energy minima but the heat of interaction with the nitrile group is $-12.6 \text{ kcal mol}^{-1}$, whereas, with the ester carbonyl group (a) it is only $-8.0 \text{ kcal mol}^{-1}$. Under equilibrium conditions, only structure (b) will exist and (a) can be safely neglected. In a similar way, the structures and energies of other typical liquid crystals were calculated and the data are shown in Figure 10.

Although in essence a very simple electrostatic model is used, the results are often counterintuitive: The calculated heats of interaction do not necessarily have a direct relationship with the dipole moment or the polarizability; for example, the computed dipole moment is 7.1 D for ME2N.F but “only” 3.9 D for PCH-3 and 3.1 D for CCP-3OCF₃ **23**. Admittedly qualitative in nature, this simple model allows a preliminary assessment of the AMD suitability of new liquid crystals before their actual synthesis.

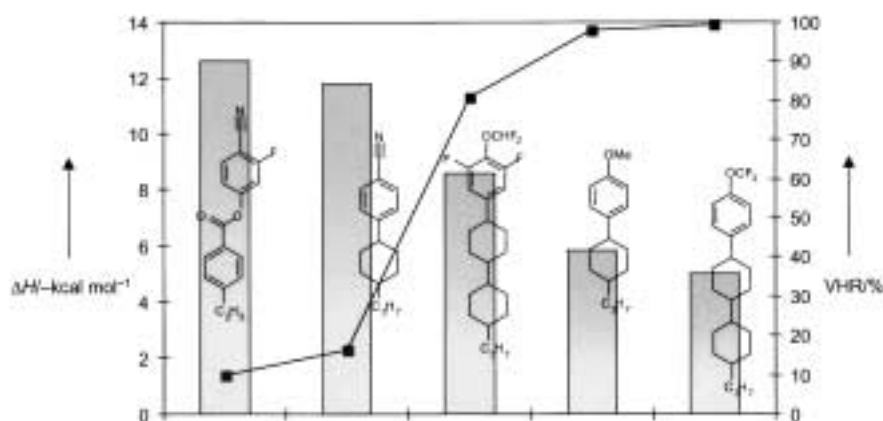


Figure 10. Calculated (AM1) heats of interaction (ΔH) and voltage-holding ratios (VHR) for some typical liquid crystals.

6. Conclusion and Outlook

During the time span between their first application in simple LCDs and their use in modern AM-LCDs, structural as well as physicochemical characteristics of liquid crystalline materials have changed drastically. The parallel development of various types of active matrix technologies, with their sometimes very different demands with regard to the physical properties of the materials, led to a broad diversification of the chemical structures and also of the synthetic methods used for their production on an industrial scale.

The increasing pace of the technological advance in the display field has created a powerful incentive for the materials manufacturers to put the design and development of new liquid crystals on a more rational basis. Although it might not be expected offhand, some important properties of the condensed liquid crystalline phase can be predicted by modeling the isolated molecules. There are relationships between molecular parameters and experimental clearing temperatures, electrooptical anisotropies can routinely be calculated for any structure, and a simple model for the solvation of ions yields results in agreement with important liquid crystal reliability parameters like VHR or residual DC. In addition to these properties specific to liquid crystals,

modeling offers important tools for the synthetic organic chemist to guide synthetic strategies by providing information on conformational space, thermodynamic stabilities, activation energies, and the ground-state and transition structures of molecules.

A decisive driving force for the future development of new AM-LCD technologies will be cost reduction in mass production. The reduction of the power consumption, such as by use of reflective displays, will be another important goal. Within the next few years, the consumer will have to choose from a broad spectrum of different devices based on AM-LCDs, ranging from large screens for TV applications to very small displays with increased resolution for viewfinders, mobile phones, or virtual reality goggles. For the design of liquid crystals, the challenge to explore materials with extreme electrooptical and viscoelastic properties will continue, with ever more stringent quality requirements than currently required.

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An Eldorado for Homogeneous Catalysis?

Gerald Dyker*

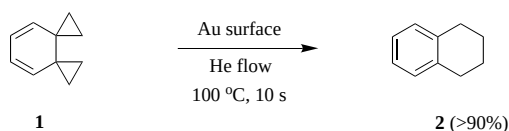
Nach Golde drängt, am Golde hängt doch alles.

Goethe: Faust I^[1]

Gold has always been the embodiment of something extremely valuable and evidently holds a fascination deep rooted in the cultural history of mankind. As is generally known, the endeavor to produce gold synthetically plays a significant role in the development of chemistry over a long period.^[2] In catalysis research gold has to date lived in the shadows, which perhaps lies in the preconceived opinion that gold is expensive and inert.

Indeed gold is the most precious metal and was considered for a long time to be extremely chemically inert particularly in reactions with nonmetals such as hydrogen, carbon, and oxygen.^[3] Through successes in heterogeneous catalysis, which are also of economic significance, this assessment has fundamentally changed.^[4a] Tetrachloroauric acid on active charcoal is currently the best catalyst for the hydrochlorination of ethyne, and gold clusters (diameter 2–5 nm) on iron oxide are particularly highly active for the oxidation of carbon monoxide.^[4b] Other possible applications in environmentally relevant fields are the oxidative decomposition of halogenated hydrocarbons and the reduction of nitrogen monoxide with carbon monoxide and hydrogen to give nitrogen, carbon dioxide, and water.^[4c,d, 5]

Surprisingly, gold can also catalyze skeletal rearrangements of hydrocarbons: For instance, the isomerization of 2,2-dimethylbutane to *n*-hexane has been achieved by Schmid with the aid of Au₅₅ clusters on titanium dioxide,^[4c, 6] and the aromatization of the dispirocyclic **1** to tetrahydronaphthalene **2** was achieved by de Meijere et al. in a reactor with gold surface at 100 °C in a few seconds (Scheme 1).^[7]



Scheme 1. Gold-catalyzed skeletal rearrangement of a strained hydrocarbon.

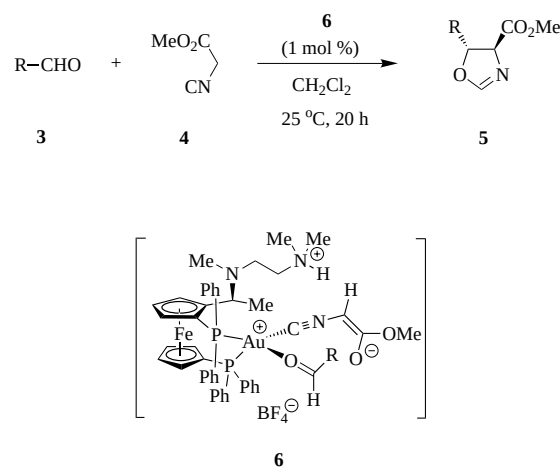
[*] Prof. Dr. G. Dyker

Fachbereich 6, Organische/Metallorganische Chemie
Gerhard-Mercator-Universität Duisburg
Lotharstrasse 1, 47048 Duisburg (Germany)
Fax: (+49) 203-379-4192
E-mail: dyker@uni-duisburg.de

In the field of homogeneous catalysis only a few gold-catalyzed processes are known to date, which, however, are characterized by the need for extremely small amounts of gold salts. In view of the high catalytic activity of gold salts, therefore the higher price for gold salts is relativized in comparison to that for the corresponding palladium and ruthenium compounds.

The new gold chloride catalyzed C–C and C–O couplings by Hashmi et al.^[8] provide a fitting opportunity to highlight the current status^[9] of homogeneous catalysis with gold salts and to show the potential for further development.

A first landmark in gold catalysis was made by Ito and Hayashi when they carried out an asymmetric aldol reaction in 1986.^[10] Aldehydes **3** were treated with isocyanoacetate **4** to give oxazolines **5** (Scheme 2). The active catalyst is a

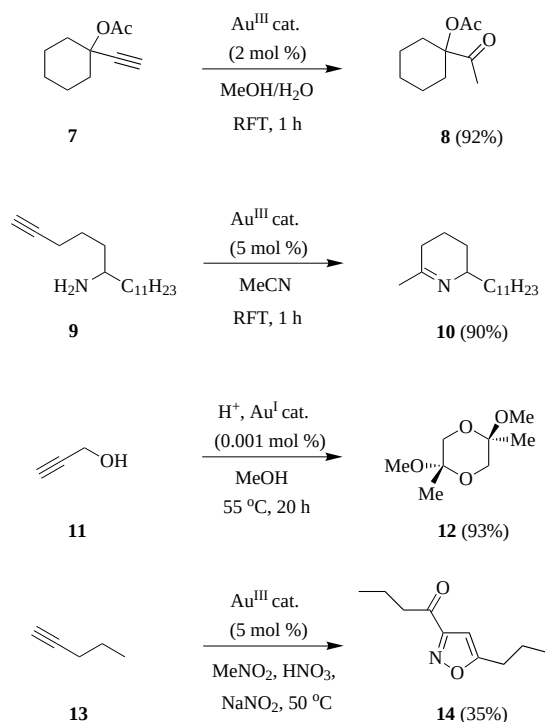


Scheme 2. Synthesis of oxazolines by a gold-catalyzed asymmetric aldol reaction.

ferrocenylphosphane-gold(I) complex (structure **6** sketches the complex with coordinatively bound reactants). In general near quantitative yields and diastereo- and enantioselectivities of greater than 90 % in favor of the 4*S*,5*R*-configured *trans* product **5** are achieved in this reaction. For instance, this method facilitates a two-step synthesis of the naturally occurring amino acid *threo*-3-hydroxylysine from 4-phthalimidylbutanal as the aldehyde component **3**.^[10d]

Also in the activation of alkynes for nucleophilic attack, gold salts prove to be soft, exceptionally carbophilic Lewis

acids, as confirmed by the examples in Scheme 3.^[11] According to Utimoto and Fukuda both the addition of water as well as of amines to alkynes are catalyzed by gold(III) salts, in particular sodium tetrachloroaurate; ketones such as **8** and

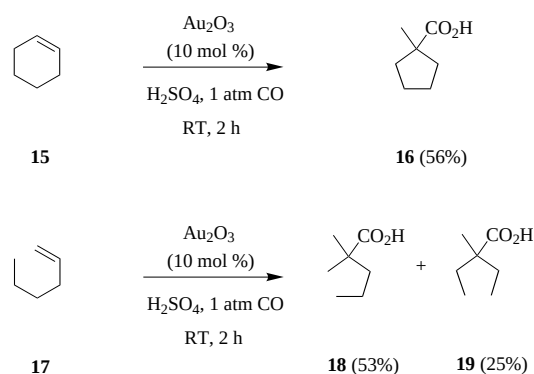


Scheme 3. Gold-catalyzed additions to alkynes. Ac = Acetyl, RFT = reflux temperature.

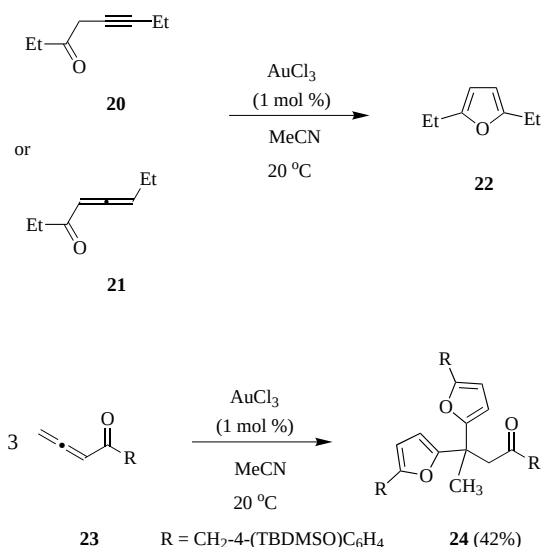
imines such as the ant toxin **10** are obtained as products in excellent yields.^[11a–e] In the cyclization to give the 1,4-dioxane **12** developed by Teles et al., the short reaction time and use of extremely small amounts of catalyst, in this case methyl(tri-phenylphosphane)gold(I), are particularly impressive.^[11d,e] In the isoxazole synthesis according to Gasparrini et al. gold salts catalyze the addition of HNO₃ to alkynes; under the oxidative reaction conditions nitrile oxides are formed, which undergo a cycloaddition to give final products such as **14**.^[11f]

In the gold-catalyzed carbonylation of olefins according to Xu et al., gold(I) carbonyl complexes are considered as active catalysts; this reaction proceeds already at room temperature in concentrated sulfuric acid at a CO pressure of 1 atm and leads after acid-catalyzed skeletal rearrangement to tertiary carboxylic acids such as **16**, **18**, and **19** (Scheme 4).^[12]

In a more recent publication Hashmi et al. reported that propargyl ketones such as **20** and allenyl ketones such as **21** can be cyclized to furans such as **22** in reactions catalyzed by gold(III) chloride (Scheme 5).^[8] This type of reaction had already been described as a silver(I)-catalyzed process by Marshall et al. (typical reaction conditions: 20% catalyst and a reaction time of several hours);^[13] however, gold salts are evidently significantly more active catalysts, which is confirmed by the fact that quantitative yields are obtained after a few minutes at room temperature and that only 1 mol% catalyst is required.



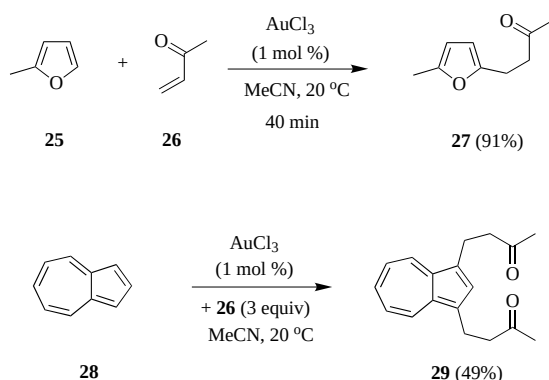
Scheme 4. Gold-catalyzed carbonylation of alkenes.



Scheme 5. New gold-catalyzed C–C and C–O coupling reactions developed by Hashmi et al. TBDMS = *tert*-butyldimethylsilyl.

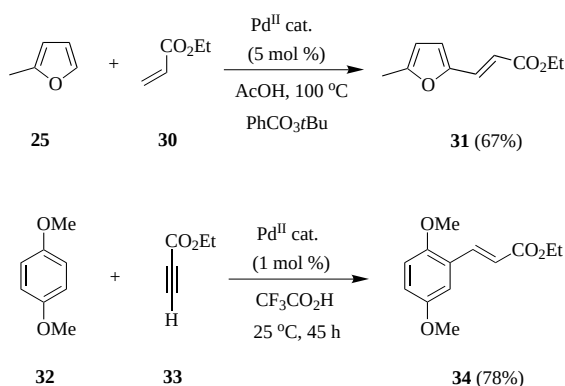
Of particular interest is the observation that in certain cases domino products such as **24** are formed: After the formation of the furan, evidently a double Michael-like addition of these intermediates to the remaining starting material **23** can take place at the unsubstituted 5-position.

Preliminary experiments to investigate the potential and limits of such additions in the presence of gold salts also confirm the applicability to the functionalization of other electron-rich arenes (Scheme 6): Besides furans, azulene **28** and di- and trialkoxybenzene are suitable as nucleophiles for the reaction with α,β -unsaturated carbonyl compounds.^[14] For instance, 2-methylfuran (**25**) reacts at the reactive 5-position with methyl vinyl ketone **26** to give the addition product **27**, and for azulene **28** twofold alkylation occurs to give **29**. In contrast to the catalysis of these reactions with hydrochloric acid and other protonic and Lewis acids,^[15] gold(III) chloride in acetonitrile guarantees sufficiently mild reaction conditions so that the otherwise typical decomposition and polymerization reactions, particularly in the case of furans, are suppressed. On the basis of the results obtained so far in these investigations, it is considered that the acidic hydrate of gold(III) chloride or tetrachloroauric acid function as protic catalysts.^[14]



Scheme 6. Michael-like additions of electron-rich arenes to methyl vinyl ketone.

Similar products are obtained from reaction sequences consisting of electrophilic metalation of electron-rich arenes and subsequent C–C coupling by carbometalation, which to date was a domain of palladium catalysis (Scheme 7).^[16, 17] As



Scheme 7. Palladium-catalyzed C–C coupling reactions by C–H activation of electron-rich arenes.

shown by the coupling reaction of 2-methylfuran (**25**) with the acrylate **30** developed by Tsuji and Nagashima,^[18] owing to the pronounced tendency of alkylpalladium intermediates for β -H elimination the unsaturated product **31** is obtained; the generally associated reduction of the active palladium(II) catalyst necessitates the addition of an oxidizing agent such as a peroxy acid ester. Very recently Fujiwara et al.^[19] reported that corresponding coupling reactions with alkynes such as **33** in trifluoroacetic acid as solvent proceed already at room temperature with reaction times of several hours.

Whether gold salts can also metalate electron-rich arenes under C–H activation will require detailed reactivity studies. It has already been shown that gold salts display considerable catalytic activity under moderate conditions; thus an extensive development of gold catalysis with numerous new applications is anticipated.

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Molecular Gold—Multinuclear Gold(I) Complexes

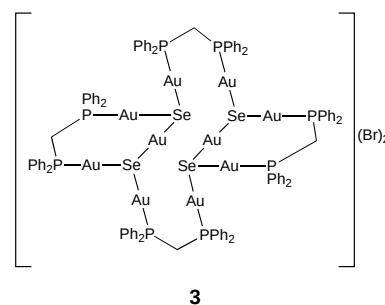
Vivian W.-W. Yam* and Eddie C.-C. Cheng

Gold, being an emblem of wealth, has been deeply involved in our daily lives for thousands of years. Its chemistry, however, has developed fairly slowly, until the last few decades, which may to a certain extent be attributed to its high elemental stability. The recent growth of interest in the study of polynuclear gold(I) compounds has stemmed from the findings that gold(I) atoms with a closed-shell electronic configuration show a strong tendency to form aggregates through aurophilic interaction. These compounds usually possess interesting structural properties and may serve as model systems for the understanding of structure and bonding in solid-state nano-materials.^[1]

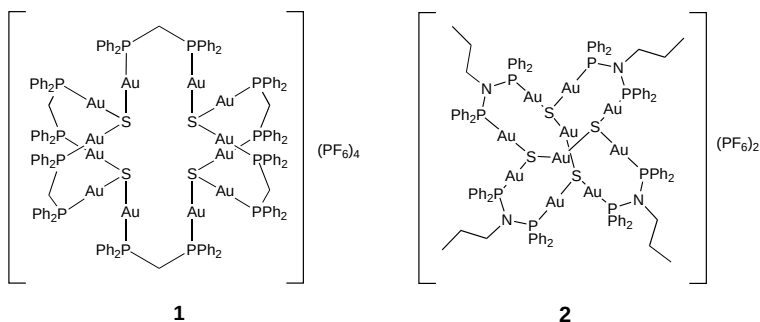
Gold(I) chalcogenide clusters represent one such class of compounds that has amused chemists and offers a synthetic challenge. High-nuclearity gold(I) chalcogenide clusters are rare despite the numerous examples of clusters of the lighter congeners, that is, copper(I) and silver(I).^[2] For copper(I) and silver(I), an usual coordination number of four can be easily achieved, whereas for gold(I), a coordination number of two is usually observed. The more restricted coordination chemistry of monovalent gold is in part responsible for the less prevailing nature of these clusters. The difficulty is further complicated by the tendency of monovalent gold to form insoluble binary chalcogenides or polymeric materials, a tendency which has to be circumvented or avoided by stabilization with phosphane ligands. This poses further limitations on the availability of coordination sites for chalcogenides. In spite of all these problems and difficulties examples of gold(I) chalcogenide clusters are known, such as the cubane-type clusters $[\text{Au}_{12}\text{S}_8]^{4-}$ and $[\text{Au}_{12}\text{Se}_8\text{Na}]^{3-}$, and the phosphane-containing aggregates $[(\text{AuPR}_3)_3(\mu_3\text{-S})]^+$ and $[(\text{AuPR}_3)_4(\mu_4\text{-S})]^{2+}$.^[3, 4] However, examples involving the use of bridging phosphane ligands, which may open up new avenues for the construction of high-nuclearity clusters, are extremely rare.^[5] Recently, two sulfido-based gold(I) clusters, $[\text{Au}_{12}\text{S}_4(\text{dppm})_6](\text{PF}_6)_4$ (**1**) and $[\text{Au}_{10}\text{S}_4(\text{Ph}_2\text{PN}(\text{nPr})\text{PPh}_2)_4]$

$(\text{PF}_6)_4$ (**2**), were reported with the diphosphane ligands, bis(diphenylphosphanyl)methane (dppm) and bis(diphenylphosphanyl)-*n*-propylamine ($\text{Ph}_2\text{PN}(\text{nPr})\text{PPh}_2$).^[6]

Cluster **1** has a “cross-road” sign structure whereas **2** is propeller-shaped. These complexes have also been shown to exhibit rich luminescence properties.^[6] Fenske et al. added to the scanty list of high-nuclearity gold(I) clusters with the isolation of the gold(I) selenido clusters, $[\text{Au}_{10}\text{Se}_4(\text{dppm})_4]\text{Br}_2$ (**3**) and $[\text{Au}_{18}\text{Se}_8(\text{dppe})_6]^{2+}$ (**4**, Figure 1; dppe = bis(diphenylphosphanyl)ethane).^[7]



Although clusters **2** and **3** share a similar molecular formula, they have completely different structures in the solid-state. While in **2** the four sulfur atoms are oriented in an alternating up-and-down fashion forming a tetrahedron, the four selenium atoms in **3** are coplanar. The delicate balance between the nuclearity of the metal atoms and the nature of diphosphane ligands is revealed in the dramatic difference in the molecularity and the structure of the clusters **3** and **4**, with just a change of the diphosphane ligand from dppm to dppe. Cluster **4** shows an interesting structure in which the gold atoms are arranged in two major layers (Figure 1), with a Au_6Se_2 distorted cubane core at the center and the remaining twelve Au atoms, which are attached to the dppe ligands, in the outer shell. The inner core and outer shell are interconnected by six Se atoms. A gold-sulfide cluster, $[\text{Au}_{18}\text{S}_8(\text{dppe})_6] \cdot \text{H}_2\text{O}$ (**5**), isostructural to **4**, was reported in 1987 by the Waters group and also mentioned in a review by Pyykkö.^[8] However, in the X-ray crystal structure determination, the anion could not be located, a similar problem was encountered in the structural solution of **4**. From the published $\text{Au}^{\text{I}}\text{—Au}^{\text{I}}$ bond lengths (2.909(5)–3.330(6) Å), it would be reasonable to suggest that **5**, like **4**, is a multinuclear gold(I) cluster, rather than a $\text{Au}^{\text{I}}\text{—Au}^0$ mixed-valent species. In **5** the anions could not be identified and located, whereas in **4** the cocrystallized CH_2Cl_2 molecules are disordered. Such problematic issues and difficulties are commonly encountered in large clusters of this sort. Further complications and difficulties come from the loss of solvent of crystallization from these clusters; solvents have been found to play an important role in the crystallization of closed-shell d^{10} systems.



[*] V. W.-W. Yam, E. C.-C. Cheng
Department of Chemistry
The University of Hong Kong
Pokfulam Road, Hong Kong SAR (China)
Fax: (+852)2857-1586
E-mail: wwyam@hku.hk

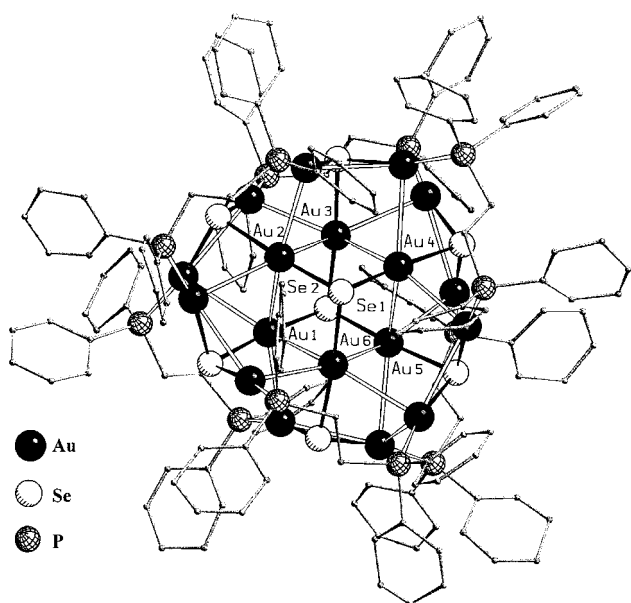
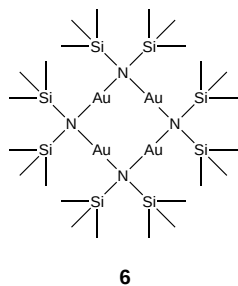


Figure 1. Molecular structure of **4**. The H atoms are omitted for clarity. (Reprinted from ref. [7].)

Electrospray ionization (ESI), a “soft” ionization source for mass spectrometry, has proved to be an extremely powerful and versatile method for the characterization not only of multinuclear metal clusters but also in the identification of coordination compounds and organometallic complexes. It provides a convenient and effective way to produce intact ions from thermally labile complexes and from multiply charged metal clusters.^[9] Clusters **1–4** have been characterized by ESI mass spectrometry and shown to be intact in solution. Details on the fragmentation pattern, dissociation behavior, as well as bond energies can be readily estimated using collision-induced-dissociation studies.^[9]

Apart from the soft chalcogenide ligand, hard nitrogen donor ligands have been shown to bridge gold(I) centers in a limited number of complexes. The best-known example is $[(\text{AuPPh}_3)_3(\mu_3\text{-NR})]^+$ with a μ_3 -bridged imido ligand;^[10] base-stabilized gold(I) amido complexes such as $[\text{Au}(\text{PMe}_3)\text{-N}(\text{SiMe}_3)_2]$ and $[\text{Au}_2(\text{PET}_3)_2\text{N}(\text{SiMe}_3)_2]^+$ are also known.^[11] However, it was only very recently that the first base-free gold(I) amido complex $[\text{Au}[\mu\text{-N}(\text{SiMe}_3)_2]]_4$ (**6**) was isolated by Rees, Jr and co-workers.^[12]

Gold(I) is widely accepted as a soft Lewis acid, and will form stable compounds with soft Lewis bases. However, amides are hard Lewis bases and this may account for the scarcity of gold(I) amido complexes. The cluster **6** is tetrameric in nature, forming a molecular square with N–Au–N edges. Aurophilic contacts of 3.0100(3)–3.0355(3) Å have been observed. Analogous copper(I) and silver(I) complexes have been synthesized, by Miele et al. and Hitchcock et al., respectively.^[13] The copper analogue did show some metal–metal interaction but not the silver analogue. $[\text{Cu}[\mu\text{-N}(\text{SiMe}_3)_2]]_4$ was reported



to phosphoresce at 512 nm with a long radiative lifetime of 30 μs .^[14] A related tetrameric complex of nitrogen donor ligand, $[\text{Au}_4\text{Cl}_4(\text{pip})_4]$ (pip = piperidine), has also been shown to show intense phosphorescence.^[15]

The extension of gold(I) chemistry to mixed-metal clusters has attracted much attention in the last decade. The incorporation of other metal centers can increase the structural diversity and variety of the final complexes formed. The employment of the metalloligand approach brings structural chemistry into a new era. Laguna et al. reported the syntheses and characterization of a series of novel polynuclear gold(I)–copper(I) and gold(I)–silver(I) mixed-metal organometallic clusters with unsupported gold–metal bonds.^[16] The isolation of $[\text{M}\{\text{Au}(\text{mes})\}_5]\text{X}$; (M = Cu, X = PF_6 **7**; M = Ag, X = ClO_4 **8**; mes = 2,4,6-trimethylphenyl (mesityl)) was accomplished by the reaction of the electron-rich $[\text{Au}(\text{mes})]_5$ complex with the corresponding metal ion precursors in an appropriate mole ratio. When the 2,4,6-triisopropylphenyl group (trip) was employed in place of the mesityl group, a “cart-wheel” like cluster, $[\text{Ag}\{\text{Au}(\text{trip})\}_6]\text{CF}_3\text{SO}_3$ (**9**) was isolated.^[16] Figure 2 shows the X-ray crystal structure of the cation of **9**. The

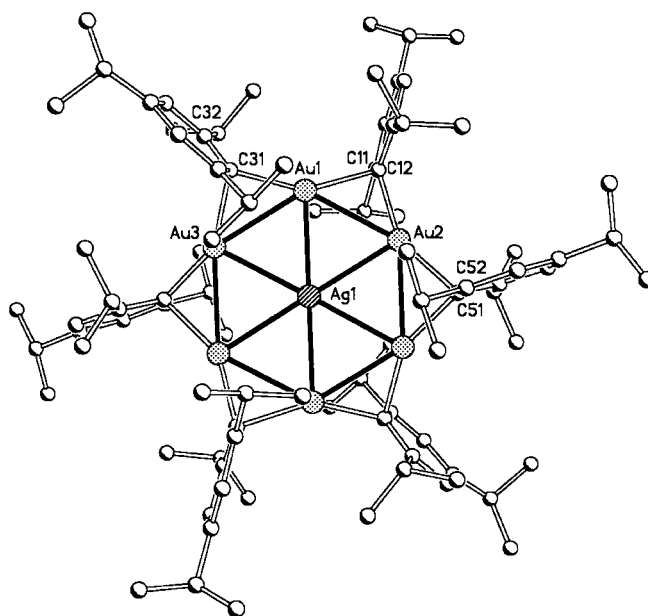


Figure 2. Molecular structure of the cation of complex **9**. The H atoms are omitted for clarity. (Reprinted from ref. [16].)

silver(I) atom is situated at the center of a regular hexagon of gold(I) atoms to form an almost planar AgAu_6 core, with the six gold(I) atoms disposed to donate electron density to the central silver atom. Short intermetallic interactions have been demonstrated which might account for the stability of the heptanuclear heterometallic clusters. This may open up a new generalized synthetic route for the synthesis of other mixed-metal clusters.

Gold, because of its inherent and unique features, has made a significant contribution to the study and design of new classes of polynuclear metal complexes and metal clusters. A more thorough and insightful understanding of binary or ternary solids such as the recently reported alkali metal auride

aurates(i) $[\text{MAu}_4][\text{M}_3\text{AuO}_2]$ ($\text{M} = \text{Rb}, \text{Cs}$), formed by the disproportionation of gold into Au^+ and Au^- ,^[17] requires the synthetic cluster chemists to extend their work to the construction of nanoscale high-nuclearity homo- and hetero-metallic clusters. Through the strategy of employing bridging ligands, whether soft such as sulfide or selenide or hard such as amides, the nuclearity of the gold clusters can be increased easily, in a uniform, systematic, and controllable fashion. The versatility of the synthetic design strategies can be further elaborated by using the metalloligand approach. With such an ability and the potential to assemble new multinuclear supramolecular arrays, gold has important implications for the future design and development of advanced nanomaterials.

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Single Fullerene Molecules and the Wave – Particle Dualism

K. O. Greulich

In the past, new developments in physics often have fostered progress in applied chemistry. Now it is applied chemistry which boosts physics in one of its central questions: wave – particle dualism. C_{60} and C_{70} molecules, fullerenes, help solve two of the most important questions of physics: “Is the wave – particle dualism also valid for large objects?” and “Can particles interfere with themselves?”

C_{60} molecules have a molecular weight of approximately 1000 Dalton. This corresponds to a peptide of 8–9 amino acids or an oligonucleotide of 3–4 monomers. The linear

dimension of such a fullerene is equivalent to four carbon atoms (since $4^3 = 64$), namely, three C–C bonds or five Ångströms. Objects of this size can be visualized with good scanning – tunneling or atomic force microscopes. Most readers would probably agree that these are true particles.

If it were possible to show that fullerenes of this size also have wave properties, this would represent a major breakthrough in one of the most basic questions of physics. Exactly this is reported by the group of Anton Zeilinger, Vienna.^[1]

Recall that when a (plane) wave encounters a double slit, part of it will move through slit A, the other one through slit B. When the slit width as well as the distance between the slits are of the order of the wavelength, both slits are the origin of spherical waves which interfere with each other, that is, they generate regions of amplification and regions of extinction. A screen in the path of such interfering waves will register an intensity pattern with maxima and minima. One

[*] Prof. Dr. K. O. Greulich
Institut für Molekulare Biotechnologie
Postfach 100813, 07708 Jena (Germany)
www.imb-jena.de/greulich
Fax: (+49) 3641-656-410
E-mail: kog@imb-jena.de

can model this process easily in a basin with still water: Holding the thumb and ring finger of one hand in a fixed position at a water surface, oscillation of the hand will model the two spherical waves emanating from two slits. A clear interference pattern is seen with regions of heavy wave motions and regions of quiescence. Double-slit experiments with light (Young, Huygens) have, in the 19th century, proven that light is a wave. A more general rule is: Whenever two or more paths are possible and interference patterns result, this is evidence for waves. So far, everything is straightforward—only the mathematics describing the process is perhaps somewhat difficult.

Problems arose with the advent of quantum mechanics: The latter postulates, that interference occurs also when a beam of particles, instead of waves, passes the double-slit arrangement. This occurs even when the beam is attenuated so much that only one particle at a time can pass the apparatus. This is no longer interference of a partial beam travelling through slit A with a partial beam travelling through slit B. Some hidden information must organize this process. Alternatively, single particles interfere with themselves, as it was mentioned above.

For decades, most experiments of this kind were performed with light, which, for this purpose, was regarded as a beam of photons, and photons were thought to be strict particles. Phrases such as “when light is attenuated so much, that only one photon at a time is in the apparatus” can be found in many textbooks, even in quite new ones. However, such an experiment has never been made. The reason is that a light source, be it a laser or a cheap light bulb, always generates wavetrains. Since in such wavetrains, for example with green light, the temporal distance between two photons is 1.6 femtoseconds and techniques which might resolve such short times^[2] have never been used in a double slit experiment, one can never say if there were really single photons or if two or more photons were in the apparatus, which might have interfered classically, as described. Even so-called single-photon detectors can never ascertain if they have observed a single photon or just the first photon of a more-or-less short wavetrain. Therefore, all double-slit experiments which were used to discuss the above-mentioned quantum mechanical questions are disputable.

Less disputable are the Zeilinger experiments with fullerenes: There is little doubt that we have a true single-molecule experiment. Nevertheless, when fullerenes are sent through a double slit (or, for practical reasons, through a grid with more than two slits), molecule by molecule, particle by particle, the result is the one shown in Figure 1a: Obviously interference!

The Experiment

Commercial fullerene C_{60} molecules (purity 99.5%) were purchased. After heating in an oven to 900 or 1000 K, those molecules which migrate in the wanted direction are collimated in vacuum (5×10^{-7} mbar) by two $10 \mu\text{m}$ slits 1.04 m

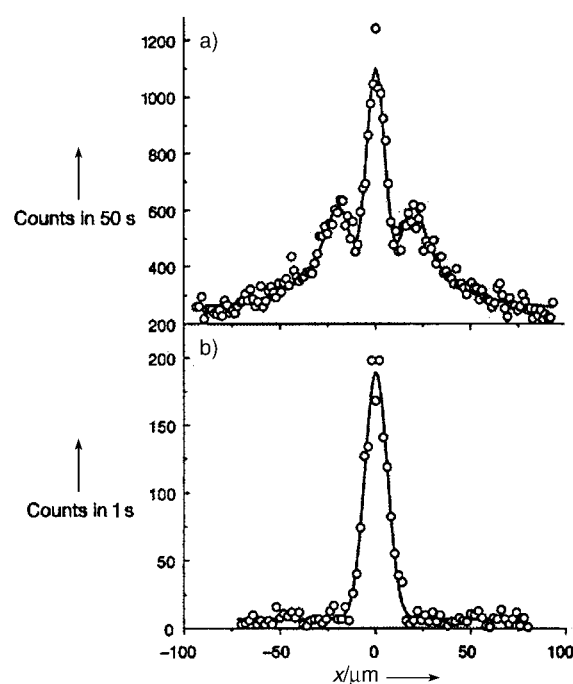


Figure 1. Experimental interference results, collected with (a) and without (b) the grid present. Note that the count rate scales are different. The solid line is calculated from theory. (Reproduced from ref. [1].)

apart from each other (Figure 2a). Immediately after the second collimation slit, a grating with 50 nm wide slits and a grid constant of 100 nm was inserted into the path. The detection system was positioned 1.25 m further (Figure 2b). The fullerenes were first ionized with a focused 24 W argon-

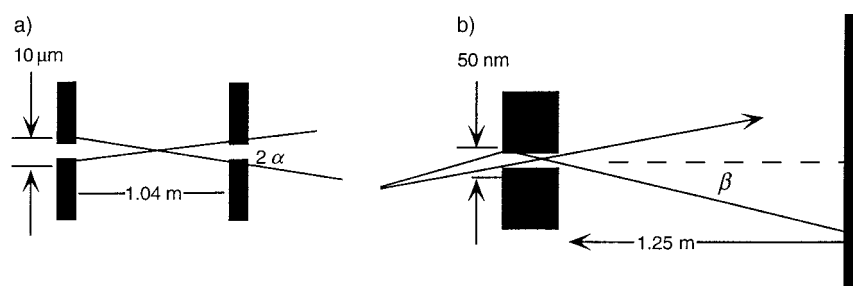


Figure 2. Experimental set-up for the collimation (a) and the collection (b) geometries, showing the collimation and collection angular deviations, α and β , respectively.

ion laser and then detected with a beryllium–copper conversion electrode combined with a channeltron detector. The dark count rate for this system is less than one count per second.

Are “Trivial” Interpretations Possible?

In such an important scientific question as discussed here, we should thoroughly check if some trivial interpretation is perhaps possible. In fact, we should ask a few obvious questions. In the collimator, a small angular divergence remains. One can easily verify that the numerical value of this divergence α is $10 \mu\text{m}$ per 1.04 m or about 10^{-5} . On the other hand, the width of the single slits of the grid is 50 nm.

Even with the comparably stable material of which the grid is made (SiN_x), the grid must be some micrometers thick. Figure 2b shows that, in this case, the walls of the slit might act as reflectors for the 0.5 nm large fullerene molecules. In the detector plane, such reflected molecules should be detected [$10\text{ }\mu\text{m} \times (1.25\text{ m}/1.04\text{ m}) =] 12.5\text{ }\mu\text{m}$ on the right and the left of the central peak, which would be generated by unreflected molecules passing the device. In fact, the side peaks occur at approximately that position (Figure 1a).

Interference of fullerenes—just a trivial geometric problem? Not so! With reasonable assumptions for geometric details, which are, however, not directly evident from the experimental description, the side peaks should be at least an order of magnitude weaker than they are. Thus the trivial explanation seems to be improbable.

More problematic is that in the diffraction experiment only a fraction of all fullerene molecules are counted. The scales in Figure 1a and 1b are very different. The peak of the control experiment has an intensity of 200 counts per second or 10000 counts per 50 seconds (Figure 1b). The intensity of the central peak in Figure 1a is 1100 counts per 50 seconds. Even when considering that the diffraction pattern is broader, a rough estimate indicates that at most 20% of fullerene molecules are counted when the grid is introduced into the path of the fullerene molecules. In a pure wave model, introducing a grid into the experiment should not “swallow” such a large percentage of waves.

We cannot solve this point here, but the data can be explained with high accuracy by Kirchhoff's diffraction theory (the solid line through the data points). It is quite improbable that this agreement would result by chance, with other physical reasons for generation of this pattern. Particularly so, the relative intensities are hardly to be generated by chance. So, with little small remaining doubt, we can accept that the experiment in fact has seen (self-)interference and thus wave properties of single fullerene molecules.

Some Speculation: Are Fullerene Molecules “Soft”?

What is the consequence of interference? A naive answer is that fullerenes are predominantly wavelike and soft! What most of us have accepted for photons, electrons, neutrons, and perhaps single atoms, is obviously also true for these little solid-state bodies consisting of 60 to 70 atoms. Moreover,

since we can be quite sure that we had only one fullerene in the apparatus at a given time, we have self interference.

Theoreticians will certainly develop some explanation or they will just tell us “that is nature, we have to accept it!”. But let us be somewhat less pessimistic, let us make a speculative assumption: In a way we still have to understand fullerenes may divide into two parts, travelling different paths, and finally reunite, as one can imagine for a droplet of oil when sliced by a knife-like structure.

Is this assumption really so naive that we have to dismiss it immediately? Perhaps not! Perhaps our perception of a particle is incomplete. Everybody accepts that hard cosmic radiation consists of particles. For soft radio waves nobody would dare to suggest this. But the whole edifice of physics would collapse when these two forms of electromagnetic radiation, distinguished only by their wavelengths, were essentially different. Perhaps gamma radiation only appears particle-like since we use objects with a smaller energy density (atoms, nuclei) to detect them. Correspondingly, we perceive radio waves as soft since we use metal rods (antennas) for their detection. Here, we have clear examples that it is rather a matter of perception and not an absolute truth if we see an object as a particle or as a wave. At least elementary particles are no billiard balls.^[2] There is a second reason for not immediately dismissing the soft-molecule idea: The droplet model for the atomic nucleus is well accepted and Jensen received the 1963 Nobel Prize for Physics for this idea.

After the 20th century, in which we have learned that particles are waves and Zeilinger's experiment has shown that this is true even for comparably large particles, we should now start to ask a surprisingly simple and yet so difficult question: What is a particle? This goal is now becoming experimentally feasible with the rapid development of single molecule techniques (see, for example, ref. [4]), which allows us to observe and handle single molecules, and applied chemistry is providing the ideal particles to tackle this challenge.

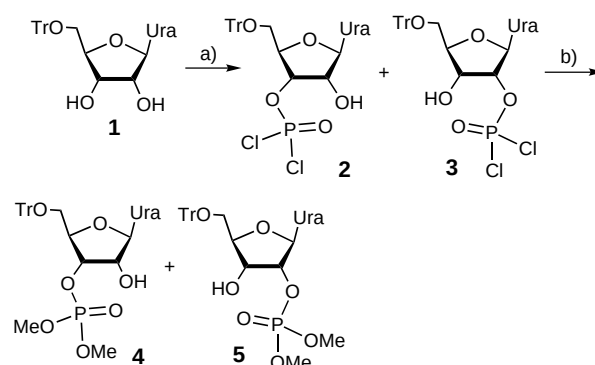
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Reexamination of the Evidence for Solvent-Induced Intramolecular Electrophilic Catalysis by a *cis* Vicinal Hydroxyl Group in Ribonucleoside Phosphorylation Reactions

Paul M. Cullis,* Martin J. P. Harger,* and Michael Lee

The mechanisms of enzymatic and chemical hydrolysis of RNA have been the subject of intense interest for many years. Early demonstration of a dramatically enhanced hydrolytic reactivity of RNA compared to DNA established a crucial mechanistic role for the 2'-hydroxyl group.^[1] Hitherto, it has been accepted that the major role of the 2'-hydroxyl group is nucleophilic catalysis and the 2',3'-cyclic phosphate intermediate is well established. The comparatively recent discovery of RNA catalysis (ribozymes)^[2] has stimulated renewed interest in the potential role(s) of the 2'-hydroxyl group in an increasingly diverse range of reactions. Thus, the report by Roussev et al.^[3] of the *electrophilic catalysis* of displacement reactions at a neighboring phosphoryl center by the *cis* vicinal hydroxyl group, if it is correct, offers a potentially novel mechanism that could be of significance to ribozymes. The importance of this claim prompted the present investigation.

Roussev and colleagues compared the reactions of 5'-protected ribonucleosides and 5'-protected-2'-deoxyribonucleosides with an excess of phosphorus oxychloride and pyridine in CH₂Cl₂ followed by treatment with an excess of an alcohol.^[3] In the case of the ribonucleosides the two-stage sequence led to a mixture of the corresponding 2'- and 3'-phosphate triesters (Scheme 1) while in the case of the 2'-deoxyribonucleosides the 3'-phosphate triester was produced. Importantly the substrates with a free 2'-hydroxyl group showed much greater reactivity in the second stage, which is the alcoholysis of the intermediate chlorophosphates. This was rationalized in terms of the *cis* vicinal hydroxyl group hydrogen bonding to the P=O group of the ribonucleoside phosphorodichloridate **2** or **3**, thereby assisting the subsequent nucleophilic displacements of chlorine by electrophilic catalysis. Crucial to this rationalization was the identification of the intermediate as a mixture of the regioisomeric phosphorodichloridates **2** and **3**. This assignment was based on the ³¹P NMR spectrum of the reaction mixture. The relevant signals in the case of 5'-O-trityl uridine were reported



Scheme 1. Reaction scheme proposed by Roussev et al.^[3] for the two step synthesis of the 5'-protected ribonucleoside phosphate triesters. Reagents: a) POCl₃ and pyridine in CH₂Cl₂; b) MeOH.

as $\delta = 22.40$ (d, $^3J_{\text{P,H}} = 12$ Hz) and $\delta = 20.03$ (d, $^3J_{\text{P,H}} = 10$ Hz). The cyclic phosphorochloridate structure **6a/b** was assigned to a minor resonance (6–7%) at $\delta_{\text{P}} = 20.01$ (t, $^3J_{\text{P,H}} = 9.2$ and 8.4 Hz), and this signal apparently did not increase with time (1 h). Reaction of the 5'-O-trityl-2'-deoxyuridine under the same conditions gave the phosphorodichloridate **7** with a ³¹P chemical shift of $\delta = 6.7$ (d, $^3J_{\text{P,H}} = 11$ Hz). Treatment of the intermediates with an excess of methanol led to the nucleoside dimethylphosphate triesters **4/5** ($\delta_{\text{P}} = 1.11$ and 0.92) and **8** ($\delta_{\text{P}} = 0.65$), respectively.

Two aspects of these observations seemed to warrant closer examination. Firstly, the resonance signals attributed to structures **2** and **3** are substantially further downfield than would be expected for an acyclic phosphorodichloridate,^[4] whereas, the ³¹P NMR chemical shift of the corresponding phosphorodichloridate **7** derived from 5'-O-trityl-2'-deoxyuridine is in line with expectation. To account for the large downfield shift for **2** and **3** relative to **7** ($\Delta\delta_{\text{P}} = 13$ –15) the authors again invoke hydrogen bonding between the P=O group and the *cis* vicinal hydroxyl group. Noteworthy, however, is that a comparable hydrogen bond could exist in the product phosphate triesters **4** and **5** ($\delta_{\text{P}} = 1.11$ and 0.92) and yet the chemical shifts for these differ little from that of the corresponding 2'-deoxytriesters **8** ($\delta_{\text{P}} = 0.65$). Secondly, if the species responsible for the minor resonance at $\delta_{\text{P}} = 20.01$ is indeed the cyclic phosphorochloridate **6a/b** it is not clear

[*] Prof. Dr. P. M. Cullis, Dr. M. J. P. Harger, M. Lee
Department of Chemistry
University of Leicester
Leicester, LE1 7RH (UK)
Fax: (+44) 116-2523789
E-mail: pmc@le.ac.uk

why more cyclization of **2** and **3** does not take place, since presumably **2** and **3** will be intermediates in the formation of **6a/b**. The only way around this would be to suggest that the mixture represents a thermodynamic equilibrium. At odds with this, however, are the many examples where cyclic phosphorochloridates are prepared in good yield from 1,2-diols and POCl_3 ,^[5] and the demonstration of very favorable cyclization onto phosphorus when the product is a five-membered ring.^[6]

In our hands, when 5'-O-trityl uridine **1** was treated with excess POCl_3 in dichloromethane in the presence of pyridine the ^1H -decoupled ^{31}P NMR spectrum was closely similar to that reported by Roussev et al.^[3] Two resonance signals were observed at $\delta = 22.20$ and $\delta = 21.00$ in a ratio of approximately 60:40, apparently corresponding to the two resonances previously reported. Interestingly we did not observe the third smaller resonance that was attributed to the cyclic phosphorochloridate **6**. However, the ^1H -coupled ^{31}P NMR spectrum differed markedly from that previously reported.^[3] The downfield signal at $\delta_{\text{P}} = 22.20$ appeared as a doublet of doublets with J values of 3.5 and 12.4 Hz (Figure 1). One of

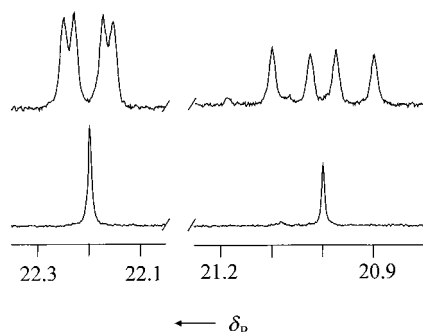
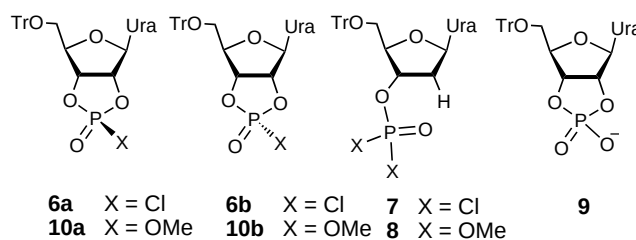


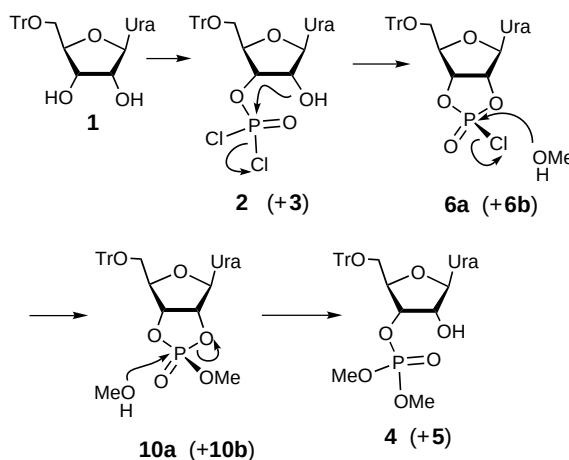
Figure 1. ^{31}P NMR spectra (162 MHz; Bruker ARX400, lower trace) of the intermediates in the reaction of 5'-O-trityl uridine with POCl_3 , showing the proton coupling (upper trace) consistent with the cyclic phosphorochloridate compounds **6a** and **6b**.

these coupling constants is small and, with line broadening, the signal may appear as a doublet. The upfield resonance at $\delta_{\text{P}} = 21.0$ also appeared as a doublet of doublets, with J values of 20.3 Hz and 12.1 Hz, Figure 1, and in this case it is difficult to see how it could be interpreted as just a doublet with a J value of 10 Hz. Roussev et al. noted that this signal was close to the additional resonance assigned to the cyclic phosphorochloridate **6** ($\Delta\delta_{\text{P}} = 0.02$) so there would have been significant overlap of the ^1H -coupled ^{31}P NMR resonances which must have made the assessment of the multiplicity more difficult.^[3] The straightforward explanation of our observations is that the species with resonance signals at $\delta_{\text{P}} = 22.20$ and 21.00 are the diastereoisomeric cyclic phosphorochloridate compounds **6a** and **6b**. Each of these would be expected to give rise to a doublet of doublets in the ^1H -coupled ^{31}P NMR spectrum because of coupling with both the 2'- and 3'-protons. The size of the coupling constants would, of course, depend on the conformations of the rings. Examination of the ^1H - ^{31}P 2D NMR spectrum confirmed that both the 2' and 3' protons are indeed coupled to both of the signals $\delta_{\text{P}} = 22.20$ and 21.00 . Such coupling is incompatible with structures **2** and **3**, but

would accord well with the diastereoisomeric cyclic phosphorochloridates **6a** and **6b**.^[7] The values of δ_{P} for the phosphorochloridates are consistent with structures involving a phosphoryl group in a five-membered ring so the need to invoke hydrogen-bond induced shifts of remarkable proportions is avoided.^[8] Further evidence that these two intermediates are indeed the two cyclic phosphorochloridates **6a** and **6b** was obtained from hydrolysis of the mixture using an excess of water. The resulting cyclic phosphate diester **9** is dramatically more resistant to further nucleophilic attack than are the cyclic phosphate triesters **10a/b**, and gave rise to a singlet



$\delta_{\text{P}}(\text{H}_2\text{O}-\text{MeOH}, 9:1) = 19.89$ in the ^1H -decoupled ^{31}P NMR spectrum and a doublet of doublets, ($^3J_{\text{PH}} = 6$ and 12 Hz) in the ^1H -coupled spectrum. The mass spectrum of the hydrolysis product confirmed the cyclic structure **9** (m/z (-ES) 547 [M^-]). Finally we confirmed that the intermediate phosphorochloridates now assigned structures **6a** and **6b** do indeed give 5'-O-trityl uridyl 2'- and 3'-dimethylphosphates **4** and **5** on treatment with an excess of methanol.^[9] However, in contrast to Roussev et al. we were able to detect transient species ($\delta_{\text{P}} = 20.20$ and 19.80); these are almost certainly the diastereoisomers of the cyclic phosphate triesters **10a/b** which on subsequent ring opening give 5'-O-trityl uridyl 2'- and 3'-dimethylphosphate compounds **4** and **5** (Scheme 2). Furthermore, when methanol was added in portions it was possible to demonstrate by ^{31}P NMR spectroscopy the simultaneous disappearance of the cyclic phosphorochloridates **6a** and **6b** and the appearance of the cyclic phosphate triesters **10a** and **10b**.



Scheme 2. Revised reaction scheme in accord with the identification of the intermediates with resonance signals at $\delta_{\text{P}} = 22.20$ and 21.00 as the cyclic phosphorochloridate compounds **6a** and **6b**.

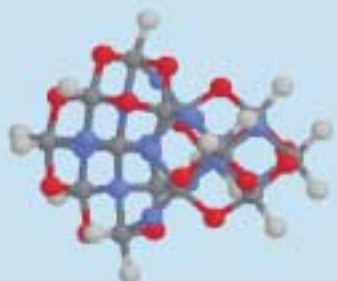
Noteworthy is that reaction mixtures containing the cyclic phosphorochloridate compounds **6a** and **6b** showed signs of a secondary reaction characterized by a very small downfield shift of the signals $\delta_P = 22.20$ and 21.00 . After about 24 h this secondary reaction appeared to be complete. It seems likely that the uracil ring is reacting with POCl_3 /pyridine at the 3,4-positions transforming the $-\text{NHCO}-$ moiety into $-\text{N}=\text{CX}-$ ($\text{X} = \text{Cl}$ or $\text{C}_5\text{H}_5\text{N}^+$). The mass spectrum of the hydrolysis product now suggested structure **9** but with the uracil 3,4-NHCO group replaced by $-\text{N}=\text{CX}-$ ($\text{X} = \text{C}_5\text{H}_5\text{N}^+$; m/z (+ES) 610 [$M+\text{H}^+$]; m/z (−ES) 644 , 646 [$M+\text{Cl}^-$]). Roussev et al. suggest that reaction with POCl_3 /pyridine affords a convenient method for the introduction of phosphoryl groups in nucleoside chemistry; it is now clear that reaction times must be kept short if the integrity of the heterocyclic base is to be maintained.

From this reinvestigation it is clear that the reaction of 5'-O-trityl uridine with an excess of POCl_3 and pyridine in CH_2Cl_2 followed by an excess of methanol gives the corresponding uridyl 2'- and 3'-dimethylphosphates **4** and **5** via the stereoisomeric cyclic phosphorochloridates **6a** and **6b** (Scheme 2) rather than the regioisomeric 2'- and 3'-phosphorodichloridate compounds **2** and **3**. The rate increase seen in the formation of **4** and **5** as compared to the corresponding reaction sequence for the 5'-O-trityl 2'-deoxyuridine arises from the well documented acceleration of nucleophilic displacement reactions at phosphoryl centers held within five-membered rings.^[10] Since the early work of Westheimer^[10] it has been established that this rate acceleration can be up to 10^8 . The initial reaction of the diastereoisomeric cyclic phosphorochloridates **6a/b** with methanol leads to the corresponding cyclic phosphate triesters **10a/b** but these are very susceptible to further nucleophilic attack by alcohol leading to the uridyl 2'- and 3'- dimethylphosphate compounds **4** and **5** (Scheme 2). None of the observations requires the recently postulated novel electrophilic catalysis of nucleophilic displacement reactions at the phosphoryl group of ribonucleosides by the vicinal *cis* hydroxyl group.^[3]

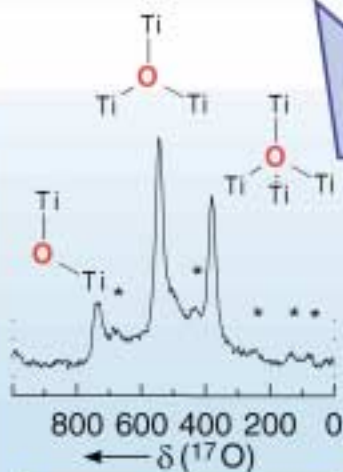
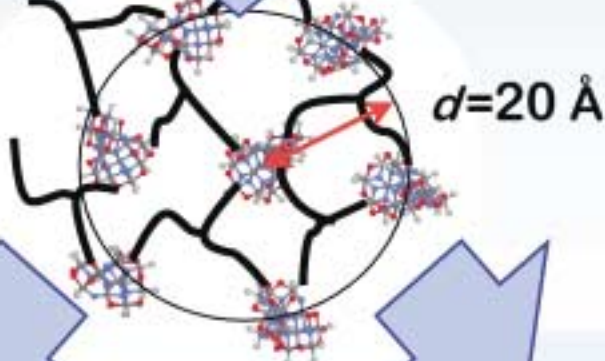
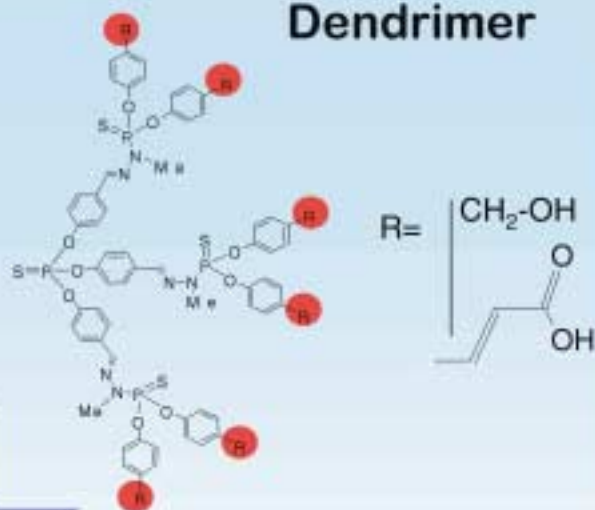
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- [9] Isolated by chromatography (silica, chloroform:methanol, 95:5) as the mixture of regioisomers; **4** (60%) ^{31}P NMR (121.5 MHz, CDCl_3): $\delta_P = 0.01$ (d × sept, $J_{\text{P,H}} = 6, 11$ Hz); ^1H NMR (300 MHz, CDCl_3 , 20°C , TMS) $\delta_{\text{H}} = 9.25$ br (s, NH), 7.70 (d, $J_{\text{H,H}} = 8$ Hz, H^6), 7.4–7.15 (m, Ph_3C), 5.91 (d, $J_{\text{H,H}} = 4$ Hz, $\text{H}^{1'}$), 5.28 (d, $J_{\text{H,H}} = 8$ Hz, H^5), 4.86–4.79 (m, $\text{H}^{3'}$), 4.41 (dd, $J_{\text{H,H}} = 4, 5$ Hz, $\text{H}^{2'}$), 4.26 (m, $\text{H}^{4'}$), 3.72 (d, $J_{\text{P,H}} = 11.5$ Hz, OMe), 3.66 (d, $J_{\text{P,H}} = 11$ Hz, OMe), and 3.52–3.37 (m, $2\text{H}^{5'}$); **5** (40%) ^{31}P NMR (121.5 MHz, CDCl_3): $\delta_P = 0.59$ (d × sept, $J_{\text{P,H}} = 7, 11.5$ Hz); ^1H NMR (300 MHz, CDCl_3 , 20°C , TMS): $\delta_{\text{H}} = 8.56$ (s, NH), 7.84 (d, $J_{\text{H,H}} = 8$ Hz, H^6), 7.4–7.15 (m, Ph_3C), 5.98 (d, $J_{\text{H,H}} = 2.5$ Hz, $\text{H}^{1'}$), 5.24 (d, $J_{\text{H,H}} = 8$ Hz, H^5), 4.86–4.79 (m, $\text{H}^{2'}$), 4.52 (dd, $J_{\text{H,H}} = 6.5, 4.5$ Hz, $\text{H}^{3'}$), 4.09 (m, $\text{H}^{4'}$), 3.77 (d, $J_{\text{P,H}} = 11.5$ Hz, OMe), 3.76 (d, $J_{\text{P,H}} = 12$ Hz, OMe), and 3.52–3.37 (m, $2\text{H}^{5'}$); ES-MS: m/z (+ES) 617 [$M+\text{Na}^+$] 100% and 1211 [$2M+\text{Na}^+$], 75%.
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The assembly of well-defined nano building blocks yields novel mesostructured hybrid compounds.

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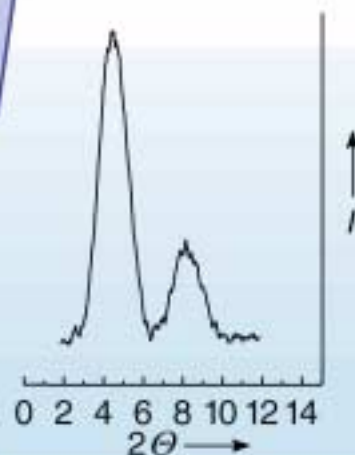
$[\text{Ti}_{16}\text{O}_{16}(\text{OEt})_{32}]$
Cluster



^{17}O MAS NMR
*Integrity of the
Ti-oxo brick*



FT-IR
Connectivity



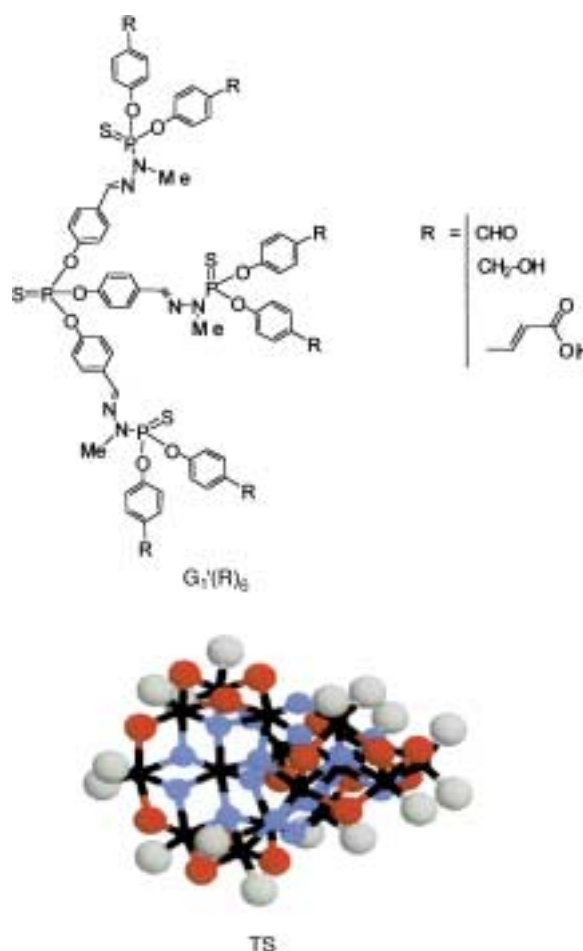
XRD
Mesostructure

New Mesotextured Hybrid Materials Made from Assemblies of Dendrimers and Titanium(IV)-Oxo-Organo Clusters**

Galo J. de A. A. Soler-Illia, Laurence Rozes, Mary Kate Boggiano, Clément Sanchez,* Cédric-Olivier Turrin, Anne-Marie Caminade, and Jean-Pierre Majoral*

The discovery that micellar and lyotropic liquid-crystal phases can direct the assembly of periodic inorganic or hybrid organic–inorganic materials heralded a new class of materials with hierarchical structures and complex forms.^[1] The chemical construction and patterning of such materials remains an important challenge in the science of organized matter chemistry.^[1d] A number of imaginative routes to prepare new hybrid materials have been recently reviewed in some excellent articles.^[2] The chemistry of silica-based hybrid systems is quite well understood and a large amount of work has been devoted to these materials, which have been formed as mesoscopic powders, films, and fibers.^[3] On the contrary, the organic–inorganic interfaces built from non-silicate precursors (transition metals, Al or Sn) are more difficult to control; fine tuning of the hydrolysis/condensation and the assembly processes is more difficult to achieve. For many transition metals, their higher reactivity towards hydrolysis^[4] precludes the reproducible synthesis of ordered hybrid phases. These problems can be overcome by resorting to less reactive, preformed transition metal–oxo building blocks and controlling their connection into organized nano- or mesostructures. Such strategies, based on the assembly of nano building blocks (ANBB)^[5] with well-defined structures, can provide new pathways to design new chemical systems with novel properties or combinations of properties.

Herein we report, what is to the best of our knowledge, the first synthesis and characterization of new mesoscopically ordered hybrid materials built through the ANBB. New functional dendrimers^[6] (hereafter called $G_1(\text{COOH})_6$ or $G_1(\text{OH})_6$; see Scheme 1) and structurally well-defined Ti-oxo-organo clusters, $[\text{Ti}_{16}\text{O}_{16}(\text{OEt})_{32}]^{[7-9]}$ (TS, Scheme 1) were used as nano building blocks. The preparation of both building blocks (BBs) and the hybrids is described in the Experimental Section. Hybrids are named TS- $G_1(\text{OH})_6$ -Z or



Scheme 1. Representation of the organic and inorganic building blocks: dendrimers $G_1'(\text{CH}_2\text{OH})_6 = G_1(\text{OH})_6$, $G_1'(\text{CH}_2\text{CH}=\text{CHCOOH})_6 = G_1(\text{COOH})_6$, and $G_1'(\text{CHO})_6 = G_1(\text{CHO})_6$ (top) and TS (bottom). For TS the Ti cations are represented in black, μ_n -O groups in blue, and the EtO groups in red (bridging) or white (terminal).

TS- $G_1(\text{COOH})_6$ -Z, in which Z represents the cluster-to-dendrimer molar ratio, TS/ G_1 , with $0.25 < Z < 4$.

Upon mixing colorless solutions of TS and dendrimer $G_1(\text{OH})_6$ or $G_1(\text{COOH})_6$ in THF, a deep yellow sol is obtained. This color becomes more intense as the solvent evaporates and yellow-orange glassy solids are finally obtained after solvent removal (the color is more pronounced for TS- $G_1(\text{COOH})_6$ -Z than TS- $G_1(\text{OH})_6$ -Z). The original organic conjugate system shows an intense $\pi \rightarrow \pi^*$ absorption at 325 nm; the complexation to Ti(IV) centers gives rise to a bathochromic shift of about 40 nm.^[10] The GC analysis performed on the cold-trapped solvent evaporated from the TS- $G_1(\text{COOH})_6$ -Z systems shows the presence of ethanol, the quantity of which increases with the value of Z. The number of ethanol molecules released by the cluster is, in all cases, lower than the total number of acidic functions added. This suggests that the TS skeleton is not destroyed. Moreover, we note that the cluster is linked to between three to five acidic functions. Once obtained, the solids are insoluble in organic solvents such as THF or toluene. Yellow gels can also be obtained within 1 h when more concentrated solutions ($[\text{TS}] \approx 50 \text{ mM}$) are kept in a closed vessel. All these results

[*] Dr. C. Sanchez, Dr. G. J. de A. A. Soler-Illia, Dr. L. Rozes, M. K. Boggiano
Laboratoire de Chimie de la Matière Condensée
UMR 7574, Université Pierre et Marie Curie
4, place Jussieu, 75252 Paris cedex 05 (France)
Fax: (+33)1-44-27-47-69
E-mail: clems@ccr.jussieu.fr

Dr. J.-P. Majoral, C.-O. Turrin, Dr. A.-M. Caminade
Laboratoire de Coordination, CNRS
205 route de Narbonne, 31077 Toulouse cedex 4 (France)
Fax: (+33)561-553-003
E-mail: majoral@lcc.toul.occ-toulouse.fr

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are consistent with the occurrence of nucleophilic reactions between the functional tips of the dendrimers and the titanium centers of the TS clusters. Ultimately a network of titanium-oxo-organo clusters, cross-linked through functional dendrimers forms. The key features, which are the control of the hybrid interface between the organic template and the inorganic building unit, the preservation of the $\text{Ti}_{16}\text{O}_{16}$ oxo core, and the mesotexturation of the resulting hybrid are discussed below.

Titanium alkoxides are known to be prone to very fast transalcoholysis reactions,^[11] as has been shown through $^{47,49}\text{Ti}$ NMR spectroscopy.^[12] Such reactions were also reported to occur with titanium oxo-alkoxo clusters, such as $[\text{Ti}_{16}\text{O}_{16}(\text{OEt})_{32}]$; it has been shown that the titanium-oxo cores of these clusters are preserved in the presence of alcohols.^[8, 9, 13] In the present work, similar transalcoholysis reactions occur between the ethoxy groups of the cluster and the benzyl alcoholic groups of the dendrimers tips, to lead to the formation of the TS-G1(OH)₆ hybrid materials. In the solid state, the covalent bonding between the dendrimers and the TS clusters was shown by ^{13}C NMR MAS (MAS = magic angle spinning) experiments, these show a new resonance signal at $\delta = 66.2$, which corresponds to the benzylic carbon of a $\text{C}_6\text{H}_5\text{-CH}_2\text{-O-Ti}$ group. The integrity of the titanium-oxo core in the final hybrid solid was checked through solid-state ^{17}O NMR spectroscopic measurements on clusters that were selectively ^{17}O enriched in the oxo bridges.^[8, 9, 13, 14] Figure 1a shows the typical ^{17}O NMR solid-state spectrum of enriched crystalline TS. Solid-state ^{17}O NMR spectrum of a typical dry TS-G1(OH)₆ hybrid ($Z = 0.4$, Figure 1b) exhibits three sets of

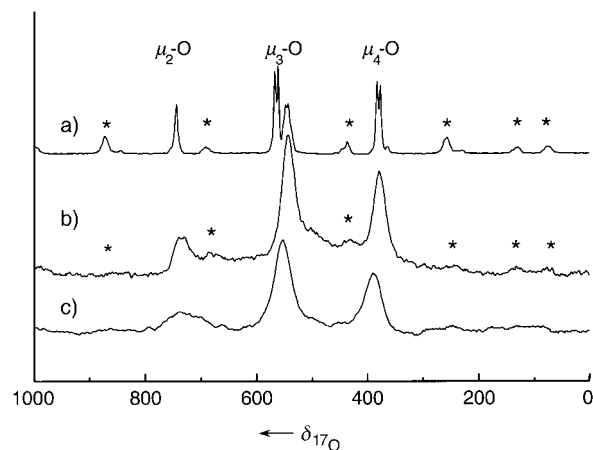


Figure 1. ^{17}O MAS NMR spectra recorded at $\nu(^{17}\text{O}) = 40.67$ MHz (spin rate 12.5 kHz) of a) TS particles and the b) TS-G1(OH)₆ ($Z = 0.4$), and c) TS-G1(COOH)₆ ($Z = 4$) hybrids. The asterisks denote rotation bands. The oxo resonances are indicated.

resonance signals located at $\delta = 734$, 542, and 377, corresponding to the $\mu_2\text{-O}$, $\mu_3\text{-O}$, and $\mu_4\text{-O}$ oxo species present in the TS cluster.^[13] There is a significant peak broadening compared to the crystalline TS, which indicates a loss of symmetry. The chemical environments of the titanium-oxo ligands suffer subtle changes upon the interaction of the clusters with the dendrimers, leading to a distribution of sites. These results confirm that the $\text{Ti}_{16}\text{O}_{16}$ titanium-oxo core is conserved in the

presence of mildly nucleophilic species, such as the alcoholic tips of the G1(OH)₆ dendrimers.

FT-IR spectroscopy is a sensitive tool with which to monitor the reaction of the G1(COOH)₆ dendrimers and TS. The most important bands of G1(COOH)₆ (Figure 2a) are located at

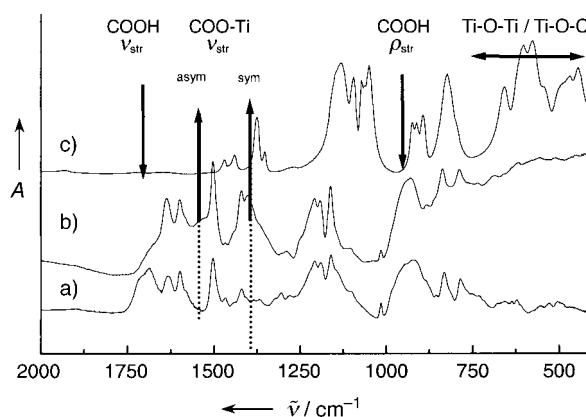


Figure 2. FT-IR spectra for the different BBs and hybrid systems: a) G1(COOH)₆ dendrimer; b) TS-G1(COOH)₆ ($Z = 2$); and c) the TS cluster. The most significant peaks are labeled.

921 (COOH dimer, out of plane stretching ρ_{str} and $\nu_{\text{P-O}}$) and 1687 cm^{-1} ($\nu_{\text{C=O}}$ of an α, β unsaturated acid^[15]). The TS clusters display characteristic sharp $\nu_{\text{Ti-OR}}$ bands in the $400\text{--}750\text{ cm}^{-1}$ region, as well as a broad absorption arising from the $\delta_{\text{Ti-O-Ti}}$ modes, as shown in Figure 2c. The $\nu_{\text{TiO-C}}$ vibrations of the surface ethoxy groups are also present (1049 , 1076 , 1094 , and 1129 cm^{-1}).^[11] These bands decrease in the spectra of the hybrid compounds (Figure 2b), which exhibit all the bands expected for the inorganic and dendritic species. The strong absorption of the dendrimer dominates this region. There are though, drastic changes upon TS addition for the bands arising from the carboxylate dendrimer (921 and 1687 cm^{-1}), which weaken in systems with increasing quantities of TS; the $\nu_{\text{C=O}}$ absorption corresponding to acidic tips in fact disappears when $Z = 2$; these results agree well with the GC results (see above). At the same time, two bands appear at 1402 and 1541 cm^{-1} and their intensity increases with Z , although their observation is hindered by the strong dendrimer C-H signals. The reported positions are characteristic of the symmetric and asymmetric vibrations of bridging coordinated carboxylate species,^[16] as is the band splitting of $\Delta\nu = 139\text{ cm}^{-1}$ which has been reported in many Ti-carboxylates.^[17–19] The appearance of this band from a bridging carboxylate group, coupled with the loss of the peaks typical for the acidic dendrimer is evidence of the formation of the hybrid interface by Ti-O-C:O-Ti bonds. The ^{13}C NMR spectra of the hybrid systems (e.g. TS-G1(COOH)₆ $z = 0.25$, not shown) shows less-intense resonance signals ($\delta = 172$) for the carboxylate groups than observed for the dendrimer alone. At the same time, a resonance appears at $\delta = 168$ which is assigned to coordinated carboxylate units. Moreover, the presence of the remaining surface EtO groups belonging to TS clusters (not clearly seen in the IR spectra) is confirmed by the resonance signals at $\delta = 71$ and 72 (for $\text{TS-O-CH}_2\text{-CH}_3$) and $\delta = 18$ (for $\text{TS-O-CH}_2\text{-CH}_3$). Based on the collected NMR and FT-IR spectroscopic

evidence, we can postulate that a fraction of the bridging EtO moieties is removed by the attack of the carboxylate dendrimer tips; in this way, nucleophilic substitution coupled to a single proton transfer from the acid to the alcohol suffices to substitute two coordination positions in vicinal Ti atoms. The integrity of the $\text{Ti}_{16}\text{O}_{16}$ inorganic core was also demonstrated through ^{17}O NMR spectroscopy (Figure 1c). The characteristic three sets of broad resonance signals (μ_n -O oxo-species) of the cross-linked TS clusters appears for the TS-G1(COOH)₆-4 system. When the *Z* ratio is decreased, further broadening of these resonance signals is observed, indicating that for higher carboxylate:titanium ratios (*Z* < 0.1) a fraction of the $\text{Ti}_{16}\text{O}_{16}$ cores is cleaved.

To understand the reactions involved in the first steps solution ^{17}O NMR spectroscopic experiments were performed on the initial mixtures. TS-AcOH (Ac = acetyl) model systems were studied to assess the behavior of the TS cluster surface towards a relatively strong nucleophile. ^{17}O NMR spectra measured in a TS:toluene solution^[20] show that the main resonance signals of the $\text{Ti}_{16}\text{O}_{16}$ core (μ_2 -O δ = 750; μ_3 -O δ = 563 and 555; μ_4 -O δ = 383) are slightly modified upon addition of small amounts of acetic acid ($H = [-\text{COOH}]:[\text{TS}]$ ratio < 8); all the signals undergo a slight broadening. A decrease in intensity of the μ_3 -O (δ = 563) resonance is also observed. This change is in conjunction with the appearance of a new μ_3 -O resonance signal (δ = 565) which indicates that the early substitution of ethoxy groups by acetato ligands occurs at some specific sites of the cluster. For a higher amount of acetic acid ($H > 8$), a fast cleavage of the $\text{Ti}_{16}\text{O}_{16}$ core is observed. In the case of TS-G1(COOH)₆ systems, all the characteristic resonances of the μ_n -OTi units in the TS cluster (*n* = 2, 3, 4) are still observed by solution ^{17}O NMR spectroscopy, although an appreciable signal broadening for low *Z* ratios can be seen. This feature is also associated with a decrease in the overall intensity of the NMR signal. Both effects are in fact typical of systems for which gelation processes take place; in this case, gelation is caused by the quick cross-linking of the clusters by the polyfunctional dendrimers. An increase in the molecular size of species probed by NMR spectroscopy can lead to broadening of the resonances and even a partial loss of the signal intensity.^[21, 22] However, solid-state ^{17}O NMR spectroscopy of the final dry TS-G1(COOH)₆ hybrids shows that the cluster signature remains even for *Z* = 0.25 hybrid systems, which correspond to H = 24. For such a high acid to cluster ratio, and in the presence of small monofunctional organic acids such as HAcO, TS is destroyed. The rigid medium quickly attained in our cross-linked systems kinetically hinders the cleavage of the $\text{Ti}_{16}\text{O}_{16}$ core.

Diffraction patterns for TS-G1 hybrids were obtained using graphite-filtered $\text{CuK}\alpha$ radiation (λ = 1.5406 Å). The XRD patterns of the TS-G1(OH)₆ hybrids only exhibit one very broad diffraction peak at correlation distances (*d* values) between 1.5–1.8 nm, indicating that a local order is attained: the higher the polymer contents, the higher the *d* value. These distances are in agreement with the mean diameter of the Ti-oxo core.^[23] For TS-G1(COOH)₆ systems, two diffraction lines are observed at lower two theta values (1.9 nm < *d* < 2.1 nm), indicating a better degree of ordering (Figure 3); the ratio of

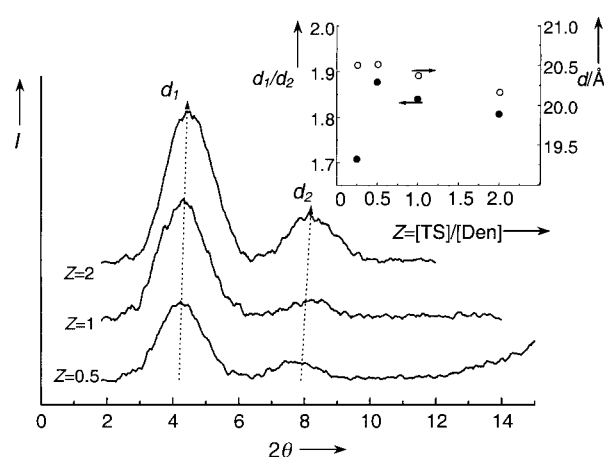
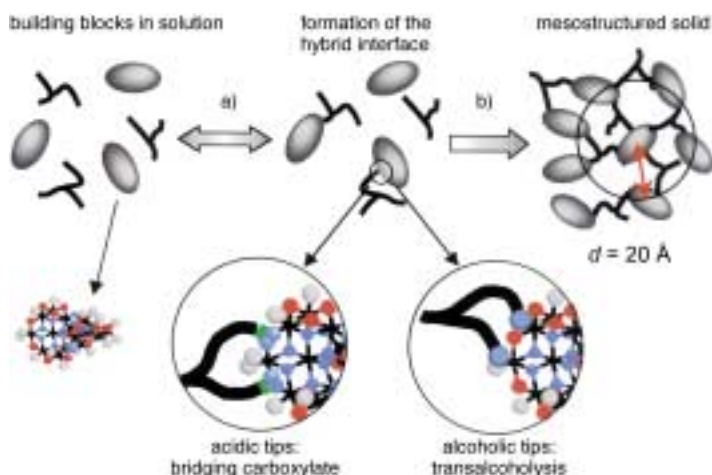


Figure 3. XRD diagrams for typical TS-G1(COOH)₆ phases; the particle to dendrimer ratio (*Z*) values are given in the Figure. Inset: variation of the distance ratios *d*₁/*d*₂ (closed dots) and the correlation distances, *d* (open dots) with *Z*, for TS-G1(COOH)₆ phases.

the diffraction peaks is in the range 1.7–1.9 (Figure 3, inset). These lines are more intense for solids that are richer in TS because the inorganic building blocks have a higher scattering power. The correlation distances are greater for lower *Z* values (see inset in Figure 3); this phenomenon also takes place in TS-G1(OH)₆ solids. This suggests that in both cases, the dendrimers act like spacers in the array of clusters. An analysis of the peak breadth by a simple Scherrer equation^[24] allows an estimated coherent scattering domain (CSD)^[25] of 5–6 nm in all cases. A local ordered array of TS clusters is thus formed in a volume of about 125 nm³, that is, 2–3 times the correlation distance along each direction. The order blurs at longer distances. The symmetry of these arrangements cannot be ascertained, but the distances found could correspond to the [100] and [110] reflections of a closely packed arrangement. Noteworthy is that under our “dry” conditions, glasslike cluster–polymer hybrids are easily obtained with polymers (such as PEO-PPO-PEO, or PEO-C_nH_{2n+1}; PEO = poly(ethylene oxide), PPO = poly(propylene oxide) that can establish covalent interactions with the clusters. However, in the latter case, ordered phases are not obtained; XRD diagrams display flat patterns characteristic of amorphous hybrids. We can thus conclude that the symmetry of the organic template, as well as the ability of the cluster to anchor the dendrimer, are essential features in obtaining organized dispersions.

New mesotextured hybrid materials comprising covalently bonded dendrimers and [Ti₁₆O₁₆(OEt)₃₂] clusters can be easily synthesized by using preformed organic and inorganic building units. Moreover, the shape of the organic blocks dictates a pyramidal local symmetry, which is repeated to form a longer-range ordered texture. “Dry” synthesis conditions, as well as controlled polymer volume fractions (≈ 30–60 %) hinder the formation of a continuous inorganic phase. The route to these TS-G1 hybrids (TS-G1(COOH)₆, TS-G1(OH)₆ with different *Z* ratios) is sketched in Scheme 2, and is composed of three steps: 1) “snap-on” reactions at the surface level (in dilute solution), leading to hybrid bricks, 2) cross-linking of these hybrid moieties, spurred by solvent evaporation, 3) formation

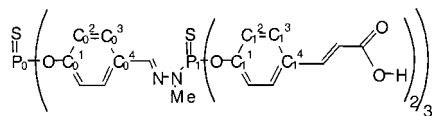


Scheme 2. Pictorial representation of the formation of the hybrid phase a) nucleophilic substitution; b) solvent evaporation; see text for full details.

of a bicontinuous hybrid gel, in which TS clusters are regularly spaced by the polymers. The combination of the surface chemistry of the clusters and the versatility of the dendrimeric tips permits these tailored phases.

Experimental Section

Numbering for dendrimers G1(COOH)₆ (for G1(OH)₆, the numbering of the aromatic rings is the same):



G1(COOH)₆: A mixture of dendrimer G1(CHO)₆^[26] (1423 mg, 1 mmol), malonic acid (1442 mg, 15 mmol), and piperidine (110 μL, 1.5 mmol) were heated at 95 °C in pyridine (15 mL) for 4 h and then refluxed for 15 min to complete carbon dioxide removal by using a routine Doebner-like procedure.^[27] The yellow brownish solution was cooled in an ice bath and poured onto 10 M HCl (31 mL). The precipitate of dendrimer was then washed with water and dried under vacuum. The crude white powder was ultimately washed with diethylether and dried by evaporation to afford G1(COOH)₆ as an amorphous white powder, yield 93 %. ³¹P{¹H} NMR ([D₆]DMSO): δ = 50.2 (s, P₀), 61.0 (s, P₁); ¹H NMR ([D₆]DMSO): δ = 3.46 (bd, ³J_{H,P} = 10.0 Hz, 9H, P₁-N-CH₃), 6.58 (d, ³J_{H,H_{arom}} = 17.1 Hz, 6H, C₆H₄-CH), 7.3–7.9 (m, 39H, CH_{arom} and CH=N), 7.65 (d, ³J_{H,H_{arom}} = 17.1 Hz, 6H, CH-COOH), 12.3–12.8 (bs, 6H, COOH); ¹³C{¹H} NMR ([D₆]DMSO): δ = 34.6 (d, ²J_{C,P1} = 12.4 Hz, P₁-N-CH₃), 121.0 (s, C₆H₄-CH), 123.0 (d, ³J_{C,P10} = 4.5 Hz, C₀, C₁²), 130.2 (s, C₀³), 131.5 (s, C₁³), 133.3 (s, C₁⁴), 134.3 (s, C₀⁴), 142.4 (d, ³J_{C,P1} = 13.5 Hz, CH=N-N-P₁), 144.3 (s, CH-COOH), 152.2 (d, ²J_{C,P0} = 7.5 Hz, C₀¹), 152.8 (bd, ²J_{C,P1} = 6.7 Hz, C₁¹), 169.1 (s, COOH). IR (KBr): ν̄ = 1686 cm⁻¹ (ν_{C=O}); elemental analysis calcd (%) for C₇₈H₆₆N₆O₂₁P₄S₄ (1675.56): C 55.91, H 3.97, N 5.01; found: C 56.05, H 3.89, N 4.93.

G1(OH)₆: BH₃·SMe₂ (1.65 mL, 3.3 mmol, 2 M solution in CH₂Cl₂) was added to a solution of dendrimer G1(CHO)₆ (0.6 g, 0.422 mmol) in THF (20 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 4 h and then allowed to reach room temperature overnight. The sticky precipitate formed was dissolved with dry ethanol (1 mL) and the crude material was evaporated to dryness. The crude white foamy solid was washed with a mixture of pentane/ether (1/1, 50 mL) to afford G1(OH)₆ as a white powder, 98 % yield. ³¹P{¹H} NMR ([D₆]DMSO): δ = 52.3 (s, P₀), 62.6 (s, P₁); ¹H NMR ([D₆]DMSO): δ = 3.45 (bd, ³J_{H,P} = 10.6 Hz, 9H, P₁-N-CH₃), 4.57 (s, 12H, CH₂), 5.25 (bs, 6H, OH), 7.23–8.09 (m, 39H, CH_{arom} and CH=N); ¹³C{¹H} NMR ([D₆]DMSO): δ = 33.2 (d, ²J_{C,P1} = 11.9 Hz, P₁-N-CH₃), 62.4 (s, CH₂), 120.7 (d, ³J_{C,P1} = 3.7 Hz, C₁²), 121.6 (bs, C₀²), 128.0 (s, C₁³), 128.7 (s, C₀³), 133.0 (s, C₀⁴), 139.9 (s, C₁⁴), 140.4 (d, ³J_{C,P1} = 13.5 Hz, CH=N-N-P₁),

148.9 (d, ²J_{C,P1} = 6.0 Hz, C₁¹), 150.6 (bd, ²J_{C,P0} = 6.6 Hz, C₀¹); elemental analysis calcd (%) for C₆₆H₆₆N₆O₁₅P₄S₄ (1435.43): C 55.23, H 4.63, N 5.85; found: C 55.39, H 4.72, N 5.79.

Hybrid materials were obtained using G1(COOH)₆ and G1(OH)₆ (carrying acidic or alcoholic ends respectively) and TS^[7–9] both dissolved in THF, the concentrations were kept below 10⁻² mol L⁻¹ to avoid the quick formation of a gel. The dissolved TS was added to the dendrimer in THF; this gave rise to orange-yellowish solutions. To avoid degradation of the [Ti₁₆O₁₆(OEt)₃₂] cluster the final solutions were placed in open vessels or Petri dishes into a desiccator under inert gas (P_{H₂O} < 10 ppm). After solvent removal, under nitrogen flow, transparent yellow, glassy solids are obtained.

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Functionalized Fullerene as an Artificial Vector for Transfection

Eiichi Nakamura,* Hiroyuki Isobe, Naoki Tomita, Masaya Sawamura, Shigeki Jinno, and Hiroto Okayama

Since our discovery that a photo-excited water-soluble fullerene oxidatively cuts the DNA duplex,^[1] the interaction of organofullerenes with DNA has continued to attract the interest of chemists. Base-sequence-selective cutting has also

been achieved with fullerene/DNA conjugates,^[2] and a number of studies on structural variations of the reagent,^[3] the mechanism of the photo cleavage,^[4] and the applications to photodynamic therapy^[5] have been reported. The lack of acute toxicity ($\text{LD}_{50} \gg 500\text{ mg kg}^{-1}$) and the pharmacokinetics of organofullerene have been recorded.^[6] We report herein an entirely new aspect of fullerene/DNA interaction, namely, the use of a functionalized fullerene as an agent for gene transfer (transfection). A newly designed “two-handed” fullerene ($\pm$)-**1** (see Figure 1) binds to duplex DNA, condenses it, and allows the complexed DNA to be delivered into and to be transiently expressed in the target cell.

The two-handed fullerene **1** is a unique amphiphile possessing [60]fullerene as the central core, on which a C₂-symmetric S-shape hydrocarbon structure, bearing two side chains, is attached. The basic C₂-core skeleton has been synthesized through the tether-directed regioselective double [3+2] cycloaddition by the route detailed in the Supporting Information.^[7] The diamine side chains are separated by 1.2 nm from each other, they are protonated at pH 7,^[8] and will exert strong electrostatic interactions with the parallel phosphate backbones of a DNA duplex (which, along the major groove, are also separated from each other by 1.2 nm). The phosphate charges on DNA will be neutralized at a reagent/base pair ratio (R) of 0.5. The central fullerene core provides a hydrophobic binding force for the condensation of DNA and probably also for the delivery of the fullerene/DNA complex to the interior of the target cell.

Various organic molecules have been developed for transfection,^[9] and commercialized under a generic name of “lipofection” reagents such as Transfectam (**7**, also known as “DOGS”, was used as the reference in these studies).^[10] The central concept of lipofection is the conversion of a negatively charged DNA into a positively charged lipid-like entity through complexation with the reagent. Although these lipofection reagents have been extensively used in laboratories and in some cases in vivo,^[11] the efficiency of transfection is by no means perfect and the reagents exhibit considerable cell toxicity, caused most likely through perturbation of the cell membrane by the lipophilic reagent. In designing a new strategy for gene delivery, we abandoned the idea of lipid mimicry and counted on the extremely high hydrophobicity of the fullerene core, with which the reagent/DNA complex may be delivered from aqueous medium into the cell.

For a given compound to act as a transfection vector, it must first bind tightly to DNA. In the absence of previous studies on a fullerene-based gene transfer approach, we needed to screen various molecules so that we could identify structural features necessary for tight DNA binding. To start the investigation, we synthesized the two-handed tetramine **1** as well as various related compounds **2–5** (Figure 1), each of which lacks some element of the structural features of **1**. The tetramine **2** lacks the fullerene core and the “one-handed” diamine **3** has only one cationic side chain. The dicarboxylic acid **4** is “two-handed”, but is negatively charged in a neutral buffer solution. The affinities of these reagents for DNA duplexes were probed by several independent sets of experiments performed on calf thymus DNA, plasmid pBR322 DNA, and linear DNA fragments. The experiments were

[*] Prof. Dr. E. Nakamura, Dr. H. Isobe, N. Tomita, Dr. M. Sawamura
Department of Chemistry
The University of Tokyo
Hongo, Bunkyo-ku, Tokyo 113-0033 (Japan)
Fax: (+81) 3-5800-6889
E-mail: nakamura@chem.s.u-tokyo.ac.jp
Dr. S. Jinno, Prof. Dr. H. Okayama
Department of Biochemistry and Molecular Biology
The University of Tokyo
Hongo, Bunkyo-ku, Tokyo 113-0033 (Japan)

Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

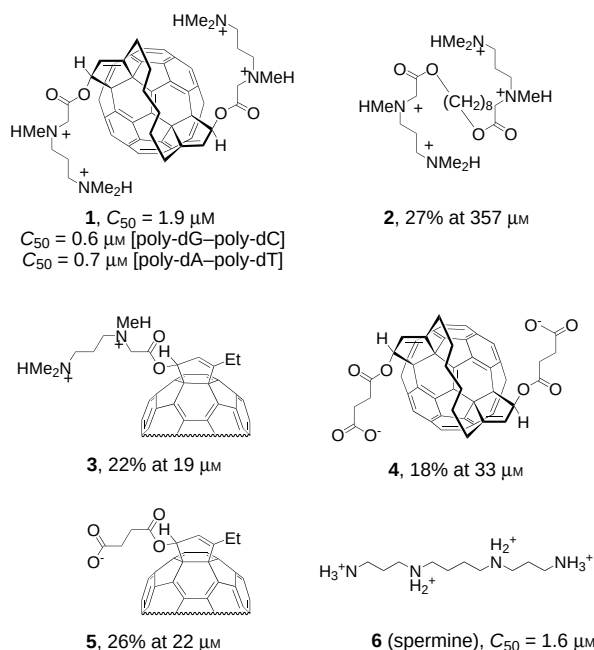


Figure 1. Tetramine **1** and reference compounds. The binding affinities (C_{50}) were measured by ethidium bromide displacement assay for calf thymus DNA (unless noted) and poly-dG–poly-dC and poly-dA–poly-dT. For **2–5**, the drug concentrations that effected maximum % displacement are shown.

performed in the range $R = 2.5 \times 10^{-4}$ –2.6. Spermine was also examined as a DNA binding reference compound.

The screening with the ethidium bromide displacement assay demonstrated a very clear structure/activity relationship for the compounds as shown in Figure 1. The two-handed fullerene tetramine **1** showed a C_{50} ^[12] as small as $1.9 \mu\text{M}$. This value rivals the data of spermine ($C_{50} = 1.6 \mu\text{M}$) determined under the same conditions. The C_{50} values for poly-dG–poly-dC and poly-dA–poly-dT were equally small with 0.6 and $0.7 \mu\text{M}$, respectively, which indicates that **1** binds to DNA without recognition of specific bases. In contrast to **1**, the other reference compounds **2–5** are unable to achieve 50% displacement even when added in a large excess.

In addition to the difference in the binding assay, we also found a marked difference between **1** and **2–6** in their ability to induce phase separation of the reagent/DNA mixture at a higher R value range. Thus, when we increased the amount of **1** from $R = 0.5$ to 2.6, the amount of DNA in the aqueous phase decreased precipitously with visibly observable phase separation. No such phase separation took place with **2–6** even at a high R range (up to 2.6). For instance, experiments using a mixture of four ^{32}P labeled linear DNA fragments of different GC contents (44–57% GC) showed that an excess amount of **1** ($R = 2.0$) converted DNA into the material that stays at the origin of electrophoresis gel (Figure 2, lane 2). The formation of the insoluble material is reversible. Thus, when the mixture was extracted with chloroform, in which **1** is soluble, the duplex DNA was released into the aqueous phase without a change in the ratio between the fragments (Figure 2, lane 3). The above comparison between **1** and **2–6** demonstrate the strong binding power of **1** through the synergetic

effects of the large hydrophobic core and the cationic side chains.

The experiments described above indicated that the two-handed fullerene tetramine **1** is unique among other fullerenes in its ability to bind to duplex DNA in a reversible manner (cf. chloroform extraction experiment). With such data in hand, we started our studies on transfection.

To optimize various parameters we initially followed the protocol used for lipofection agents (transfection for 6 h without serum, incubation for 2 d with serum). The reagent/base pair ratio (R), the amount of DNA, the transfection time, and the incubation time were the parameters examined extensively. Plasmid pGreen LANTERN-1 containing a green fluorescent protein (GFP) reporter gene was used for the determination of the transfection efficiency (TE), which is defined as the number of fluorescent cells relative to the total number of cells transfected on the plate.^[13] In the first set of experiments, the R -dependency of the transfection efficiency was examined. The experiments with transfection and incubation times fixed for 6 h and 2 d, respectively (Figure 3), show that the efficiency

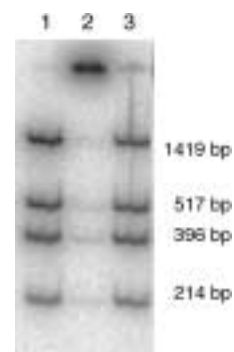


Figure 2. Precipitation of linear DNAs caused by **1** at $R = 2.0$. Addition of **1** to mixtures of four linear DNA fragments with different numbers of base pairs (bp; control, lane 1) forms immobile material (lane 2). Chloroform extraction of **1** before loading to gel liberates soluble DNAs without a change in their relative amounts (lane 3).

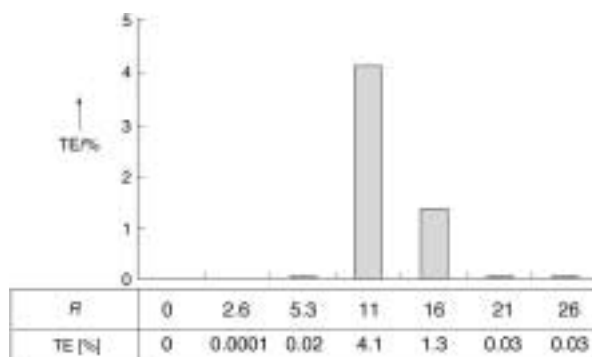


Figure 3. Dependency of transfection efficiency (TE) on R as examined for the GFP vector (COS-1 cells = 1×10^5 , DNA = $4 \mu\text{g}$, transfection time = 6 h, incubation time = 2 d). Effective transfection takes place only within a narrow range of R values. See text for full details.

increased toward $R = 11$ and then dropped with further excess of **1**. A similar R -dependent profile has been reported for other lipofection reagents. With the R value fixed at 11, the efficiency reached the maximum value of 4.1% at the DNA amount of $4 \mu\text{g}$ (Figure 4). The cell viability relative to the control experiment at the optimum transfection condition was 26%, which is comparable to a 33% value with Transfectam (**7**). The cell death was caused at least partly by the dimethylformamide (DMF) used to dissolve **1** in aqueous solution (see below).

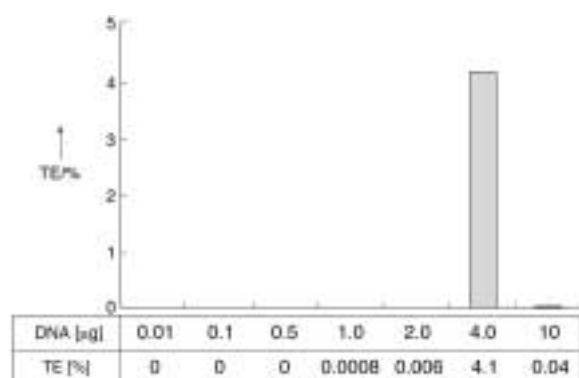


Figure 4. Dependency of transfection efficiency (TE) on the amount of the GFP vector (COS-1 cells = 1×10^5 , $R = 11$, transfection time = 6 h, incubation time 2 d). Efficient transfection takes place with a relatively large amount of the DNA.

The effects of transfection time and incubation time were examined, the TE increased up to the transfection time of 24 h (Figure 5), but after 6 h the relative cell viability fell down precipitously to $\sim 10\%$ (data not shown). The TE also depends upon the incubation time. The TE was very low on day 1 and increased rapidly until day 5 (Figure 6) when the maximum TE of 14% was achieved; fluorescence was detected in 0.4% of cells even after incubation for 12 days. In contrast, lipofection with Transfectam (7) under similar conditions (transfection time = 6 h, DNA = 4 µg, $R = 4.0$) showed the maximum efficiency of 29% after incubation for 2 days, and the efficiency dropped to 11% at day 5. Since our vector plasmid (pGreen LANTERN-1) has no ability to self-replicate (no SV40 origin contained),^[14] the five-day-long persistent increase in the number of GFP-expressing cells is attributable to the continuous release of the DNA from the 1/DNA complex taken up by the cell and its delivery into the nuclei (see above). The presence of fluorescent cells even after 12 days incubation suggests the occurrence of stable transfection.

In spite of the photoreactivity of (weakly DNA binding) fullerenes,^[1] no differences were noted when the transfection experiments with 1 were carried out under ambient light or black light. The cytotoxicity of 1 was found to be quite small, cells grew exponentially in the presence of 1 (Figure 6). In addition, tetrazolium assay indicated that the cytotoxicity of 1

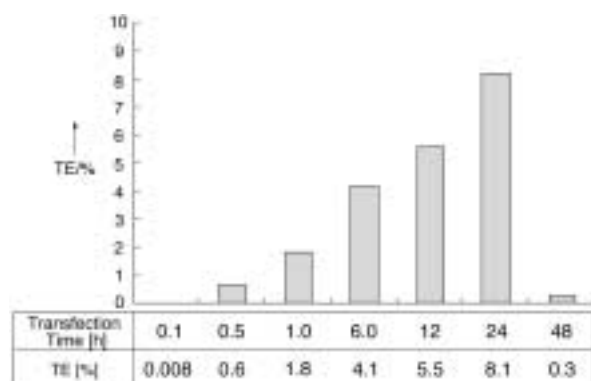


Figure 5. Dependency of transfection efficiency (TE) on the transfection time (COS-1 cells = 1×10^5 , $R = 10.6$, DNA = 4 µg, incubation time 2 d).

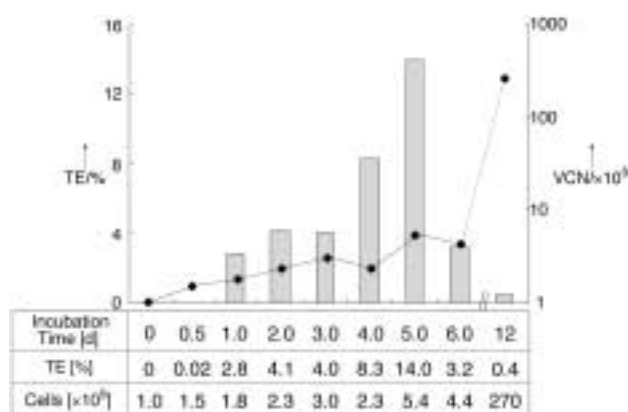


Figure 6. Dependency of transfection efficiency (TE; bar graph) and the number of viable cells (VCN = viable cell number; line graph) on the incubation time (COS-1 = 1×10^5 , $R = 11$, DNA = 4 µg, transfection time 6 h). After 4 days incubation, cells were approximately 100% confluent and re-plated in a 35-mm plate and incubated again in 10% FBS/DMEM (this operation caused deviation from exponential cell growth). FBS = fetal bovine serum, DMEM = Dulbecco modified Eagle medium.

in DMF or DMSO is largely caused by the solvent toxicity (IC_{50} (median growth inhibitory concentration) = 1.8% (v/v) and 2.5% (v/v), respectively), since the IC_{50} values of the same order of magnitude were obtained for the solution of Transfectam (7) in these solvents. Due to the toxicity of the DMF used to dissolve 1 in aqueous solution, cell viability relative to the control experiment at $R > 21$ became so small (1–3%) that the data for $R > 21$ is rather unreliable.

The transfection process with the fullerene 1 (brown) could be directly observed with a microscope. Figure 7A is a differential interference contrast (DIC) micrograph taken 1 h after the addition of an aqueous DMF solution of the 1/DNA complex to COS-1 cells from which most of the solvent

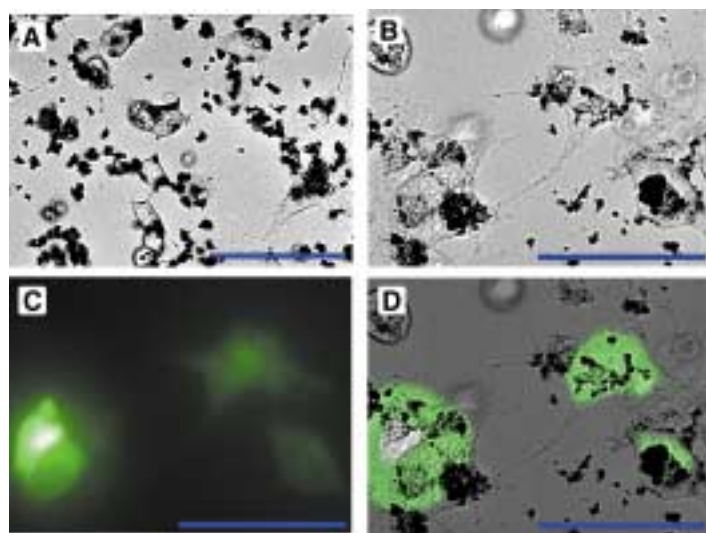


Figure 7. Transfection of the GFP plasmid into COS-1 cells by using 1. Inset blue bars show 100 µm. A) DIC micrograph of COS-1 cells and small particles (2–10 µm) of the fullerene/DNA complex after 1 h transfection time; B) DIC micrograph of COS-1 cells and taken-up fullerene/DNA complex after 2 days incubation time; C) fluorescence micrograph of (B); D) superimposed photograph of (B) and (C), the round black clumps of 2–10 µm observed within the fluorescent cells (as in the one center right) contain fullerene material(s).

has been removed (this experiment generated the data presented in Figure 5). The micrograph shows a plate surface on which there are several cells and many black spots of the fullerene/DNA complex. These black spots, of irregular outline, are more densely distributed on the cells than on the bare plate surface. Figures 7B and C are the DIC and fluorescence micrographs of the cells taken in the same series of experiments after 2 days incubation with **1**. As seen more clearly in Figure 7D, which is the superimposition of Figure 7B and 7C, the morphology and the distribution of the black objects in the fluorescent cells are different from those of the objects outside the cells. These black globules of 2–10 μm in diameter are located within the cytoplasm of the fluorescent cells, indicating that the fullerene/DNA complexes have been taken into the cells during incubation.

Departing from the conventional lipid-mimicking strategy for designing transfection reagents, we have synthesized an entirely new class of transfection agent (**1**) through tailor-made functionalization of [60]fullerene. The structure/activity relationship of the DNA binding properties indicate that the structural synergy in **1** plays an important role in binding. The efficiency of gene transfer by this first-generation fullerene-based transfection reagent is already comparable to that of commercial reagents. The morphology of large aggregates of the fullerene/DNA complex in the GFP-expressing cells indicates that the complex is taken up by cells through phagocytosis. The fullerene-based transfection reagent is unique among known transfection reagents in its ability to effect sustained protein expression for a prolonged period, suggesting that plasmid DNA in fullerene complex is stable inside phagosomes.

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Ternary Complexes Between DNA, Polyamine, and Cucurbituril: A Modular Approach to DNA-Binding Molecules**

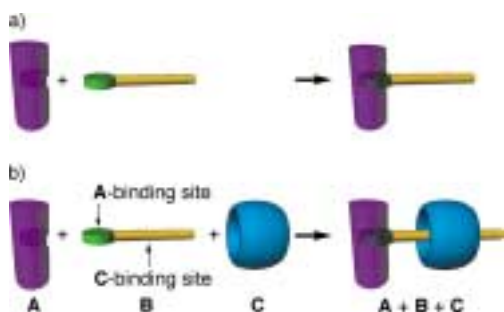
Hiroyuki Isobe, Naoki Tomita, Jae Wook Lee, Hee-Joon Kim, Kimoon Kim,* and Eiichi Nakamura*

The design of a rational method for the delivery of a molecule to a target site of a biomolecule is an issue of general interest for chemists. Although a conventional targeting scheme relies on the covalent installation of a binding site on the molecule to be delivered (Scheme 1a), another intriguing approach is a modular strategy relying on a non-covalent many-body assembly of molecules as illustrated in Scheme 1b.^[1] We report herein a chemical prototype of such supramolecular chemistry in the binding of a small molecule (**C**, that can not bind to DNA) to DNA (**A**) with the aid of a

[*] Prof. Dr. E. Nakamura, Dr. H. Isobe, N. Tomita
Department of Chemistry
The University of Tokyo
Hongo, Bunkyo-ku, Tokyo 113-0033 (Japan)
Fax: (+81)3-5800-6889
E-mail: nakamura@chem.s.u-tokyo.ac.jp

Prof. Dr. K. Kim, Dr. J. W. Lee, Dr. H.-J. Kim
Department of Chemistry
Division of Molecular and Life Sciences and the National Creative Research Initiative Center for Smart Supramolecules (CSS)
Pohang University of Science and Technology (POSTECH)
Pohang 790-784 (South Korea)
Fax: (+82)54-279-8129
E-mail: kkim@postech.ac.kr

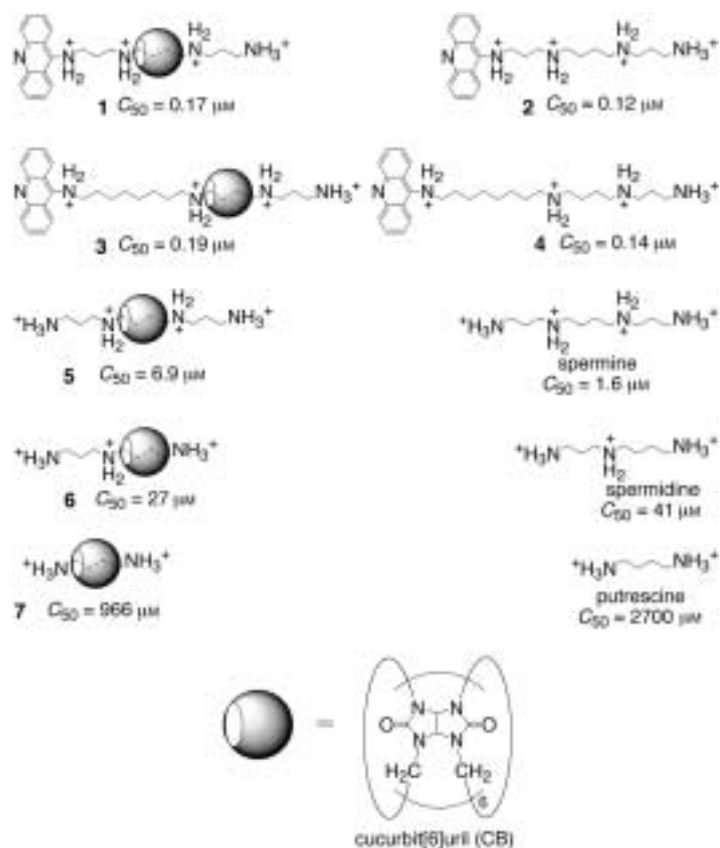
[**] We thank Prof. B. C. Baguley for the detailed information on the ethidium displacement assay. This work was supported by a Grant-in-Aid for Scientific Research from Monbusho and the Creative Research Initiative Program of the Korean Ministry of Science and Technology.



Scheme 1. a) Conventional targeting scheme. b) Modular strategy: Molecule **C**, which cannot bind to the target molecule **A**, can bind with the aid of the linker molecule **B**.

linker molecule (**B**) that has two different types of binding sites. The $A + B + C$ strategy relies on non-covalent assembly between DNA and acridine, and between spermine and cucurbit[6]uril (CB).

CB is a large-cage compound composed of six glycoluril units interconnected with twelve methylene bridges. First prepared nearly 100 years ago,^[2] this cyclic molecule has been attracting much attention because of its unique ability to tightly bind to diamines through the formation of a pseudo-rotaxane structure such as **5**.^[3] To test our targeting scheme we



designed linker molecules **2** and **4**.^[4] These bifunctional linkers are composed of a spermine moiety attached covalently to an acridine unit. Since acridine binds specifically to double stranded DNA between the nucleobases by intercalation, and spermine binds specifically to CB in a thread-in-

hole fashion, we expected that the acridine/spermine hybrids **2** and **4** would chemospecifically link DNA and CB at the respective binding sites. The known CB/polyamine binary complexes **5–7**^[3] were employed as reference compounds and were prepared by simply mixing CB with the respective polyamines in an aqueous solution. The acridine/spermine hybrids **1** and **3** were prepared from the linkers **2** and **4** in the same manner.

The binding ability of a DNA intercalator can be assessed through competition with ethidium bromide, and is shown in a value known as C_{50} .^[5] The C_{50} values in the binding with calf thymus DNA for the parent aminoacridine compounds **2** and **4** were determined to be 0.12 and 0.14 μM , respectively. The binary complexes **1** and **3** were found to be slightly less tightly binding with $C_{50} = 0.17$ and 0.19 μM , respectively.^[6, 7]

For comparison, we also examined the binding properties of CB/polyamine binary complexes **5**, **6**, and **7** that lack the acridine moiety.^[8] The C_{50} values were found to be 6.9, 27, and 966 μM , respectively. CB itself was unable to induce 50 % displacement even on the addition of a large excess of the reagent (11 % displacement at 255 μM).^[9] These values for the binary complexes as well as the C_{50} values for the parent polyamines (1.6, 41, and 2700 μM) indicate the following: 1) CB binds DNA extremely weakly if at all. 2) The low binding ability of the diamine complex **7** suggests that the CB/diamine part in **1**, **3**, **5**, and **6** does not contribute very much toward DNA binding. 3) The DNA binding ability increases in the order of $7 > 6 > 5$ suggesting that the presence of the free ammonium group(s) increases DNA binding. 4) In general, CB may attenuate the binding properties of spermine (and its analogues) as suggested from the data for **1**, **3**, and **5**.

The small C_{50} values obtained for **1** and **3** at μM concentration, however, do not necessarily guarantee the high binding power of these complexes, the polyamine moiety could have slipped out of the CB and become bound by itself to DNA and thus give the small C_{50} values. If a free polyamine were indeed a major contributor to the observed ethidium displacement with **1**, **3**, and **5–7**, addition of excess free CB to the test mixture would reduce the concentration of the free polyamine and hence increase the observed C_{50} value. However, essentially the same C_{50} data ($\pm 20\%$, for **1**, **3**, and **5–7**) were obtained in the presence of a large excess of free CB (20 μM). Thus, the contribution of the free polyamine must be negligible under these conditions.^[10]

Gel electrophoresis experiments (Figures 1 a and b) afforded conclusive evidence that the binary complex **1** binds tightly to the DNA duplex to form the expected **1**/DNA ternary complex (not the **2**/DNA complex). Thus, **1** was incubated with DNA duplex (studied simultaneously for three representative topologies, supercoiled (*S*), circular (*C*), and linear (*L*)) and the mobility of the resulting intercalated DNA was examined. As shown in Figure 1 a (lanes 2 and 3), the DNAs migrated as single bands and the degree of retardation differed between **1** and **2**,^[11, 12] clearly indicating that the **1**/DNA complex is different from the **2**/DNA complex and hence that **1** maintains its integrity upon binding to DNA.

Further evidence of ternary complex formation was obtained in the experiments where the effect of CB–polyamine complexation was gauged by the rate of nuclease-induced

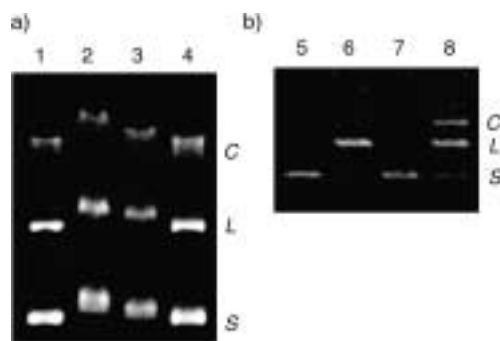


Figure 1. a) Change of gel mobility caused by complexation of DNA duplexes to **1** and **2**. Lanes 1 and 4: DNA, lane 2: DNA + **2**, lane 3: DNA + **1**. b) Retardation of enzymatic DNA hydrolysis by **1** and **2**. Lane 5: intact plasmid DNA pBR322 (S); lane 6: complete cleavage of S to L with *Ban* II restriction enzyme; lane 7: complete inhibition of the DNA cleavage by prior treatment of pBR322 with **2**; lane 8: partial inhibition of the DNA cleavage by prior treatment with **1** leading to production of only 72% of L, 26% of C (because of single strand cleavage) and 2% of S left unchanged. S = supercoiled, C = circular, and L = linear DNA.

hydrolysis of DNA strands. For this purpose, we employed the restriction enzyme *Ban* II that cuts plasmid DNA pBR322 at specific sites,^[13] the results are shown in Figure 1 b. When we allowed excess acridine **2** to intercalate to the supercoiled DNA (reagent/base pair (bp) ratio, $R=2.6$; lane 5), we observed complete inhibition of the nuclease action (lane 7) under the conditions where, without **2**, the DNA was completely hydrolyzed to linear DNA (lane 6). When we performed the same experiment but with **2** replaced by the same amount of **1** ($R=2.6$), we found considerable retardation of the strand cleavage.^[14] Thus, the C_{50} data, the gel mobility shift assay, and the nuclease inhibition experiments have shown that the acridine/spermine linker module can effectively deliver CB to the target DNA through ternary supramolecule as illustrated in Scheme 1 b. Further studies on the sequence preference of ternary complex formation and the effect of CB complexation on the polyamine activities are ongoing.

Experimental Section

Ethidium bromide displacement assay:^[5] The displacement assay was carried out in a buffer containing 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES; 2 mM), ethylenediaminetetraacetate (EDTA; 10 μ M), and NaCl (9.4 mM), pH 7.0. Ethidium bromide was dissolved (1.26 μ M) in the buffer and a solution of calf thymus DNA (Sigma) was added to provide a base pair concentration of 0.5 μ M. A reagent solution (0.1–1.0 mM) was added in microliter portions. Fluorescence emission was measured at 595 nm (slit width 0.75 nm) with excitation at 546 nm on a Hitachi 650-40 spectrometer at 25 °C. The final reagent concentration that induced 50% reduction of the original fluorescence intensity (based on more than three runs) was taken as the C_{50} value of the reagent. Experimental error under these conditions is $\pm 26\%$.

Change of gel mobility caused by complexation of DNA duplexes: Gel mobility shift assay was carried out on agarose gel for pBR322 partially digested with *Sph* I (a mixture of S, C, and L DNA, [bp] = 38 μ M). A mixture of S, C, and L was incubated with **1** or **2** at $R=0.5$ in a HEPES–Mg buffer (40 mM HEPES–KOH and 10 mM $MgCl_2$; pH 7.0) for 1 h. Electrophoresis was performed by using 1% agarose gel and the DNA bands are subsequently visualized by ethidium bromide (Figure 1 a).

Retardation of enzymatic DNA hydrolysis: Plasmid pBR322 DNA ([bp] = 38 μ M) was incubated with **1** or **2** (at $R=2.6$) and *Ban* II restriction enzyme

(0.02 U μ L⁻¹; TaKaRa) in an H buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 100 mM NaCl; pH 7.5) at 37 °C for 1 h. After purification of DNA through phenol treatment and ethanol precipitation, the reaction mixture was analyzed by agarose gel electrophoresis (Figure 1 b).

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- [6] In DNA/9-aminoacridine intercalation complexes, the amine residue of the 9-aminoacridine is located in the minor groove, and the binding constant value has been reported as $K=4 \times 10^5$ M⁻¹; see ref. [5], and references therein.
- [7] The quenching values (ref. [5]) of intercalative compounds (**1–4**) were found to be small ($Q_{0.1}$ values (percentage quenching of ethidium fluorescence at $R=0$): 1.3% for **1**, 1.6% for **2**, 1.0% for **3**, and 1.0% for **4**), which combined with the small C_{50} values, suggests that the binding constants of these compounds are larger than that of ethidium bromide ($K=3 \times 10^6$ M⁻¹).
- [8] Unlike the acridine/ethidium competition, the competition between spermine (groove binder) and ethidium (intercalator) is not a true competitive experiment because their binding sites are different. Nonetheless, C_{50} values for groove binders (such as spermine, $K=1 \times 10^6$ M⁻¹) have widely been taken as a qualitative measure of DNA binding ability, but their magnitude cannot be directly compared with those for intercalator (such as **1**): K. D. Stewart, T. A. Gray, *J. Phys. Org. Chem.* **1992**, *5*, 461–466.
- [9] The experiments with CB were carried out with an aqueous solution of CB (447 μ M) in an aqueous 10 mM Na_2SO_4 .
- [10] The reported dissociation constant for the spermine/CB complex **5** (7.6×10^{-8} M) was measured in a H_2O/HCO_2H (hence acidic) solution under competition between spermine and a weaker amine (ref. [3]). The present results suggest that the dissociation constant under the buffer conditions is much smaller. This point may need further studies. We thank a referee for pointing out the issues concerning the dissociation constants.
- [11] The mobility of the **1**/DNA and the **2**/DNA complexes differ from each other over a wide range of the reagent/base pair ratio ($R=0.1–1.5$). The same degree of difference in the gel mobility shift was also detected for the pair **3** and **4** (data not shown).
- [12] To ascertain that the binding of **1–4** to DNA observed under electrophoretic conditions is caused by acridine intercalation and not to the spermine/DNA interaction, spermine and its CB complex **5** were examined under the same gel electrophoresis conditions. The spermine/DNA or **5**/DNA complexes showed the same mobility as the intact DNA (data not shown), indicating that these positively charged

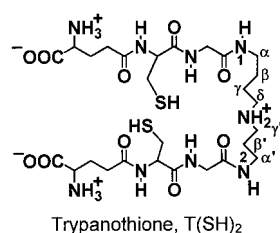
polyamine binders were removed from the negatively charged DNA duplex under the applied electric field used for electrophoresis.

- [13] Restriction enzyme *Ban* II cuts pBR322 at a GA(G)GCT(C)C sequence (locations 471 and 485).
- [14] A referee suggested that the observed retardation of hydrolysis could have been caused by a small amount of free amine **2** liberated from **1** (used in a 100 μ M concentration), such an equilibrium would liberate **2** in a 2.7 μ M or less concentration (ref. [10]). A series of experiments with various concentrations of **2** indicated that no inhibition of the nuclease activity occurs with such a small concentration of **2**, indicating in turn that the free amine is not responsible for the observed retardation with **1**.

Complexation of Antimony(III) by Trypanothione**

Siucheng Yan, Keyang Ding, Li Zhang, and Hongzhe Sun*

In contrast to almost all other organisms, trypanosomatids can conjugate the sulfur-containing tripeptide glutathione and the polyamine spermidine to form trypanothione (N^1, N^8 -bis(glutathionyl)spermidine).^[1–3] Together with trypanothione reductase (TR), the dithiol form of trypanothione ($T(SH)_2$), which is an analogue of glutathione found in the mammalian host, provides a uniquely intracellular reducing environment in parasites. Besides its protective and regulatory roles, $T(SH)_2$ and its related enzymes are essential for growth and survival of these parasites.^[4–6] The trypanosomatid protozoan



parasite *Leishmania* is the causative of kala azar and severe forms of leishmaniasis that afflict more than ten million people worldwide. Several antimony compounds are currently the agents of choice for the treatment of leishmaniasis and have been used for decades. Analogous to arsenical drugs against African trypanosomes,^[7] one of the major targets for

antimony drugs is probably $T(SH)_2$. Overproduction of $T(SH)_2$ in cells is also related to resistance to antimonials in *Leishmania*.^[8] We show here that Sb^{III} forms a novel complex with $T(SH)_2$ by binding to two sulfur atoms and to the oxygen atom of a water molecule. This appears to be the first chemical characterization of a metal complex of $T(SH)_2$, which may play a role in the mechanism of action of the antimony antileishmanial drugs.

Trypanothione $T(SH)_2$ can be readily obtained by the reduction of trypanothione disulfide (TS_2) by tris(2-carboxyethyl)phosphane.^[8] The electrospray (ESI) mass spectrum of $T(SH)_2$ at around pH 7 exhibits a cluster of ions at m/z 724.9 corresponding to $C_{27}H_{50}N_9O_{10}S_2$ (calcd 724.3). The reaction product of an antimony compound and $T(SH)_2$ at pH 7.4 displayed a prominent cluster of ions related to the isotopic distribution of Sb and C, with an $[M^+]$ peak at m/z 842.6 corresponding to $SbS_2C_{27}H_{50}N_9O_{10}H_{48}$ (calcd for $SbT(S)_2$ complex: 843.2; see Figure S1 in the Supporting Information). This represents a 1:1 antimony(III) trypanothione complex, in contrast to the antimony(III) glutathione complex $Sb(GS)_3$.^[9]

The ^1H NMR signals of $T(SH)_2$ in aqueous solution were assigned with the aid of various 2D TOCSY, NOESY, and ROESY spectra. Signals from pairs of like residues overlap, with only minor differences attributable to the actual asymmetry of the spermidine moiety. This indicates an approximate symmetry of the two halves of $T(SH)_2$ that is similar to that of its oxidized form.^[10] Only four relatively sharp NH peaks and one very broad peak were observed at pH 4.6, and the very broad one disappeared above pH 5. The NH peaks of both Cys and Gly are significantly broadened at pH 7.4 (Figure 1). Addition of 0.5 of a molar equivalent of antimony(III) tartrate at pH 4.6 resulted in a peak at $\delta = 3.36$, while the signal of the β protons of the Cys residues of free

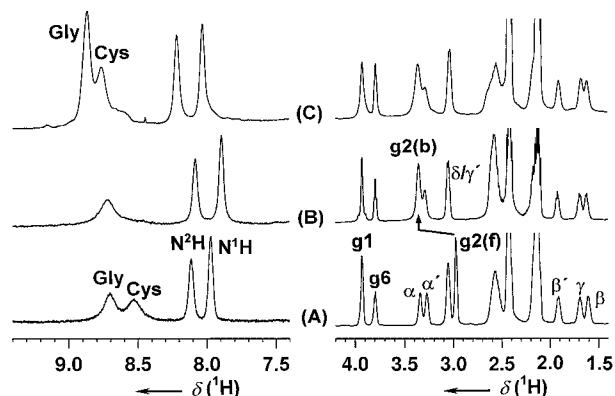


Figure 1. 500 MHz ^1H NMR spectra. A) Trypanothione $T(SH)_2$. B) $T(SH)_2$ after addition of antimony(III) tartrate (molar ratio 1:1) at 298 K and pH 7.4; C) as B) but at 278 K. Note the large low-field shifts of the peaks for Cys β -CH₂ (g2) on complexation to Sb^{III} . Labels: g1, α -CH₂ of Gly; g6, α -CH₂ of Glu.

$T(SH)_2$ at $\delta = 2.97$ decreased in intensity. Similarly, the NH signal of the Cys residue of $T(SH)_2$ at $\delta = 8.54$ decreased in intensity, while a new peak appeared at slightly lower field ($\delta = 8.59$). A 2D TOCSY spectrum confirmed that the peaks at $\delta = 4.52$, 2.97, and 8.54 are coupled to each other, as are those at $\delta = 4.68$, 3.36, and 8.59. The former set can therefore

[*] Dr. H. Sun, S. Yan, Dr. K. Ding, L. Zhang
Department of Chemistry, University of Hong Kong
Pokfulam Road, Hong Kong (P. R. China)
Fax: (+852) 2857-1586
E-mail: hsun@hkucc.hku.hk
Dr. K. Ding
Guangzhou Institute of Chemistry
The Chinese Academy of Sciences
Guangzhou (China)

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be assigned to the α -, β -, and NH protons of the Cys residues of T(SH)₂, and the latter to protons of the Cys residues of Sb^{III}-bound trypanothione. The 2D exchange spectrum (EXSY, mixing time 400 ms) showed cross-peaks between free and bound forms of both the α -CH and β -CH₂ groups of the Cys residues of T(SH)₂ at $\delta = 4.68/4.52$ and $3.36/2.97$, respectively (data not shown). This suggested that the free and bound forms were in the slow exchange on the ¹H NMR time scale at pH 4.6. A cross-peak at $\delta = 4.70/4.34$ was also observed due to the exchange between free and bound forms of tartrate.

After the addition of a total of 1.0 molar equivalent of Sb^{III}, the signals of the Cys residues of the bound form further increased in intensity, and less than 20% of the free form remained. The latter totally disappeared when the pH of the solution was higher than 5.6, and the solution was kept at pH 7.4 thereafter. No further shifts in the proton signals of trypanothione were observed when more Sb^{III} was added, and this suggests formation of a stable 1:1 SbT(S)₂ complex, in good agreement with the ESI-MS data. The large shifts of the β and α protons of Cys residues ($\Delta\delta = 0.40$ and 0.13 , respectively; Figure 1 and Table S1 in the Supporting Information) indicated that sulfur atoms are the predominant binding sites for Sb^{III}. Surprisingly, the ¹H chemical shifts of N¹H and N²H are shifted to low frequency by $\Delta\delta \approx 0.08$ and 0.03 , respectively. Shifts for the remaining protons are minor. The signals of the amino protons of Cys were too broad to be observed at 298 K, but reappeared when the temperature was lowered to 278 K (Figure 1).

The 2D ROESY experiments (mixing time 200 ms) were performed at 298 and 278 K to elucidate the structure of the complex in solution. The important results are summarized below. No amide–amide NOEs were observed, but the cross-peaks for sequence assignment were observed clearly. Unexpectedly a cross-peak between N¹H and α' -CH₂ was observed (Figure 2), which suggested the distance between these two protons is less than 5 Å. Similarly a cross-peak between γ -CH₂ and γ' -CH₂ was also observed. Surprisingly, a variety of protons have cross-peaks with water, particularly those of the hydrophobic part of spermidine, the α - and β -

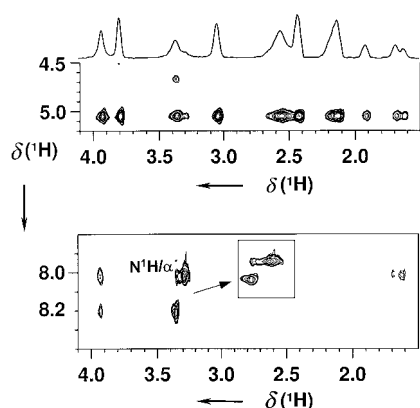


Figure 2. Expansion of the 200 ms 2D ROESY NMR spectra of SbT(S)₂ at 278 K (pH 7.4). A variety of cross-peaks were observed between water protons and the hydrophobic part of spermidine, the α - and β -protons of Cys, and the α -protons of Gly (top); a section through the 2D ROESY spectrum at the chemical shift of water is plotted above the spectrum. A weak long-range rOe between N¹H and α' -CH₂ was clearly observed (bottom).

protons of Cys, and the α -protons of Gly (Figure 2). This clearly demonstrated that the oxygen atom of a water molecule is bound to Sb^{III}, and strong hydrogen bonding of the water molecule to the carboxyl oxygen atom of Gly may account for the changes in the chemical shift of N¹H of spermidine after formation of the complex. The distances between the water protons and neighboring protons are probably around 5 Å.

Figure 3 shows a model of SbT(S)₂ which is consistent with our experimental results. For comparison, the structure of trypanothione disulfide in a TS₂ complex with trypanothione



Figure 3. A) Structure for SbT(S)₂ which is consistent with the observed NMR rOe; B) crystal structure of the disulfide form of trypanothione from a complex of trypanothione reductase and trypanothione.^[11] All hydrogen atoms are omitted for clarity. C, green; N, blue; O, red; S, yellow; Sb, purple.

reductase (TR)^[11] is also shown (Figure 3B). The Sb^{III} ion is strongly coordinated to the two sulfur atoms of T(SH)₂ (Figure 3A), and the oxygen atom of a water molecule completes the coordination sphere. The presence of an Sb^{III}-bound water molecule is necessary, since the distance between N¹H and α' -CH₂ is otherwise greater than 8 Å, as is that between γ -CH₂ and γ' -CH₂. It is known that the coordination number of Sb^{III} is normally at least three.^[12]

The strong binding of Sb^{III} to T(SH)₂ suggested that T(SH)₂ could protect trypanothione reductase from antimony drugs within whole cells under the conditions used in this work, and this is in contrast to a previous report.^[13] The inhibition of TR by Sb^{III} is probably also due to the formation of a covalent bond with the active-site cysteine residues of TR after its reduction by NADPH. Although it is thermodynamically stable, the complex SbT(S)₂ is kinetically labile under the conditions used. The exchange of Sb^{III} between free and bound forms of T(SH)₂ implies that Sb^{III} is transported by a thiolate “pool”, and Sb–TR complex could readily be formed even in the presence of high concentration of T(SH)₂. Biological investigations of SbT(S)₂ and its inhibiting action on TR are therefore warranted.

Experimental Section

Oxidized trypanothione was purchased from Bachem, and tris(2-carboxy-ethyl)phosphane and antimony tartrate from Aldrich.

¹H NMR spectra were acquired on Bruker DRX500 spectrometer operating at 500.013 MHz. All of the samples (5 mm) for NMR were in H₂O/D₂O (90/10), and several mixing times were chosen in the range of 20–75 ms (2D TOCSY), 200–400 ms (NOESY), and 200 ms (ROESY). The resonance signal for water was suppressed by the WATERGATE pulsed-field-gradient sequence.^[14] Data acquisition and processing conditions have been described previously.^[9, 15]

pH measurements were made with a Corning 440 pH meter equipped with an Aldrich micro-combination electrode calibrated with standard buffer solutions of pH 4, 7, and 10.

Electrospray mass spectrometry (ESI-MS) was performed on a LCQ spectrometer (Finnigan Corporation). The sample was infused at $3 \mu\text{L min}^{-1}$, and the ions were produced in an atmospheric-pressure ionization (API)/ESI ion source. The source temperature was 453–473 K, and the flow rate of the drying gas was 0.9 L min^{-1} . A potential of 3.5 kV was applied to the probe tip, and a cone voltage of 5–10 V over 200–2000 Da was used. The quadrupole was scanned at 100 amu s^{-1} . The mass accuracy of all measurements was within 0.5 m/z units. Data acquisition and processing were performed with the Microsoft Windows NT operating system. The mass spectrum was simulated on a PC with IsoPro 3.0.

Molecular modeling was performed by using INSIGHT II (95.0, Biosym-MSI software) with the ESFF force field under the optimized pulldown in the builder module.

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Supramolecular Modification of the Periphery of Dendrimers Resulting in Rigidity and Functionality**

Maurice W. P. L. Baars, Annika J. Karlsson, Victor Sorokin, Bas F. W. de Waal, and E. W. Meijer*

The number, size, and function of peripheral groups of dendritic macromolecules determine many of the typical dendrimer properties, such as dense-shell packing, overall shape, and multivalency.^[1] Properties related to solubility or physical state—semi-crystalline, glass, liquid crystalline, or liquid—are also strongly dependent on the nature of the dendritic end group.^[2] Finally, specific interactions of guest molecules with the dendritic hosts rely on both the core and the shell of the dendrimer.^[3–11] Most of the end-group modifications are based on covalent bonding, while the use of supramolecular interactions to obtain new dendritic peripheries is limited. Chechik and Crooks showed that ionic bonding between an amine-terminated poly(aminoamine) (PAMAM) and a fatty acid resulted in similar host–guest properties as those of the corresponding covalent amide analogues, while Tomalia and co-workers recently used ionic interactions to assemble dendrimers into higher aggregates.^[12] We anticipated that the combination of a dense packing of the shell with the possibility of tuning the functionality of the periphery is of great importance in making dendrimers that can be used as shape-persistent building blocks in nanotechnology. Herein we disclose a general methodology to modify the periphery of poly(propyleneimine) dendrimers using such a supramolecular approach. The covalently attached adamantylurea end groups of the dendrimer are used as a scaffold to reversibly bind glycylurea building blocks through strong and directional multiple interactions (Scheme 1).

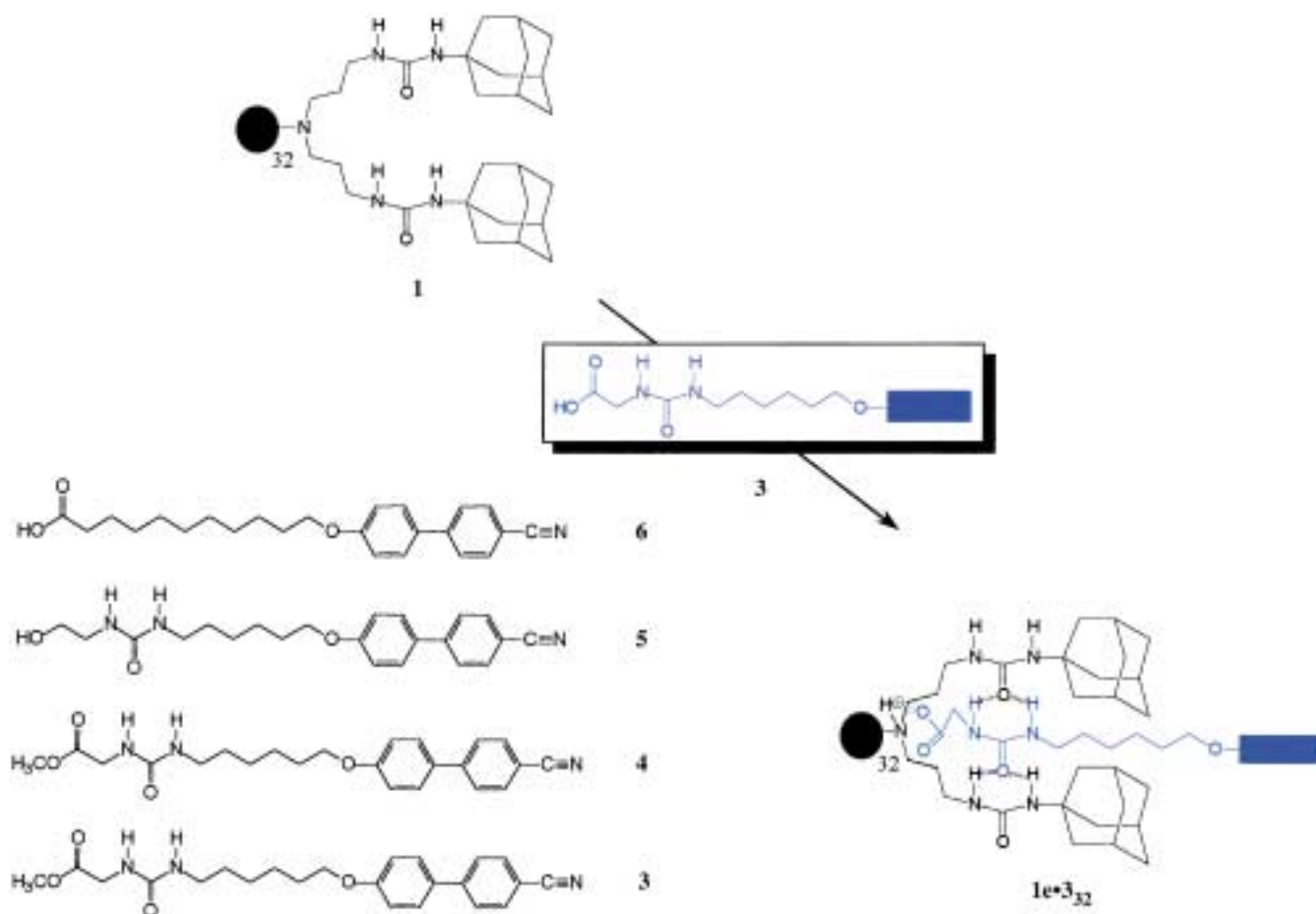
The design of the modification is given in Scheme 1 and the scaffold is based upon DAB-dendr-(NHCONH-Ad)_n (**1**, with $n = 4, 8, 16, 32$, and 64 for **1a–e**, respectively). These dendrimers were selected after studying DAB-dendr-(NHCO-Ad)_n (**2a–e**), DAB-dendr-(NHCONH-C₁₂H₂₅)_n, and DAB-dendr-(NHCO-C₁₂H₂₅)_n as well. All dendrimers were synthesized in quantitative yield from DAB-dendr-(NH₂)_n and the corresponding isocyanate or acid chloride and were fully characterized.^[13a] The concept of the supramolec-

[*] Prof. Dr. E. W. Meijer, Dr. M. W. P. L. Baars, Dr. A. J. Karlsson, Dr. V. Sorokin, B. F. W. de Waal
Laboratory of Macromolecular and Organic Chemistry
Eindhoven University of Technology
P.O. Box 513, 5600 MB Eindhoven (The Netherlands)
Fax: (+31)40-2451036
E-mail: E.W.Meijer@tue.nl

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Scheme 1.

ular modification is illustrated with the glycylurea derivative **3**, while **4–6** are used to emphasize the need for multiple interactions.^[13b]

Compound **3** is barely soluble in chloroform (0.1 mg mL⁻¹ for a saturated solution at room temperature),^[14] however, upon adding an excess of **3** to a 10 mg mL⁻¹ solution of **1e** in chloroform, most of **3** dissolves and it is evident after column chromatography on Biobeads that 32 molecules of **3** are assembled around one dendrimer molecule **1e**. ¹H NMR and IR spectroscopy were used to elucidate the structure of the complex (Figure 1).^[15]

A comparison of the ¹H NMR spectrum of **1e** (Figure 1a) with that of **1e·3**₃₂ (Figure 1c) shows there is a downfield shift from $\delta = 6.19$ and 5.44 (with an integral ratio of 1:1) to $\delta = 6.35$ and 5.70 (with an integral ratio of 2:1) for the two signals corresponding to the urea hydrogen atoms, while the methylene groups adjacent to the tertiary amines of the outermost shell are shifted from $\delta = 2.37$ to 2.83 as a result of the protonation of the adjacent tertiary amine. These shifts are in full agreement with the mechanism suggested in Scheme 1. The presence of adhered **3** in the mixture is discernable in the ¹H NMR spectrum before column chromatography on Biobeads (Figure 1b), but this is removed easily. Repeated chromatography does not decrease the number of molecules that are selectively assembled, which is illustrative of a high association constant (see below). The increased number of hydrogen-bonding interactions arising from the presence of

the urea unit within the complex is also evident from IR spectroscopy, while there is no evidence in the spectra of nonbonded hydrogen atoms in **1e**, **1e·3**₃₂, and **4** (Figure 2).

Similar experiments were performed with the other generations of **1** and in all cases a 1:1 ratio of scaffold and clicking molecule **3** was observed. Dendrimer **2** with a bis(propylamide) pincer was also able to assemble with **3**, again using a 1:1 ratio of scaffold and **3**. Although the dodecyl-modified dendrimers with either the amide or urea linkage are able to assemble with compound **3** as well, the strong intramolecular hydrogen-bonding character of the native dendrimers limits their use. It is a prerequisite, however, to have both the carboxylic acid and the urea functionality present in the molecule to be attached; all three other molecules (**4–6**) as well as **1e** are obtained as pure compounds after chromatography on Biobeads when **4–6** and **1e** are mixed in a similar way as **3** and **1e**.

Unfortunately, the low solubility of **3** in chloroform hampers a detailed determination of the association constant at different ratios and generations of **1**.^[15] However, an estimate of $K_{\text{ass}} = 10^5 \text{ M}^{-1}$ in chloroform was made by using bis(propylurea)methylamine as a model compound for complexing with **3**.^[16] Competition experiments were performed to analyze the cooperativity and the difference in the strength of the binding between **1e** and **2e**. The ¹H NMR spectra of a series of competition experiments using 1–32 moles of **3** for every mole of **1e** and **2e** showed that when **3:1e** is less than

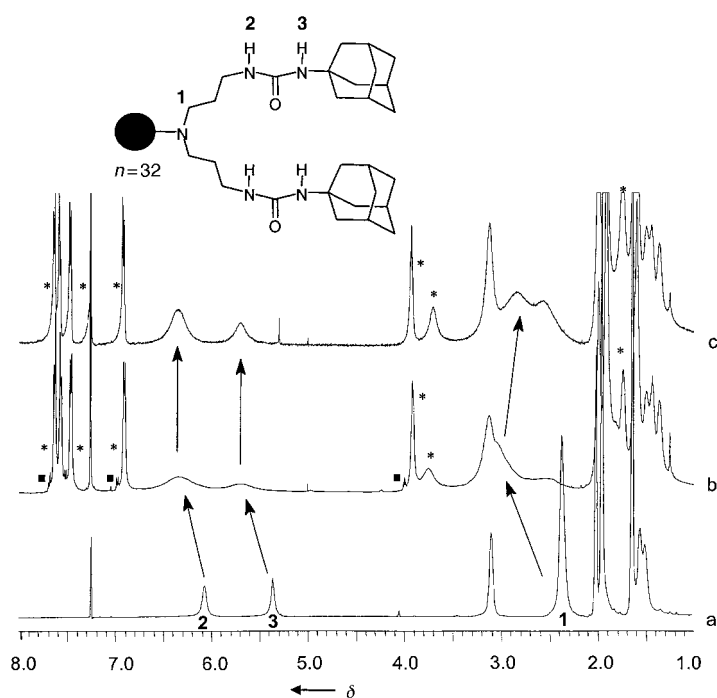


Figure 1. Top: ¹H NMR spectra (obtained at 500 MHz) of 1 mM solutions of a) **1e**, b) **1e+3_n** before column chromatography on Biobeads, and c) **1e+3₃₂** after column chromatography on Biobeads at 25 ± 0.5 °C in CDCl₃. Bottom: The shift of the methylene protons adjacent to the tertiary amines of the outermost shell (1) and of the urea hydrogen atoms (2 and 3) with an increasing number of molecules attached to **1e**.

16:1 all of **3** is bound to **1e**. When the guest:host ratio is 32:1, 24 molecules of **3** are bound to each molecule of **1e** and 8 to **2e**. These experiments show both the better fit of **3** with **1**, as well as the nonlinear association behavior.

NMR relaxation and NOE experiments in CDCl₃ solutions were performed to investigate the idea that the shell of the dendrimer should become denser as a consequence of the attachment of **3** and to investigate the location of the molecules attached. Some overlapping of the signals occurred in the ¹H NMR spectrum of the complex **1e+3₃₂** in CDCl₃, so the solution structures were further investigated for the

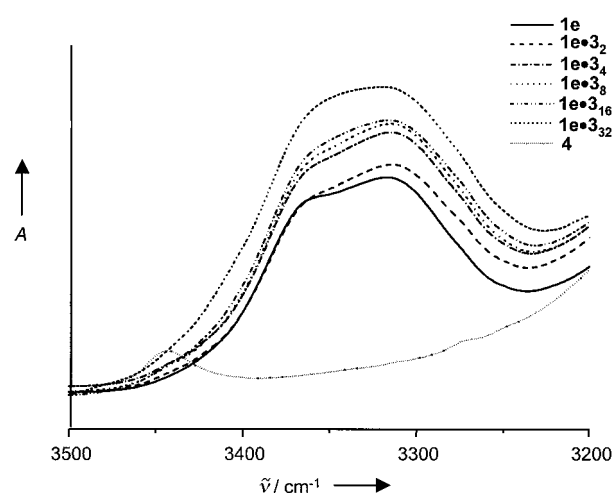


Figure 2. Partial IR spectra (showing N-H stretch vibrations) obtained from 1.5 mM solutions of **1e**, **1e+3₃₂**, and **4** in CH₂Cl₂ at 25 °C.

complex **2e+3₃₂**. The ¹H-¹H NOESY spectra (Figure 3) of the complex **2e+3₃₂** in CDCl₃ recorded at 25 °C showed clear NOE interactions as indicated.

We found that the *T*₁ relaxation times for **3** in compounds **1e+3_n** (*n* = 2, 4, 8, 12, 16, and 32) decrease upon increasing the number *n* of molecules attached (Figure 4). A decrease in the *T*₁ relaxation time is indicative of a decrease in the molecular motion of **3**. Moreover, an increase in relaxation times is observed for the atoms in the dendritic shell. Such a behavior in *T*₁ is indicative of a decrease in molecular motion, that is, an

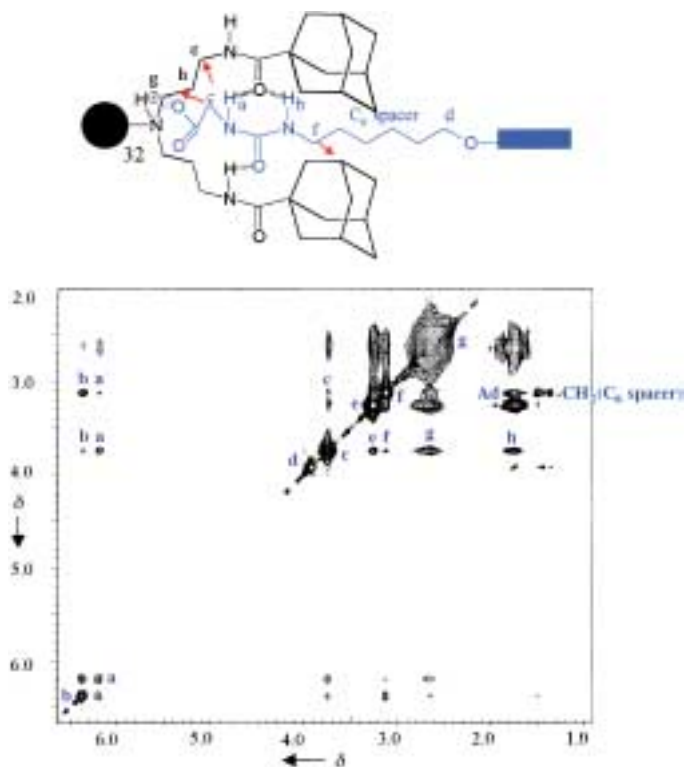


Figure 3. ¹H-¹H NOESY spectra (obtained at 500 MHz) of the complex **2e+3₃₂** in CDCl₃ recorded at 25 ± 0.5 °C.

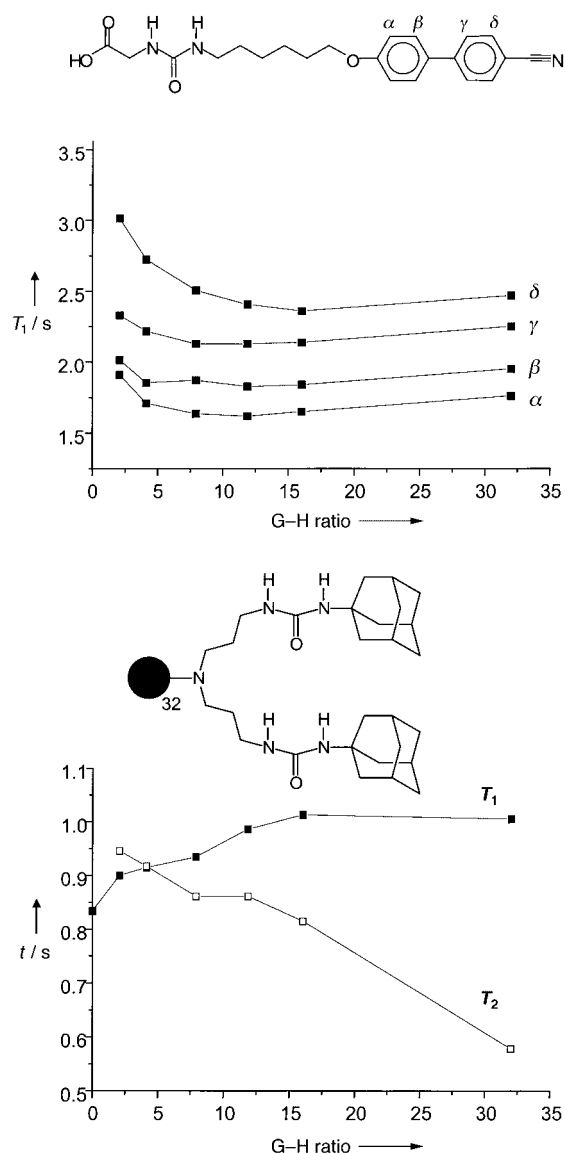


Figure 4. T_1 and T_2 ^1H NMR relaxation data versus guest–host ratio for atoms α , β , γ , δ (top), and the CH_2 group (bottom) in the bispropylurea moiety of the dendrimer in complex **1e**·**3**₃₂.

almost solid-phase behavior is obtained, similar to that obtained with the dendritic box.^[17]

Furthermore, we have investigated the changes in the packing of the dendritic shell as a function of generation in compounds **1a–e**·**3**_{*n*} (**a**: *n* = 2, **b**: *n* = 4, **c**: *n* = 8, **d**: *n* = 16, and **e**: *n* = 32). The T_1 relaxation times for the atoms close to the glycylurea unit in **3** increase for higher dendrimer generations, that is, the mobility of these atoms decreases. However, the T_1 values for the atoms in the cyanobiphenyl unit remains constant, which means that the mobility of these atoms remain unperturbed. Moreover, we found that the T_1 relaxation data for the atoms in the dendritic shell increases with dendrimer generation. These results are indicative of an almost solid-phase behavior for higher generation dendrimers, and as a result dense-shell packing in solution is proposed.

This dense-shell packing of the supramolecular assemblies is, furthermore, illustrated by the physical properties of

1a–e·**3**_{*n*}. We have shown before that, when the cyanobiphenyl unit is covalently attached to the poly(propyleneimine) dendrimers by a spacer, the resulting dendrimers are liquid crystalline as a result of the flexibility of the dendrimer itself.^[18] However, the new concept yields materials that are glasses for all generations of **1a–e**·**3**_{*n*} and that start to flow at their glass transition temperatures of 45–50 °C, as determined by polarizing microscopy and differential scanning calorimetry (DSC).

In conclusion, we have presented results on a new general methodology to self-assemble end groups in a reversible way at the periphery of poly(propyleneimine) dendrimers using multiple secondary interactions. The globular shape of the dendrimer is used to confine the ionic and hydrogen-bonded interactions in the curved two-dimensional plane of the periphery. The architectures obtained show increased rigidity at the periphery of the core, while local conformational flexibility remains at the end of the molecule that is selectively bound. We foresee a great potential for this methodology, for example, in catalysis, drug delivery, and dynamic libraries, and work along these lines is in progress.

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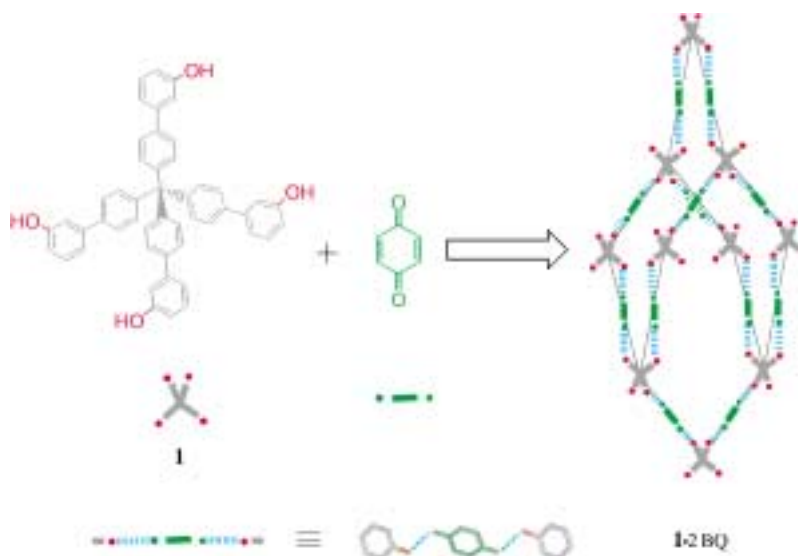
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Charge-Transfer Diamondoid Lattices: An Unprecedentedly Huge and Highly Catenating Diamondoid Network Arising from a Tetraphenol as a Tetrahedral Node and Benzoquinone as a Linear Spacer**

D. Shekhar Reddy, Takehisa Dewa, Ken Endo, and Yasuhiro Aoyama*

Diamondoid lattices have been a subject of intense recent attention in the area of crystal engineering.^[1] Organic,^[2–4] organometallic,^[5] and inorganic^[6] diamondoid materials can be obtained by connecting tetrahedral centers. Metallic or organic components of T_d symmetry as tetrahedral nodes may be assembled by covalent linkage, metal coordination, hydrogen bonding, or donor–acceptor interaction as a node connector. In particular, self-complementary multiple hydrogen bonding has been used to link tetrahedral carboxylic acid,^[2] amide (pyridone),^[3] and diaminotriazine^[7] derivatives. To the best of our knowledge, all known hydrogen-bonded organic diamondoid materials obtained so far are unimolecular species.^[2, 3] An interesting extension would be to develop multi-component organic diamondoid networks, especially from the viewpoint of functional materials design, whereas the use of linear spacers as hydrogen-bonded node connectors remains a challenge.^[8] Another point of fundamental importance is how large the superadamantane framework could be and, if the huge cavity left is to be filled by self-clathration, how high the extent of interpenetration or polycatenation would be. Here we report the crystal structure of the hydrogen-bonded 1:2 complex of tetrakis[4-(3-hydroxyphenyl)phenyl]methane (**1**) and benzoquinone (BQ). We notice the largest known (76 Å) superadamantane framework that undergoes the highest recorded (11-fold) catenation, which results in alternate donor–acceptor (phenol–quinone) charge-transfer stacking.

Slow evaporation of an ethanol solution of **1** and benzoquinone afforded brilliantly red crystals of a 1:2 adduct **1**·2BQ (Scheme 1).^[9] The crystal packing of this adduct reveals a hydrogen-bonded ($O\cdots O$ bond length 2.84 Å) phenol–quinone–phenol triad with a phenol–quinone interplanar



Scheme 1. Formation of a hydrogen-bonded superadamantane framework from **1** and benzoquinone. The benzene rings, quinone guests, and OH groups are in gray, green, and red, respectively, and hydrogen bonds are shown in blue.

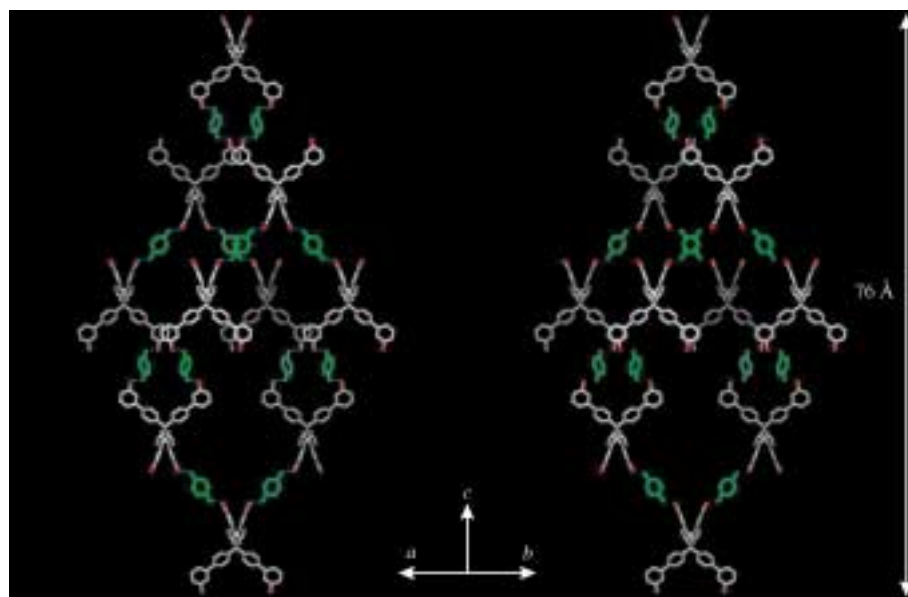


Figure 1. Stereoview of the hydrogen-bonded superadamantane framework in the crystal structure of the adduct **1**·2BQ. The colors have the same meanings as in Scheme 1.

[*] Prof. Y. Aoyama, Dr. D. S. Reddy, Dr. T. Dewa, Dr. K. Endo
CREST, Japan Science and Technology Corporation
Institute for Fundamental Research in Organic Chemistry
Kyushu University
Hakozaki, Fukuoka 812-8581 (Japan)
Fax: (+81) 92-642-2719
E-mail: aoyamay@ms.ifoc.kyushu-u.ac.jp

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angle of 55.6° (Scheme 1). Compound **1** is linked with four quinone molecules in this way. This arrangement leads to an infinite hydrogen-bonded diamondoid network with the tetraphenol **1** as a tetrahedral node and the quinone as a spacer. The resultant superadamantane framework with a tetragonal elongation, as in the case of methanetetraacetic acid,^[2b] is illustrated schematically in Scheme 1 and its actual crystal structure is shown in Figure 1. It is composed of 22 organic molecules (10 of **1** and 12 of quinone) linked together

by 24 single O–H...O hydrogen bonds and represents the largest superadamantane lattice known with a top-to-bottom length of 76 Å; the adjoining tetrahedral nodes are separated by 23 Å. The cavity is filled by self-clathration along the tetragonal *c* axis to give an 11-fold nonconnected interpenetration (catenation) of the networks separated from each other by 6.9 Å, as illustrated in Figure 2, where only one quinone column is shown for clarity. The observed 11-fold catenation is the highest degree of interpenetration known. The 9-fold catenation of [Ag(4,4'-biphenyldicarbonitrile)₂]⁺PF₆[−] was the highest previously recorded.^[10]

A set of polycatenated **1**–quinone–**1** arrays is shown in Figure 3, together with its plan view. Thus, the acceptor (quinone) column is surrounded by the framework of four donor (phenol) columns along its *c* axis (Figure 3b). In the direction perpendicular to this axis, there is alternate donor–acceptor stacking (Figure 3a) roughly of the face-to-face type (the shortest interatomic distance between the phenol and quinone units is ca. 3.3 Å, the interplanar distance is ca. 4.5 Å, and the interplanar angle 30°), which might be the origin of charge-transfer coloration.

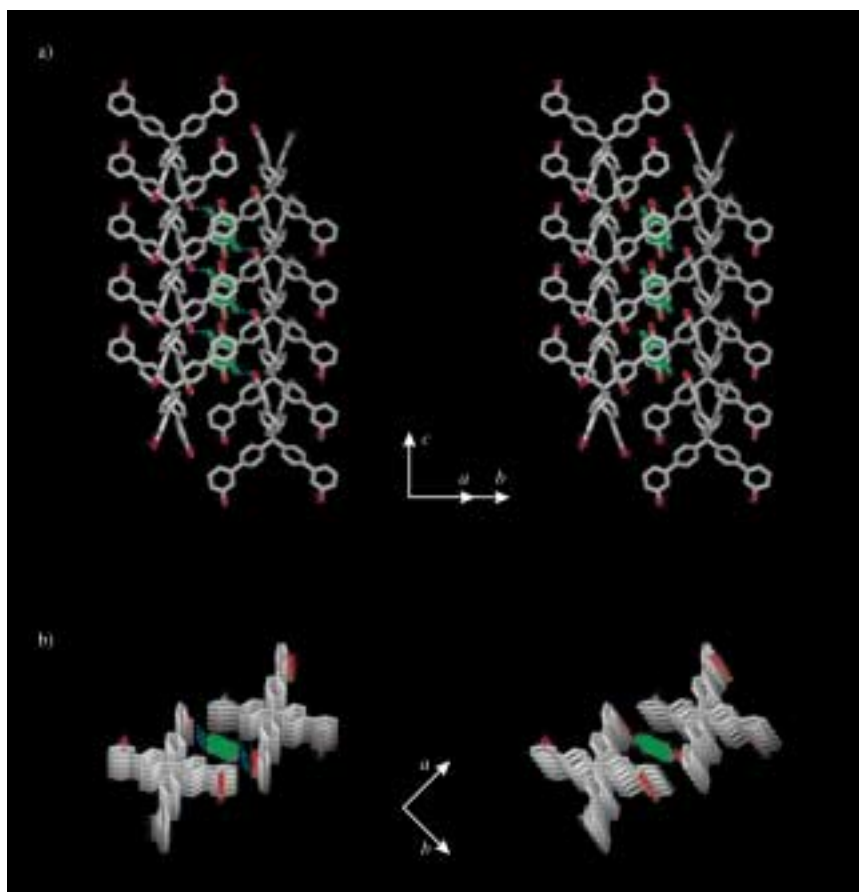


Figure 3. a) Stereoview of a set of polycatenated **1**–quinone–**1** arrays, showing alternate phenol–quinone stacking; b) plan view of (a). The colors have the same meanings as in Scheme 1.

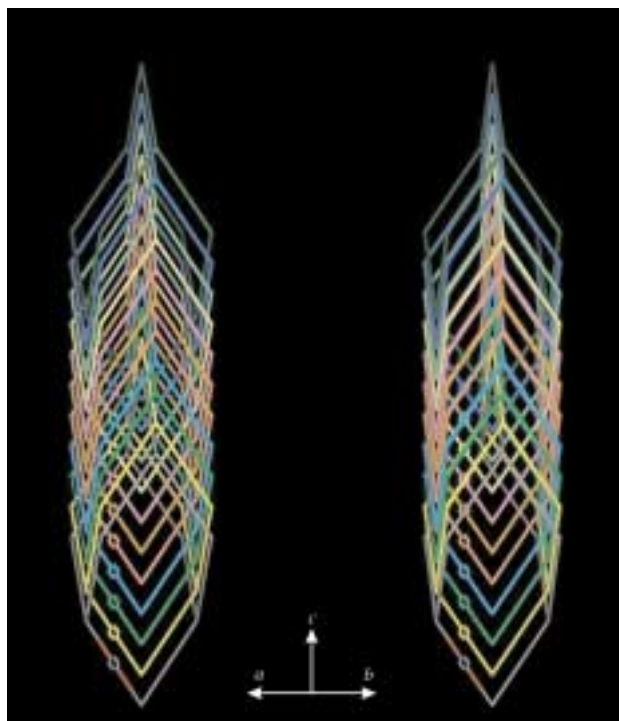


Figure 2. Schematic stereoview of the 11-fold catenation of the hydrogen-bonded superadamantane frameworks, shown in different colors, in the crystal structure of adduct **1**·2BQ, in which only one quinone column is shown for clarity.

The quinone-mediated dimerization of phenols may be compared to the dimerization of carboxylic acids. As the zero-, one-, and two-dimensional hydrogen-bonded nets found in 2:1 hydroquinone:benzoquinone,^[11a] 1:1 hydroquinone:benzoquinone,^[11b] and 1:2 anthracenebisresorcinol:benzoquinone^[12] complexes correspond to those of benzoic, terephthalic,^[13] and trimesic^[14] acids, so does the present three-dimensional net compare to that of adamantane-1,3,5,7-tetracarboxylic acid^[2a] or methanetetraacetic acid.^[2b]

Experimental Section

1: A mixture of tetrakis(4-bromophenyl)methane (3.3 g, 5.18 mmol), 3-methoxyphenylboronic acid (5.0 g, 33.3 mmol), and [Pd(PPh₃)₄] (300 mg, 0.25 mmol) in a 1/1 mixture (20 mL) of THF and 1*N* aqueous Na₂CO₃ was heated at 80 °C for 48 h. The usual workup procedure gave 3.29 g (84 %) of tetrakis[4-(3-methoxyphenyl)phenyl]methane, which was demethylated with a stoichiometric amount of BBr₃ in dry CH₂Cl₂ at room temperature for 12 h to give **1**: ¹H NMR (400 MHz, [D₆]DMSO, 25 °C, TMS): δ = 6.75 (m, 4H), 7.09 (m, 8H), 7.38 (m, 11H), 7.61 (m, 8H), 9.51 (s, 4H; OH). Adduct **1**·2BQ was obtained by slow evaporation of an ethanol solution of a 1:2 mixture of compound **1** and benzoquinone; elemental analysis for C₆₁H₄₄O₈ (904.97): calcd: C 80.96, H 4.89; found: C 80.63, H 4.97.

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Catalyst Screening Using an Array of Thermistors**

Ashley R. Connolly and John D. Sutherland*

The use of combinatorial techniques to discover and optimize chemical and biological catalysts is becoming increasingly widespread.^[1] Methodology for the production of catalyst libraries is now advanced, particularly in the case of biological catalysts with the advent of DNA shuffling and error-prone PCR.^[2] Efficient screening methodology is less advanced; although the literature abounds with ingenious screens contrived to suit a particular reaction there are few general screens. Recently IR thermography has emerged as a potentially general technique to monitor reactions associated with a temperature change.^[3] Several groups, notably those of Reetz^[3a, 3b] and Morken,^[3c] have demonstrated the power of IR thermography in catalyst screening, but the method suffers from a number of problems including relatively low resolution of temperature change (±10 mK), the requirement for visualization to be effected through IR-transparent materials, and inconvenient data analysis techniques. In pioneering work Danielsson and co-workers have demonstrated the use of thermistors to monitor temperature changes downstream of an immobilized enzyme in a continuous flow of substrate.^[4] We now report that a multiplexed array of thermistors can be used as an alternative to IR thermography for chemical and biochemical catalyst screening.

The apparatus we constructed is shown in Figure 1. A commercially available 96-well plate dispenser (Multi-

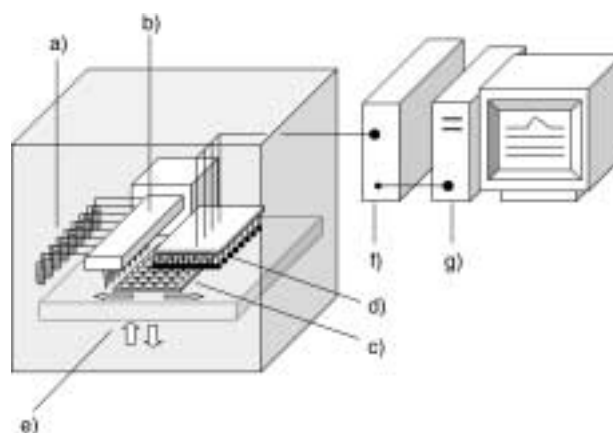
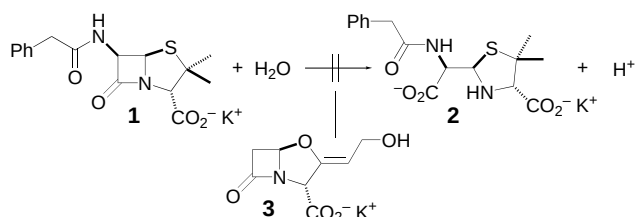


Figure 1. Thermistor array apparatus comprising: a) 8 reagent/substrate reservoirs, b) an 8-channel dispensing head, c) a 96 well plate and holder (movable in *x*-dimension), d) an 8 × 12 thermistor array, e) a base plate (movable in *z*-dimension), f) a multiplexing apparatus, g) a microcomputer.

[*] Prof. Dr. J. D. Sutherland, Dr. A. R. Connolly
Department of Chemistry
The University of Manchester
Oxford Road, Manchester M13 9PL (UK)
Fax: (+44) 161-275-4939
E-mail: john.sutherland@man.ac.uk

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drop 384 from Labsystems) is used to dispense up to eight different solutions into predetermined wells of a plate containing the other reaction components. After shaking the plate briefly to effect mixing, the plate is moved horizontally to position it under the thermistor array. Mechanical raising of the plate then causes immersion of the thermistors into the centers of all the wells and the temperature changes are detected as resistance changes in the multiplexed thermistors. The dispensing apparatus and thermistor array are housed in an incubator to enable thermal pre-equilibration of reaction components prior to mixing. We have found it convenient to monitor the temperature prior to the addition and mixing (a process, which typically takes of the order of 10 s) as well as during the subsequent reaction. Data are recorded as numerical files for each channel and can be displayed simultaneously on-screen during the reaction. Application of a zeroing procedure to the data just before mixing makes it easiest to detect active catalysts by eye; alternatively an electronic management system can be used to select those channels where temperature exceeds a pre-set threshold. These features taken with the capacity for plates to be changed robotically suggest that the method could easily be adapted to high-throughput. As a representative biological reaction we chose the β -lactamase-catalyzed hydrolysis of penicillin G (**1**) to pencilloate (**2**), a reaction known to be moderately exothermic (Scheme 1).^[5] Six wells of a microtiter



Scheme 1. β -Lactamase-catalyzed hydrolysis of **1** to **2** and its inhibition by **3**.

plate were charged with a solution of β -lactamase in phosphate buffer, with the remaining wells containing only buffer. The plate dispenser was primed with a solution of **1**, the plate was loaded, and the apparatus allowed to equilibrate at 37 °C before the addition of **1** and mixing. The output of the apparatus is shown in Figure 2: the coloured lines correspond

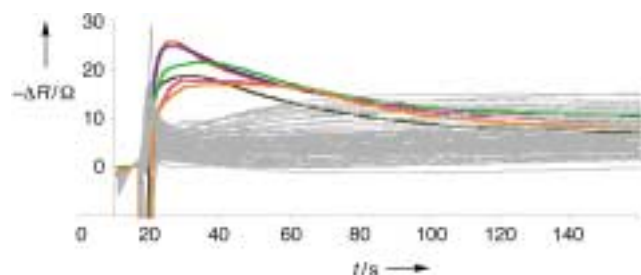


Figure 2. Time-resolved output (resistance change) of the apparatus during the screening of a plate with six wells containing β -lactamase in buffer (500 IU) and the remaining wells containing buffer alone. Reaction was initiated by the addition of **1** to all wells (to a final concentration of 0.06 M) 20 s after recording was started. After recording, the output of all channels was zeroed at a point corresponding to about 10 s before initiation.

to those wells that contain the enzyme and are clearly distinguishable from the remaining channels. Some degree of quantitation is possible by measuring the maximum temperature attained in each well. A plate containing β -lactamase at various concentrations was monitored after the addition of **1** (Figure 3). The enzyme was serially diluted left to right in

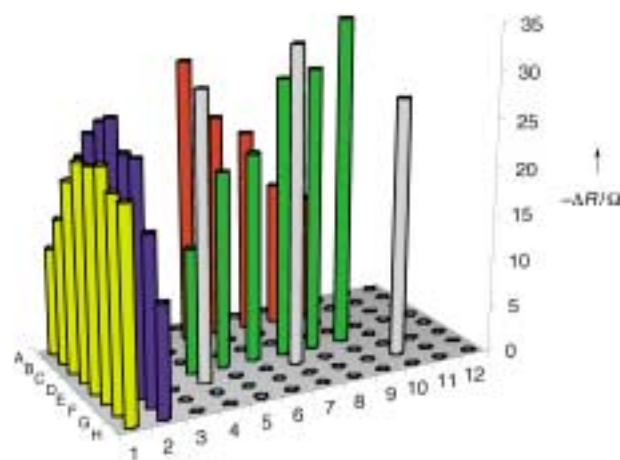


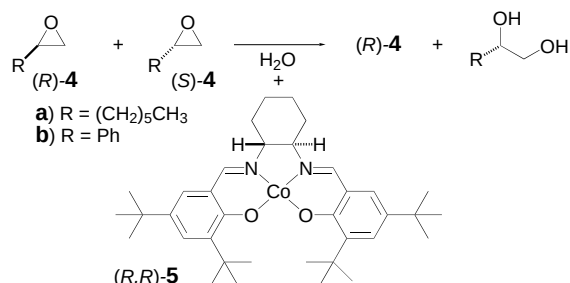
Figure 3. The maximum increase in temperature (as indicated by resistance change) for selected wells in a plate during an experiment in which the amount of β -lactamase was varied (red and green), and the effect of **3** on the hydrolysis of **1** to **2** was investigated (blue and yellow). Three positive enzyme controls are shown in gray.^[8]

row B and right to left in row E to minimize the effects of temperature gradients in the incubator. As can be clearly seen the maximum temperature attained correlates with the concentration of enzyme in each well. Screening for active catalysts amidst a background of inactive or less-active catalysts is crucial to directed evolution experiments in biology using DNA shuffling. Our results suggest that screening with thermistor arrays should be a general method for those biochemical reactions associated with at least a modest temperature change.

In drug discovery there is considerable interest in general high-throughput enzyme-inhibitor screening. To demonstrate the use of thermistor arrays in this regard we examined the inhibition of β -lactamase by potassium clavulanate (**3**).^[6] The maximum temperature attained during the hydrolysis of **1** to **2** was determined in the presence of various amounts of **3** (Figure 3). Potassium clavulanate (**3**) was serially diluted from the back to the front of the plate in column 1 and from the front to the back in column 2, again to minimize the effects of any adventitious temperature gradients in the incubator. The effects of enzyme inhibition are clearly visible and the data resemble inhibitor titration data measured by more conventional techniques. This observation suggests that the method could be used not just for inhibitor discovery but also for a crude ranking of inhibitory efficiency, thus making it a promising technique for adaptation to high-throughput enzyme-inhibitor screening.

In their seminal work on IR-thermographic catalyst screening Reetz and co-workers investigated the enantioselectivity of the hydrolysis of epoxides catalyzed by transition metal ion complexes of salen (salen = *N,N'*-bis(salicylidene)ethylenedi-

amine dianion).^[3a] To compare our thermistor array methodology with IR thermography for chemical catalyst screening we therefore decided to investigate the same chemical system. Optically pure cobalt(II)–salen complexes are efficient and highly enantioselective catalysts for the hydrolysis of terminal epoxides (Scheme 2).^[7] Initially we investigated the effect of



catalyst concentration on the hydrolysis of *rac*-1,2-epoxyoctane (*rac*-4a) by the cobalt(II)–(*R,R*)-salen catalyst ((*R,R*)-5). Different amounts of (*R,R*)-5 were added to the wells of a microtiter plate containing *rac*-4a at a fixed concentration (Figure 4). The reaction was initiated by dispensing a solution

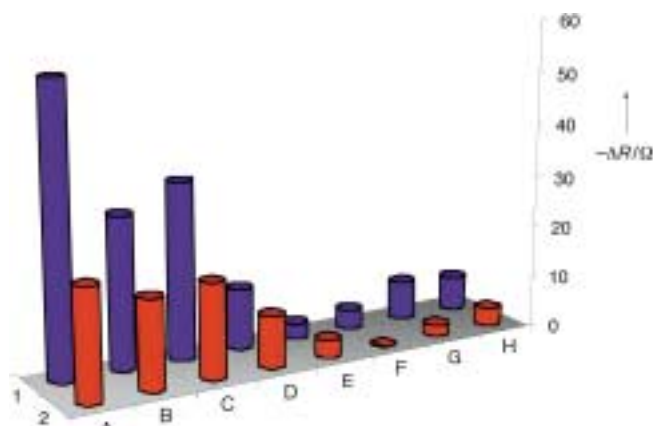


Figure 4. Effect of decreasing the concentration of catalyst (left to right, blue) and substrate (left to right, red) for the hydrolysis of *rac*-4a catalyzed by (*R,R*)-5.^[9]

of water in *rac*-1,2-hexanediol (30 % (v/v)) into each well. The temperature of each reaction was then monitored for 120 min. The maximum temperature in each well was observed after approximately 45 minutes and showed a reasonable correlation with the concentration of the catalyst (Figure 4). An experiment to investigate the effect of varying the concentration of the substrate with a fixed concentration of catalyst was also performed in the same microtiter plate. Again the apparatus was capable of distinguishing between the maximum temperatures attained in each reaction.

To demonstrate the capability of the method to monitor enantioselective catalysis (*R,R*)-5 and (*S,S*)-5 were individually mixed with each enantiomer of styrene oxide (4b; Figure 5). As expected, on addition of water, the temper-

atures increased most in the cases where the chirality of the catalyst matched that of the substrate. That reaction only took place in the matched cases was demonstrated by ¹H NMR spectroscopy. The small temperature increases observed in the nonmatched cases are possibly a result of mixing effects or thermal nonequivalence of the reaction components prior to mixing.

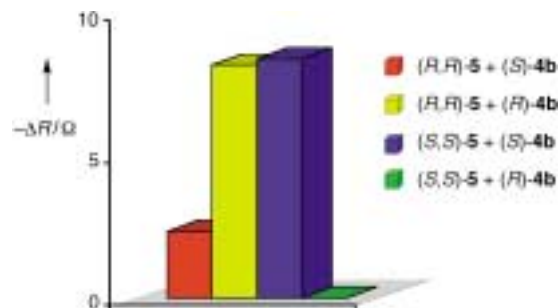


Figure 5. Demonstration of the enantioselectivity obtained using the thermistor array. Water was added to wells containing the catalyst (3.3 μM) and substrate (6.3 M) in toluene.

In conclusion we have demonstrated that thermistor arrays are an attractive alternative to IR thermography for the detection of temperature changes associated with chemical and biochemical reactions. Our method is more sensitive than IR thermography (100 μK changes can be reliably detected), can be carried out in a closed incubated system, and produces data that are easily processed and are amenable to operation at high-throughput. Furthermore it has the scope to be fully automated and the inexpensive thermistor array can easily be removed and replaced with similar arrays of probes to monitor, for example, pressure, pH, dissolved oxygen, or specific ions. Further advances in miniaturization should allow the construction of arrays of multiple probes to monitor several of these variables simultaneously.

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- [9] The plate was prepared with a solution of *rac*-**4a** at 4.7 M in *rac*-1,2-hexanediol in wells A1–H1; 4.3 M in A2; 3.7 M in B2; 3.3 M in C2; 2.7 M in D2; 2.3 M in E2; 1.7 M in F2; 1.3 M in G2, and 0.7 M in H2 and with (*R,R*)-**5** at 5.8 μM in A2–H2 and A1; 4.9 μM in B1; 4.1 μM in C1; 3.3 μM in D1; 2.5 μM in E1; 1.7 μM in F1; 0.8 μM in G1; and 0 μM in B1. Reaction was initiated by the addition of water.

[Cu(2-pyrazinecarboxylato)₂HgI₂]₂·HgI₂: An Open Noninterpenetrating Cu^{II}–Hg^{II} Mixed-Metal Cuboidal Framework Encapsulating Nearly Linear HgI₂ Guest Molecules**

Yu-Bin Dong, Mark D. Smith, and Hans-Conrad zur Loye*

Self-assembly of organic ligands and inorganic metal ions is one of the most efficient and widely utilized approaches for the construction of organic/inorganic coordination polymers. During the past decades unimetallic coordination polymers, which contain only one kind of metal center, have been the predominant synthetic target in this rapidly expanding area of research.^[1] To date, numerous unimetallic coordination polymers with impressive structural motifs have been successfully synthesized. In contrast, the chemistry of bimetallic coordination polymers has received considerably less attention, although bimetallic extended structures based on inorganic counterions such as cyanide (Prussian blue phases) and thiocyanate have been reported.^[2]

Bimetallic coordination polymers have the potential to exhibit interesting physical properties such as electrical conductivity or magnetic ordering that result from interactions between two distinct metal centers connected by a suitable linker. For example, complex magnetic behavior in Cu^{II}–Mn^{II} mixed-metal coordination polymers was reported by Kahn et al.^[3] Likewise, a series of mixed-metal molecular magnets [Cp₂⁺Z^{III}][M^{III}(ox)₃] (Z^{III} = Co, Fe; M^{III} = Cr, Fe; M^{II} = Mn, Fe, Co, Cu, Zn; Cp⁺ = η^5 -C₅Me₅) based on bridging

oxalato (ox) ligands was reported by Coronado et al.^[4] Of course, novel physical properties are not the only reason for pursuing the synthesis of bimetallic coordination polymers; propagation of the structural preferences (influenced by oxidation state and coordination predisposition) of two different metal centers in mixed-metal systems should result in a broader palette of polymer structural motifs than is achievable in unimetallic systems, and this may in turn lead to better understanding of composition–structure relationships.

We have initiated a synthetic program for the preparation of mixed-metal coordination polymers, in which the concepts of self-assembly and metal-containing building block ligands were combined to yield a series of novel bimetallic framework structures. These materials are based on [M(2-pyrazinecarboxylato)₂]₂·*n*H₂O and [M(methylpyrazine-5-carboxylato)₂]₂·*n*H₂O (M = Cu^{II}, Co^{II}, Ni^{II}, Zn^{II}; *n* = 0, 2, 3) and were synthesized by treating these metal-containing ligands with a variety of metal salts.^[5] Here we report on the first hydrothermally synthesized Cu^{II}–Hg^{II} mixed-metal framework material, namely, [Cu(2-pyrazinecarboxylato)₂HgI₂]₂·HgI₂ (**1**), which is constructed from [Cu(2-pyrazinecarboxylato)₂] building blocks and HgI₂ linkers. This neutral, cuboidal framework structure possesses regular square channels (dimensions 7.24 × 7.24 Å)^[6] in which, surprisingly, uncoordinated, nearly linear HgI₂ molecules are encapsulated. While it is quite common to find solvated species in the void spaces of coordination polymers, we believe the presence of a free inorganic salt to be quite novel.

Reaction of [Cu(2-pyrazinecarboxylato)₂] (**2**) or [Cu(2-pyrazinecarboxylato)₂]·2H₂O (**3**)^[5b, 7] with HgI₂ (1:2 molar ratio) in water under hydrothermal conditions (130 °C, 24 h), afforded the title compound as blue-green orthorhombic plate crystals in quantitative yield. Single-crystal structure analysis^[8] revealed a three-dimensional extended cuboidal framework based on [Cu(2-pyrazinecarboxylato)₂] and HgI₂ building blocks. Each Cu^{II} center has a slightly distorted [CuN₄O₂] octahedral coordination sphere, with two nitrogen donors and two oxygen donors of two 2-pyrazinecarboxylato ligands in the equatorial plane. The coordination sphere is completed by the terminal nitrogen donors of two neighboring [Cu(2-pyrazinecarboxylato)₂] building blocks in the axial positions. The framework Hg^{II} centers reside in a distorted {HgI₂O₂} tetrahedral coordination environment that is composed of two iodide ions (Hg–I 2.603(2), 2.633(2) Å; I–Hg–I 158.79(6)°) and two oxygen atoms of two adjacent [Cu(2-pyrazinecarboxylato)₂] building blocks (Hg–O 2.532(9) Å). The two copper-bound carboxylate oxygen atoms interact very weakly with the Hg^{II} centers (Hg···O 3.119(4) Å). The Cu^{II} and Hg^{II} centers are linked by the carboxylate moiety of 2-pyrazinecarboxylate in a bis-monodentate fashion to form linear [Cu(2-pyrazinecarboxylato)₂HgI₂] mixed-metal chains (Figure 1). Identical chains, rotated along the chain direction by 90°, are interconnected through Cu^{II} nodes into a three-dimensional cuboidal network. As shown in Figure 2, each cuboidal box consists of eight Cu^{II} atoms at the corners, which are connected by four long carboxylate–Hg–carboxylate (Cu···Cu 9.99(4) Å) and eight shorter pyrazine (Cu···Cu 7.24(4) Å) linkers that make up the 12 edges. This cuboidal Cu^{II}–Hg^{II} structural motif is clearly different from those of

[*] Prof. Dr. H.-C. zur Loye, Dr. Y.-B. Dong, Dr. M. D. Smith
Department of Chemistry and Biochemistry
University of South Carolina
Columbia, SC 29208 (USA)
Fax: (+1) 803-777-8508
E-mail: zurloye@sc.edu

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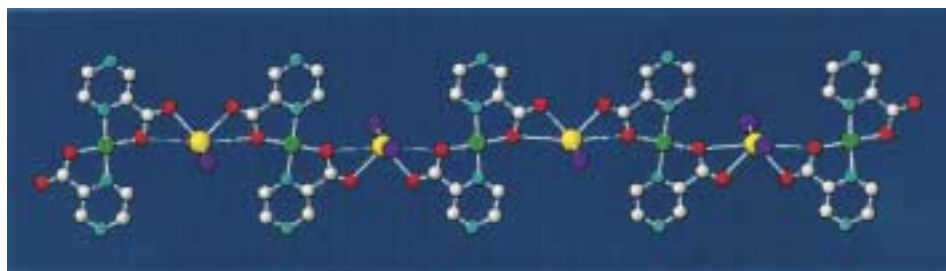


Figure 1. The $[\text{Cu}(\text{2-pyrazinecarboxylato})_2\text{HgI}_2]$ mixed-metal chain in **1**. Yellow spheres, Hg; purple, I; green, Cu; blue, N; red, O; white, C. Hydrogen atoms are omitted for clarity.

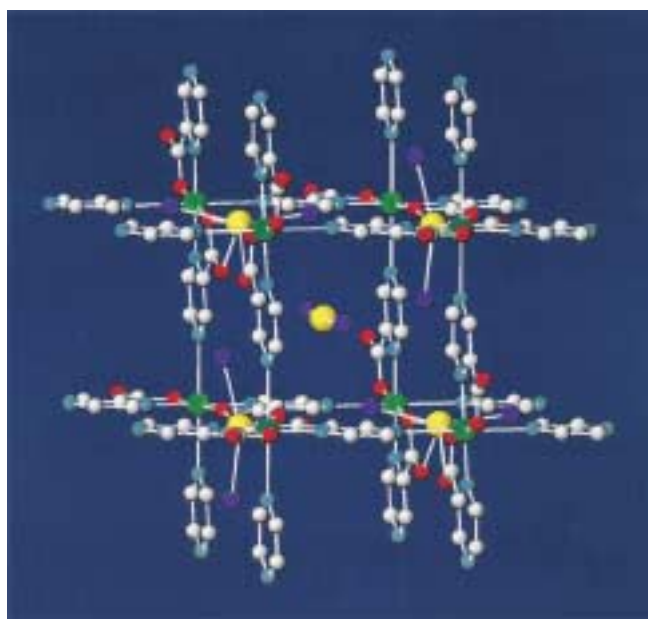


Figure 2. A single cuboid box, showing the framework around one encapsulated HgI_2 molecule. For color code, see Figure 1. Hydrogen atoms are omitted for clarity.

ReO_3 , α -polonium, and Prussian blue and analogous cyanometalate phases, all of which exhibit cubic frameworks (Figure 3).^[9] The cuboid form is one of the fundamental structural types, and **1** is the first example of a neutral organic/inorganic mixed-metal extended cuboidal framework generated from a metal/organic ligand and a metal salt.

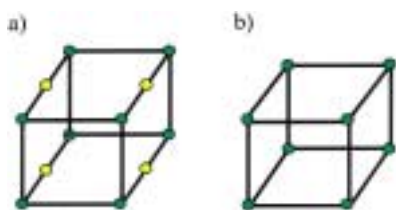


Figure 3. Comparison of the novel cuboidal unit in **1** (a) with a cubic unit (b).

Figure 4 shows a view of the full three-dimensional structure along the crystallographic b axis (along the chain direction). All $[\text{Cu}(\text{2-pyrazinecarboxylato})_2]$ units in the two equivalent chains are strictly coplanar and, moreover, perpendicular relative to each other. This is distinctly different from the orientations of the pyrazine ligands in $[\text{Ag}(\text{pyrazi-$

$\text{ne})_3](\text{SbF}_6)_3$, which forms a cationic cubic framework in which the pyrazine ligands are alternately rotated by different angles in opposite directions.^[10]

When viewed down the crystallographic b axis, very regular square channels (crystallographic dimensions, $7.24 \times 7.24 \text{ \AA}$) are evident in **1** (Figure 4). Remarkably, these channels encapsulate neu-

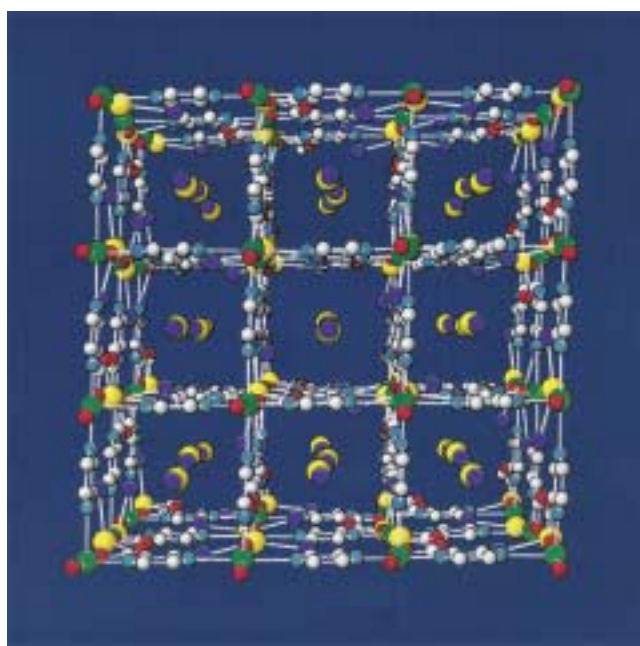


Figure 4. The extended cuboidal framework in **1**, viewed down the crystallographic b axis. For color code, see Figure 1. Hydrogen atoms are omitted for clarity.

tral HgI_2 guest molecules which are nearly linear (I-Hg-I $175.87(9)^\circ$), with equal Hg-I distances of $2.577(2) \text{ \AA}$, slightly shorter than the coordinated (framework) Hg-I bonds. Interestingly, this distance is the same as the Hg-I bond length in HgI_2 vapor ($2.57(4) \text{ \AA}$). Both framework and guest Hg-I bonds are significantly shorter than those in the low-temperature (red) form of solid HgI_2 ($2.78 \text{ \AA}$), in which mercury is tetrahedrally coordinated. The HgI_2 molecules in the intrachannel $[\cdots \text{I-Hg-I} \cdots \text{I-Hg-I} \cdots]$ columns are quite distant from one another, with intermolecular $\text{I} \cdots \text{I}$ distances of about $14.2 \text{ \AA}$, yet no other guest molecules are located in the space between these HgI_2 molecules. The mercury atoms of the HgI_2 guests are also weakly coordinated to four iodide ions in the framework, with $\text{I} \cdots \text{Hg}$ distances of $3.68(4)$, and $3.761(4) \text{ \AA}$, which are slightly shorter than the sum of the van der Waals radii of Hg and I.

A remarkable feature of **1** is the inclusion of HgI_2 guest molecules instead of the organic ligands, solvent or water molecules, or inorganic counterions commonly observed in coordination polymer voids.^[11] In addition, to the best of our knowledge, **1** is the first bimetallic coordination polymer obtained by hydrothermal synthesis,^[12] a method which has

proven to be expedient for growing crystals of numerous metal/organic compounds.^[1a]

All of the free (uncoordinated) donor atoms (two nitrogen and two oxygen donors) in the metal-containing building blocks **2** or **3** are fully utilized in the extended structure of **1** to bind the two kinds of metal ions into a polymeric structure, while retaining the original geometry of the molecular building block. In fact, both **2** and **3** can be regarded as fourfold rigid connectors for incorporation into bimetallic polymeric frameworks. The separations between the two terminal nitrogen atoms in **2** and **3** are 9.48(3) and 9.50(3) Å, respectively,^[5a, 7] significantly longer than the N...N distance (7.12(3) Å)^[13] in 4,4'-bipyridine but comparable to those of 1,2-bis(4-pyridyl)ethene (9.385(4) Å)^[14] and 1,2-bis(4-pyridyl)ethyne (9.685(4) Å).^[15] The free O...O separations in **2** and **3** are 7.86(3) and 7.96(3) Å, respectively, also slightly longer than the N...N distance in 4,4'-bipyridine.

Compound **1** is air-stable and is insoluble in water or common organic solvents. To create an open framework with an accessible void space, we examined the possibility of removing the HgI₂ guest molecules by heating a sample and monitoring the process by thermogravimetric analysis (TGA).^[16] Unfortunately, this results in the collapse of the framework structure. The TGA showed that the framework of **1** is stable up to 180 °C, and both HgI₂ moieties per formula unit (cavity and framework) were released in the temperature range 180–261 °C (observed weight loss 75.0%, calculated 74.6%).

This study again demonstrates the utility of a pre-synthesized metal-containing ligand as a precursor for constructing mixed-metal framework structures. We expect this approach to be viable for the construction of many more new and interesting mixed-metal coordination polymers, and studies toward the preparation of new mixed-metal systems containing other transition metals and/or ligands are underway.

Experimental Section

1: Either **2** or **3** (0.12 mmol), HgI₂ (0.24 mmol), and water (4 mL) were placed in an acid digestion bomb. The mixture was heated at 130 °C for 24 h, cooled to 70 °C at 1 °C min⁻¹, and held at this temperature for an additional 7 h, after which the mixture was allowed to cool to room temperature. Blue-green crystals were isolated from the bomb in quantitative yield. IR (KBr pellet): $\tilde{\nu}$ = 1651 (s), 1644 (s), 1634 (s), 1614 (s), 1593 (s), 1557 (s), 1538 (s), 1504 (s), 1468 (s), 1416 (s), 1346 (s), 1285 (s), 1181 (s), 1158 (s), 1058 (s), 1047 (s), 862 (s), 792 (s), 738 (s), 718 (s), 667 cm⁻¹ (s); elemental analysis calcd for C₁₀H₆O₄N₄I₄CuHg₂ (%): C 9.84, H 0.49, N 4.59; found: C 9.88, H 0.50, N 4.45.

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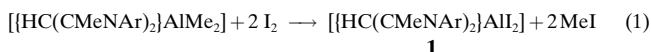
Synthesis and Structure of a Monomeric Aluminum(II) Compound $[\{\text{HC}(\text{CMeNAr})_2\}\text{Al}]$ ($\text{Ar} = 2,6\text{-iPr}_2\text{C}_6\text{H}_3$): A Stable Aluminum Analogue of a Carbene**

Chunming Cui, Herbert W. Roesky,*
Hans-Georg Schmidt, Mathias Noltemeyer,
Haijun Hao, and Fanica Cimpoesu

Dedicated to Professor Francois Mathey

There is a widespread interest in the chemistry of monovalent Group 13 species from several perspectives including synthetic methodology, structure, and theoretical implications.^[1, 2] Generally these unique species can be obtained by using bulky protecting groups and form various aggregates in the solid state. Compared to the chemistry of Al^{III} , that of low-valent aluminum compounds is much less developed. To the best of our knowledge, there are only a handful of stable neutral Al^{I} organometallic compounds, namely $[(\text{Cp}^*\text{Al})_4]$ ($\text{Cp}^* = \text{C}_5\text{Me}_5$),^[3a, 3b] $[(\text{CMe}_3)_3\text{SiAl}]_4$,^[3c] $[(\text{SiMe}_3)_3\text{CAl}]_4$,^[4a] $[\text{Cp}^*\text{Al}_3\text{AlN}(\text{SiMe}_3)_2]$,^[4b] and $[(\text{SiMe}_3)_3\text{SiAl}]_4$,^[4c] that have been structurally characterized. These compounds have tetrahedral arrangements in the solid state. The compound $[(\text{Cp}^*\text{Al})_4]$ has been reported to dissociate in solution at high temperature and in the gas phase to give monomeric Cp^*Al .^[5] At present, however, there are no structures known of monomeric Al^{I} species in the condensed phase. Herein, we report on the use of the very crowded bidentate β -diketoiminato ligand $\text{HC}(\text{CMeNAr})_2$ ($\text{Ar} = 2,6\text{-iPr}_2\text{C}_6\text{H}_3$) for the preparation of the monomeric Al^{I} compound $[\{\text{HC}(\text{CMeNAr})_2\}\text{Al}]$, a stable aluminum analogue of a carbene.

We have been particularly interested in using bulky bi- and tridentate ligands in aluminum chemistry^[6] in the hope of generating unusual species. For example, we recently isolated the first stable Group 13 compound containing the SeH ligand (that is, $[\{\text{HC}(\text{CMeNAr})_2\}\text{Al}(\text{SeH})_2]$) and its condensation product by using the bulky protecting group $\text{HC}(\text{CMeNAr})_2$.^[6c] As a continuation of our studies we have focussed on exploring aluminum chemistry further with this ligand, especially with regard to the preparation of low-aggregated Al^{I} compounds by reduction of the corresponding dihalides. Recent work in our laboratory has shown that organometallic aluminum diiodides can be more easily reduced than the related dibromides and dichlorides.^[7] Aluminum diiodide compounds can be easily obtained by reaction of the corresponding dimethyl derivatives with I_2 .^[6a] Thus, the reaction of the dimethyl compound $[\{\text{HC}(\text{CMeNAr})_2\}\text{AlMe}_2]$ ($\text{Ar} = 2,6\text{-iPr}_2\text{C}_6\text{H}_3$)^[8] with two equivalents of I_2 proceeds under mild conditions to afford the diiodide $[\{\text{HC}(\text{CMeNAr})_2\}\text{AlI}_2]$ (**1**) in high yield [Eq. (1)].

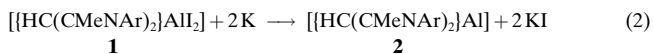


[*] Prof. Dr. H. W. Roesky, Dipl.-Chem. C. Cui, H.-G. Schmidt, Dr. M. Noltemeyer, H. Hao, F. Cimpoesu
Institut für Anorganische Chemie der Universität
Tammannstrasse 4, 37077 Göttingen (Germany)
Fax: (+49) 551-393373
E-mail: hroesky@gwdg.de

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Compound **1** forms essentially colorless crystals. It has been characterized by NMR spectroscopy, EI mass spectrometry, and elemental analysis. The EI mass spectrum shows the highest peak at $[M^+ - \text{I}]$. The formation of **1** was finally confirmed by an X-ray single-crystal analysis.^[6c]

Reduction of **1** with potassium for three days afforded the first example of a room-temperature stable monomeric Al^{I} species $[\{\text{HC}(\text{CMeNAr})_2\}\text{Al}]$ (**2**) in about 21 % yield [Eq. (2)].



Compound **2**, crystallized as red crystals from toluene, is soluble in benzene and toluene but only sparingly soluble in *n*-hexane and *n*-heptane. It is stable at room temperature under an inert atmosphere, however, significant decomposition can be observed above 150 °C. Compound **2** has been characterized by ^1H and ^{13}C NMR spectroscopy, EI-mass spectrometry, elemental analysis, and X-ray single-crystal analysis. The NMR spectra of **2** give the expected patterns, which are different from those of the reported colorless dihydride $[\{\text{HC}(\text{CMeNAr})_2\}\text{AlH}_2]$.^[6c] Single crystals of **2** suitable for X-ray analysis were obtained from toluene at -30°C . Compound **2** crystallizes in the monoclinic space group $P2_1/n$,^[10] and its structure is shown in Figure 1. It consists of well-separated $[\{\text{HC}(\text{CMeNAr})_2\}\text{Al}]$ monomers. There are no

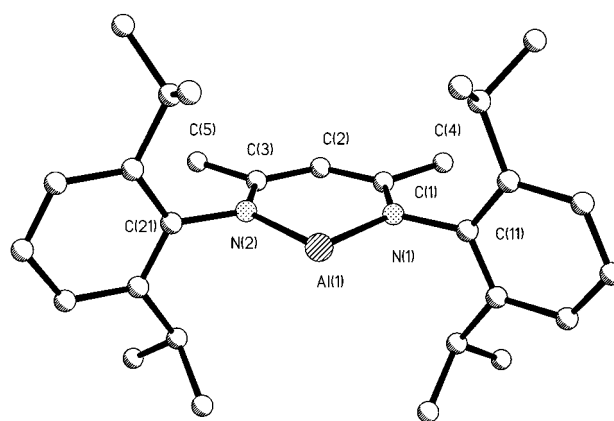


Figure 1. Molecular structure of **2** in the crystal. The hydrogen atoms have been omitted for clarity. Selected bond lengths [pm] and angles $^\circ$: Al(1)–N(1) 195.7(2), Al(1)–N(2) 195.7(2), N(1)–C(1) 134.0(3), N(2)–C(3) 134.2(3), C(1)–C(2) 139.1(3), C(2)–C(3) 139.1(3); N(1)–Al(1)–N(2) 89.86(8), C(1)–N(1)–Al(1) 128.87(15), C(1)–C(2)–C(3) 126.8(2), N(1)–C(1)–C(2) 122.8 (2).

close contacts between Al and other atoms as indicated by the closest distance of $\text{Al} \cdots \text{H}$ (*iPr*, 314.2 pm). As expected, the Al–N bond lengths (195.7(2) pm) are longer than those of the Al^{III} compounds $[\{\text{HC}(\text{CMeNAr})_2\}\text{AlMe}_2]$ (av 192.2 pm) and $[\{\text{HC}(\text{CMeNAr})_2\}\text{Al}(\text{SeH})_2]$ (av 189.9 pm). A similar lengthening has been noted in $[\text{In}(2,6\text{-Tri}p_2\text{C}_6\text{H}_3)]$ (*Tri*p = 2,4,6-*iPr*₃C₆H₂) whose In–C distance is about 10 pm longer than that of the corresponding In^{III} derivative.^[11] Similarly, the In–N distance in monomeric In^{I} tripyrazolylborates may be as much as 20 pm longer than those in related In^{III} derivatives.^[12] Furthermore the N–Al–N angle (89.86(8)°) in **2** is more

acute than those of $[\{\text{HC}(\text{CMeNAr})_2\}\text{AlMe}_2]$ ($96.18(9)^\circ$) and $[\{\text{HC}(\text{CMeNAr})_2\}\text{Al}(\text{SeH})_2]$ ($97.1(4)^\circ$). The lengthening of the N–Al bonds and the nearly perpendicular N–Al–N angle indicate that two 3p orbitals on the aluminum center are essentially involved in the bonding to the two nitrogen atoms. It has been argued that Al–N bonds in aluminum(III) amide derivatives are largely ionic, which usually leads to a relatively short Al–N distance due to the Lewis acidic Al^{III} centers.^[9] The lengthening of the Al–N bonds in **2** also indicates that these bonds are more covalent than those of the Al^{III} derivatives and the Al^{I} center is much less acidic. It is noteworthy that in **2** the Al atom and the ligand backbone (N–C–C–C–N) form an essentially planar six-membered ring, which is in contrast to the Al^{III} compounds $[\{\text{HC}(\text{CMeNAr})_2\}\text{AlMe}_2]$ and $[\{\text{HC}(\text{CMeNAr})_2\}\text{Al}(\text{SeH})_2]$, in which the Al atoms are positioned out of the planar ligand backbone.

A further notable aspect of the structure of **2** is the coordination number of two at the Al center. Compounds with three- and four-coordinate aluminum centers are well known,^[13] however, there are no compounds reported with two-coordinate aluminum atoms. The unique two-coordinate nature of the Al atom in **2** is undoubtedly due to the protection afforded by the Ar groups and the presence of a nonbonded lone pair of electrons at the Al atom.

A preliminary insight into the peculiarities of the aluminum cycle is gained from ab initio calculations,^[14] by analyzing the Laplacian of electronic density^[15] within the plane (see Figure 2). First of all, it is noteworthy that there exists a lone pair of electrons^[16] accommodated on the metal atom and arranged outside of the cycle in a quasi-trigonal-planar manner with the two Al–N bonds around the Al atom. This allows one to argue that the electrons originating from an s^2 configuration of the Al^{I} center are stereochemically active, leading to an sp-like hybrid. In this conjecture, the “off-cycle” side of the aluminum(I) atom can be considered as a Lewis base. At the same time, the charge depletion close to the Al atom in the semiplane of the cycle can be described as Lewis acid type behavior.

In summary, we have prepared and fully characterized the first example of a monomeric Al^{I} compound. The existence of a nonbonded lone pair of electrons at the aluminum center indicates a singlet carbene character of the aluminum atom. The other reported Group 13 carbene is anionic ($[\text{CHN}(\text{tBu})_2]\text{Ga}^-$,^[17]), while **2** is neutral. Compound **2** could be used in carbene-type reactions and as a Lewis base as well as a reducing reagent, which may have broad and interesting chemistry. Presently we are involved in the reactions to study the electronic equivalence of **2** for CO in metal carbonyl complexes.

Experimental Section

All operations were performed under an argon atmosphere using Schlenk line techniques.

1: Toluene (60 mL) was added to a mixture of $[\{\text{HC}(\text{CMeNAr})_2\}\text{AlMe}_2]$ (4.74 g, 10 mmol) and I_2 (5.08 g, 20 mmol) at room temperature. The deep red solution was stirred for three days after which it developed into a slightly brown-yellow solution. The solution was concentrated (ca. 20 mL) and stored at -30°C in a freezer overnight to afford slightly yellow crystals of **1** (5.8 g, 83%). M.p. $251-252^\circ\text{C}$; ^1H NMR (C_6D_6): $\delta = 7.11$ (m, 6H; Ph),

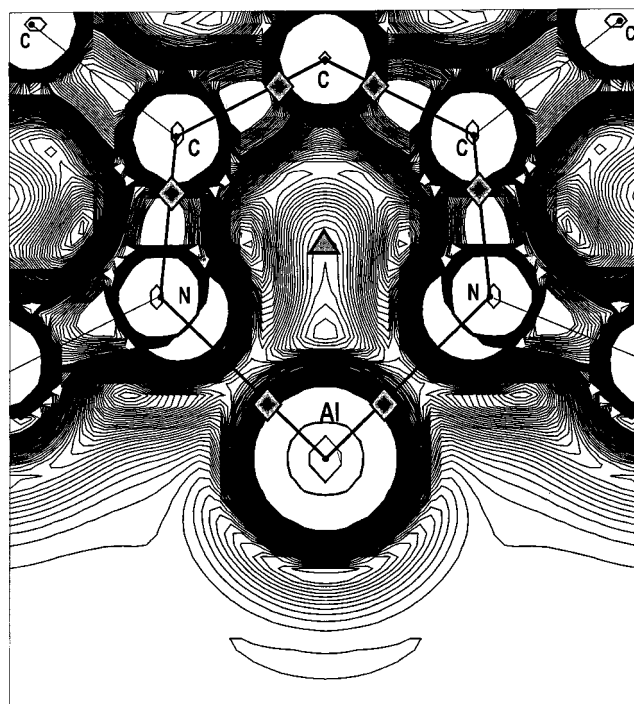


Figure 2. Contours from values of the Laplacian of electronic density ($\nabla^2\rho$) in the plane of the heterocycle of the title compound. The dark contours (areas with $\nabla^2\rho < 0$), which denote the sites of electron density accumulation, indicate the locations of the lone pair of electrons at the Al^{I} center and the deformed valence shells of the C and N atoms in the molecule. The light contours (areas with $\nabla^2\rho > 0$) denote the electron deficiency effects; those around the Al^{I} center in the direction of the cycle are remarkable. The diamonds (♦) posted on the interatomic axes show the $(3, -1)$ type of critical points, which verifies the chemical bonding, while the triangle in the center (Δ) denotes the $(3, +1)$ type of critical points, a quantum measure of the cyclic structural pattern. The π density, perpendicular to the plane, does not contribute to the depicted $\nabla^2\rho$.

5.05 (s, 1H; γ -CH), 3.58 (sept, 4H, $J = 6.75$ Hz; CHMe_2), 1.49 (s, 6H; Me), 1.43 (d, 12H, $J = 6.80$ Hz; CHMe_2), 1.08 (d, 12H, $J = 6.80$ Hz; CHMe_2); ^{13}C NMR (C_6D_6): $\delta = 172.5$ (CN), 144.9, 138.9, 125.1 (Ph), 100.4 (γ -C), 29.2 (CHMe_2), 26.6 (CHMe_2), 24.5 (CHMe_2), 24.3 (Me); ^{27}Al NMR (C_6D_6): $\delta = 83.2$ ($\nu_{1/2} = 620$ Hz); EI-MS: m/z (%): 571 (100%, $[\text{M} - \text{I}]^+$).

2: A solution of **1** (2.08 g, 3.0 mmol) in toluene (30 mL) was added to a suspension of finely divided potassium (0.25 g, 6.4 mmol) in toluene (10 mL) at room temperature. The mixture was vigorously stirred at room temperature for three days. The solution developed an orange-red color and all the potassium was consumed. The solution was filtered, and the orange-red filtrate was concentrated to about 5 mL. On standing overnight at -30°C the solution afforded X-ray quality red crystals of **2** (0.28 g, 21%). Further products in the solution could not be characterized. M.p. $> 150^\circ\text{C}$ (decomp); ^1H NMR (C_6D_6): $\delta = 7.16$ (m, 6H; Ph), 5.18 (s, 1H; γ -CH), 3.16 (sept, 4H, $J = 6.80$ Hz; CHMe_2), 1.65 (s, 6H; Me), 1.38 (d, 12H, $J = 6.80$ Hz; CHMe_2), 1.13 (d, 12H, $J = 6.80$ Hz; CHMe_2); ^{13}C NMR (C_6D_6): $\delta = 165.3$ (CN), 143.7, 142.3, 124.2 (Ph), 100.9 (γ -C), 29.0 (CHMe_2), 25.1 (CHMe_2), 23.9 (CHMe_2), 23.8 (Me); ^{27}Al NMR (C_6D_6): could not be observed; EI-MS: m/z (%): 444 (12, $[\text{M}^+]$), 429 (100, $[\text{M} - \text{Me}]^+$); elemental analysis (%) calcd for $\text{C}_{29}\text{H}_{41}\text{AlN}_2$ (444.62): C 78.3, H 9.3, N 6.3; found: C 77.9, H 9.7, N 6.3.

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Alumoxane Hydride and Aluminum Chalcogenide Hydride Compounds with Pyrazolato Ligands**

Wenjun Zheng, Nadia C. Mösch-Zanetti, Herbert W. Roesky,* Mathias Noltemeyer, Manuel Hewitt, Hans-Georg Schmidt, and Thomas R. Schneider

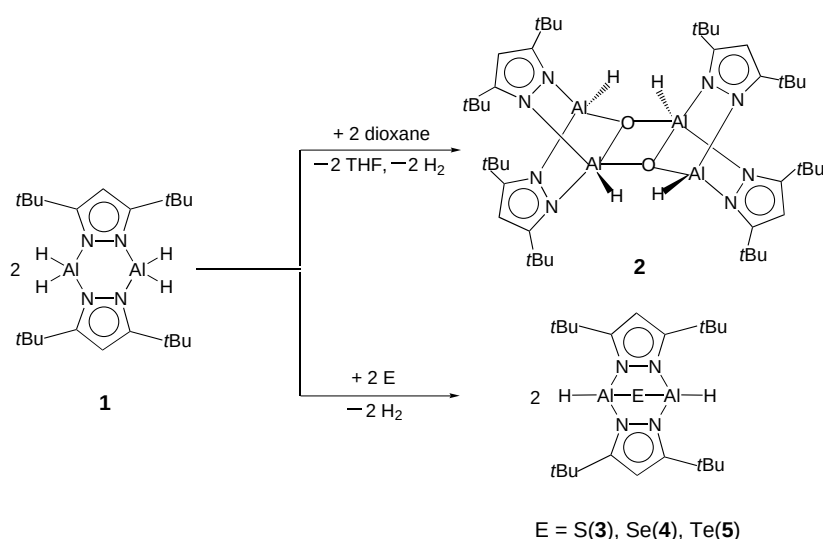
Dedicated to Professor Marianne Baudler on the occasion of her 80th birthday

Since 1980 intense interest in alkylalumoxanes has been generated primarily from the work of Sinn and Kaminsky,^[1] who found methylalumoxane (MAO) to be a good cocatalyst for the transition metal catalyzed polymerization of alkenes.^[1–3] Although the role of MAO seems to be understood,^[4] the structure of the catalytically active species of methylalumoxane remains unknown. Especially the coordination number of the aluminum center is still a question of debate. Recently, several alumoxanes ($t\text{BuAlO}$) $_n$ ($n = 6–9$) and aluminum hydroxides (aluminum oxide hydroxides) displaying a lower degree of aggregation were prepared by the reaction of R_3Al ($\text{R} = t\text{Bu}$, Me, 2,4,6- $\text{Me}_3\text{C}_6\text{H}_2$ (Mes), Ph) with water or anhydrous lithium hydroxide.^[5, 6] On the other hand, it has been shown that aluminum hydride compounds can be used as precursors for the synthesis of organoaluminum chalcogenides with the formula $(\text{RAIE})_n$ ($\text{R} = \text{organic group}$; $\text{E} = \text{O, S, Se, Te}$) upon reaction with species such as $(\text{Me}_2\text{SiO})_3$,^[7] $\text{S}(\text{SiMe}_3)_2$,^[8] $\text{Se}(\text{Te})$,^[9a, 10] or $(\text{RE})_2$ ($\text{E} = \text{Se, Te}$).^[9b] The recent results in the chemistry of 3,5-*tert*-butylpyrazolatoaluminum dihydride ($[\eta^1\text{-3,5-}t\text{Bu}_2\text{pz}(\mu\text{-Al})\text{H}_2]_2$ (**1**))^[11] prompted us to synthesize compounds of the formula $[(\eta^1\text{-3,5-}t\text{Bu}_2\text{pz}(\mu\text{-Al})\text{H})_2\text{E}]_n$ ($\text{E} = \text{O}$ (**2**) ($n = 2$); S (**3**), Se (**4**), Te (**5**) ($n = 1$)). Herein we present the molecular structures of **2–5**. To the best of our knowledge, **2** represents the first example of a structurally characterized alumoxane hydride in which two of the Al atoms are five-coordinate. Product **3** is the first compound prepared by the reaction involving aluminum hydride and elemental sulfur.

A solution of compound **1** in dry dioxane was stirred at room temperature for five days to yield **2** (Scheme 1). The X-ray crystal structure of **2** confirms the tetrameric arrangement (Figure 1),^[12] as also indicated for the gas phase by the MS(EI) spectrum (m/z 860). The Al_2O_2 core of the molecule consists of a quite regular parallelogram, and interestingly all the Al and O atoms are coplanar (the sum of angles at O is $359.98(22)^\circ$). Each oxygen atom is connected to three AlH groups to form a $\mu_3\text{-O}$ bridge. The Al atoms located outside

[*] Prof. Dr. H. W. Roesky, Dipl.-Chem. W. Zheng, Dr. N. C. Mösch-Zanetti, Dr. M. Noltemeyer, M. Hewitt, H.-G. Schmidt, Dr. T. R. Schneider
Institut für Anorganische Chemie der Universität
Tammannstrasse 4, 37077 Göttingen (Germany)
Fax: (+49) 551-393373
E-mail: hroesky@gwdg.de

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Scheme 1. Syntheses of compounds 2–5.

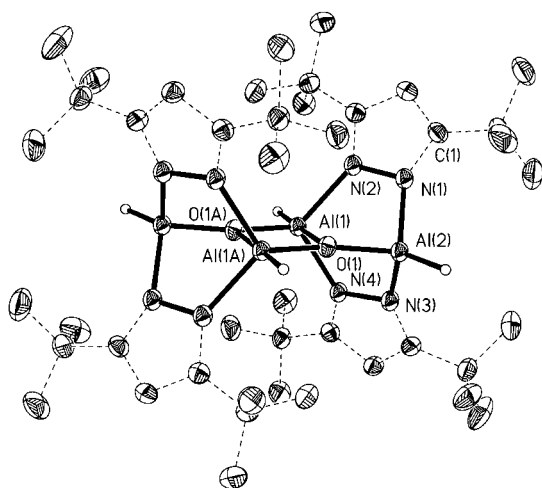


Figure 1. Molecular structure of **2**. Selected bond lengths [Å] and angles [°]: Al(1)–O(1) 1.922(2), Al(1)–O(1A) 1.786(2), Al(2)–O(1) 1.7260(14), Al(1A)–O(1)–Al(2) 155.08(8), Al(1)–O(1)–Al(2) 105.07(7), Al(1)–O(1)–Al(1A) 99.83(7), O(1)–Al(1)–O(1A), 80.17(7).

the parallelogram use two $\mu\text{-}\eta^1\text{-}\eta^1$ pyrazolato ligands to form two six-membered Al_2N_4 rings in a boat conformation. As already mentioned, the interesting feature of **2** is the coordination number five at the Al atoms in the Al_2O_2 core; this differs from other characterized alumoxanes in which the Al atoms usually show coordination numbers of three and four.^[5a,b, 6, 7] Only one structurally characterized alumoxane hydroxide containing five-coordinate Al atoms has been published.^[5c] Reports of compounds^[13] with coordination numbers five,^[13a,b] six,^[13c] and even seven^[13d] at Al have appeared in the literature, but these systems involve multi-chelating ligands such as a tetradentate Schiff base, $\text{N}(\text{CH}_2\text{CH}_2\text{OH})_3$, and crown ether. In compound **2** significantly longer bond lengths and smaller angles are observed at the five-coordinate Al(1) compared to the four-coordinate Al(2) (Figure 1). The bond lengths of Al(1)–O(1A) (1.786(2) Å) and Al(2)–O(1) (1.7260(14) Å) are shorter than the sum of the covalent radii.^[14] The Al(1A)–O(1)–Al(2) angle of 155.08(8)° is significantly larger than the Al–O–Al angle in

$[\text{Al}_6\text{tBu}_6(\mu_3\text{-O})_4(\mu_3\text{-OH})_4]$ (103.7(9)°)^[5c] and in $(\text{Mes}^*\text{AlO})_4 \cdot 2\text{C}_6\text{H}_6$ (151.32(13)°; $\text{Mes}^* = 2,4,6\text{-tBu}_3\text{C}_6\text{H}_2$).^[7]

The ^1H NMR spectrum of **2** shows only one set of signals ($\delta = 1.32, 5.80$), implying the existence of dynamic processes in solution. The GC-MS(EI) investigations on the reaction solution of **2** indicate the formation of THF. Accordingly, we assume that the formation of **2** proceeds by nucleophilic attack at the aluminum center of **1** by one of the oxygen atoms of dioxane and then insertion into an Al–H bond, leading to the degradation of dioxane and formation of an Al–O bond in **2**. However, the use of other oxygen-transfer reagents such as $n\text{Bu}_2\text{SnO}$ leads to small amounts of **2** including several by-products.

Treatment of **1** in toluene with the congeners of oxygen affords three chalcogenide compounds of type $[\{\eta^1\text{-}3,5\text{-tBu}_2\text{pz}(\mu\text{-Al})\text{H}\}_2\text{E}]$ ($E = \text{S}$ (**3**), Se (**4**), Te (**5**); Scheme 1). It is important, especially for the preparation of **3**, to use strictly equivalent amounts of the reactants and the mixed solvent system (THF/toluene 1/9), otherwise an insoluble polymeric material is formed. This might explain why the reaction of aluminum hydride with sulfur has not yet been reported. All compounds, especially **4** and **5**, are very susceptible to oxygen. Small amounts of air immediately lead to the decomposition of **4** and **5**. An attempt to substitute all the H atoms at the Al atoms of **1** with two equivalents of Se results in the homoleptic compound $[\text{Al}(\eta^2\text{-}3,5\text{-tBu}_2\text{pz})_3]$.^[15]

X-ray crystallography reveals the compounds **3**–**5** to be isostructural. They are represented by the illustration of **3** in Figure 2.^[12] Compound **3** consists of a boat-shaped, six-membered Al_2N_4 ring. A sulfur atom forms a bridge between the two Al atoms and one Al–H bond remains at each Al center. Two pyrazolato ligands are located on the same side of the molecule due to the constraint of the sulfur atom. The

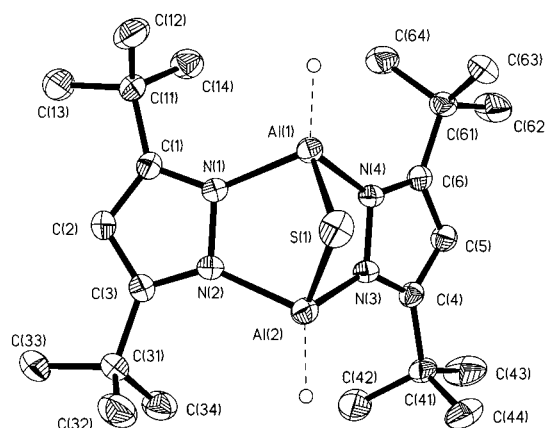


Figure 2. Molecular structure of **3**. The structures of **4** and **5** are analogous with Se(1) (**4**) and Te(1) (**5**) in place of S(1). Selected bond lengths [Å] and angles [°]: **3**: S(1)–Al(1) 2.2080(6), S(1)–Al(2) 2.2117(7), Al(1)–N(1) 1.9469(12), Al(1)–S(1)–Al(2) 79.58(2); **4**: Se(1)–Al(1) 2.3449(10), Se(1)–Al(2) 2.3574(11), Al(1)–N(1) 1.932(2); Al(1)–Se(1)–Al(2) 75.78(3); **5**: Te(1)–Al(1) 2.5763(11), Te(1)–Al(2) 2.5621(12), Al(1)–N(1) 1.944(3); Al(1)–Te(1)–Al(2) 69.41(3).

average Al–E bond length (**3**: Al–S 2.1099(7); **4**: Al–Se 2.3511(10); **5**: Al–Te 2.5692(11) Å) is not only slightly shorter than those in (Mes*AlS)₂ (2.2095(11) Å),^[8b] [Me₃NAl(SeEt)₃] (2.357(8) Å),^[9a] and [Me₃NAl(TePh)₃] (2.585(2) Å),^[9a] but also shorter than the sum of the covalent radii. The Al–E–Al angles in **3** (79.58(2)°) and **4** (75.78(3)°) are close to those in (Mes*AlS)₂ (78.09(3)°)^[8b] and [[N(SiMe₃)C(Ph)C(SiMe₃)₂–Al(μ-Se)]₂] (77.58(3)°),^[10a] whereas the Al–Te–Al angle in **5** (69.41(3)°) is significantly smaller than that in [[N(SiMe₃)C(Ph)C(SiMe₃)₂Al(μ-Te)]₂] (76.88(4)°).^[10a] The Al–E–Al unit (E = O (Al(1)–O(1)–Al(2)), S, Se, Te) shows a more acute angle upon going from the lighter to the heavier congeners.

The reaction leading to the formation of **3–5** probably proceeds via an Al–E–H intermediate. This species then reacts with one of the H atoms at another Al center, and subsequently forms the Al–E–Al bridge and H₂ (Scheme 1). This assumption is supported by the recent results on fully characterized Al–SeH compounds.^[10b]

The higher aggregation of **2** compared to **3–5** is probably due to the increased nucleophilicity of oxygen versus the heavier congeners. Therefore compound **2** might function as a molecular alternative to MAO. This work is presently in progress.

Experimental Section

2: A solution of **1** (0.83 g, 2.0 mmol) in dry dioxane (50 mL) was stirred for five days at room temperature and then filtered through celite. The filtrate was reduced to about 10 mL, and compound **2** was isolated as colorless crystals at room temperature (3–4 weeks, 15–25%). Single crystals suitable for X-ray diffraction analysis were obtained from dioxane at room temperature. M.p. 240–243 °C (decomp); ¹H NMR (200 MHz, CDCl₃): δ = 1.32 (s, 72H; *t*Bu), 4.8 (very br, 4H; AlH, *w*_{1/2} = 80 Hz), 5.80 (s, 4H; CH); ¹³C NMR (125 MHz, CDCl₃): δ = 31.19 (s; Me), 32.29 (s; CMe₃), 101.09 (s; CH), 163.31 (s; *Ct*Bu); MS (EI): *m/z* (%): 860 ([M⁺], 100).

3: Toluene and THF (50 mL, 9/1) were added to equivalent amounts of **1** (0.83 g, 2.0 mmol) and sulfur (0.064 g, 2.0 mmol; dried before use), and the suspension was stirred at 50 °C for 15 h and then filtered through celite. The filtrate was concentrated to about 10 mL, and THF (2 mL) was added to precipitate **3** as colorless crystals at room temperature (0.52 g, 58%). M.p. > 230 °C (decomp); ¹H NMR (200 MHz, C₆D₆): δ = 1.33 (s, 36H; *t*Bu), 5.97 (s, 2H; CH), 5.6 (very br, 2H; AlH); ¹³C NMR (125 MHz, C₆D₆): δ = 31.17 (s; Me), 32.40 (s; CMe₃), 102.25 (s; CH), 165.30 (s; *Ct*Bu); MS (EI): *m/z* (%): 446 ([M⁺], 98), 445 ([M⁺ – H], 100); elemental analysis (C, H, N) correct for C₂₂H₄₀Al₂N₄S.

4: Toluene (50 mL) was added to equivalent amounts of **1** (0.83 g, 2.0 mmol) and Se (0.16 g, 2.0 mmol; used as black powder as received), and the suspension was refluxed for 2–3 h until the black selenium disappeared. The resulting solution was filtered through celite and concentrated to about 10 mL to afford colorless crystals at 4 °C (0.61 g, 62%). M.p. 272 °C; ¹H NMR (200 MHz, C₆D₆): δ = 1.33 (s, 36H; *t*Bu), 5.98 (s, 2H; CH), 5.7 (very br, 2H; AlH); ¹³C NMR (125 MHz, C₆D₆): δ = 31.08 (s; Me), 32.45 (s; CMe₃), 102.57 (s; CH), 165.25 (s; *Ct*Bu); MS (EI): *m/z* (%): 494 ([M⁺], 80), 491 ([M⁺ – 3H], 100); elemental analysis (C, H, N) correct for C₂₂H₄₀Al₂N₄Se.

5: Toluene (50 mL) was added to equivalent amounts of **1** (0.83 g, 2.0 mmol) and Te (0.26 g, 2.0 mmol; used as black powder as received), and the suspension was refluxed for four days until most of the Te disappeared. The solution was filtered through celite and concentrated to about 10 mL to give colorless crystals at 4 °C (0.58 g, 54%). M.p. 250–251 °C; ¹H NMR (200 MHz, C₆D₆): δ = 1.35 (s, 36H; *t*Bu), 5.98 (s, 2H; CH), 5.7 (very br, 2H; AlH); ¹³C NMR (125 MHz, C₆D₆): δ = 30.94 (s; Me), 32.58 (s; CMe₃), 103.24 (s; CH), 165.05 (s; *Ct*Bu). MS (EI): *m/z* (%): 542 ([M⁺], 100); elemental analysis (C, H, N) correct for C₂₂H₄₀Al₂N₄Te · 0.85 toluene.

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$c = 18.031(3) \text{ \AA}$, $\beta = 106.295(14)^\circ$, $V = 3316.1(9) \text{ \AA}^3$, $Z = 4$, $\rho_{\text{calc}} = 1.270 \text{ Mg m}^{-3}$, $F(000) = 1312$, $\mu(\text{Mo K}\alpha) = 0.972 \text{ mm}^{-1}$. A total of 7932 reflections were measured in the range $7.02 \leq 2\theta \leq 50.04^\circ$, of which 5847 were unique. Final R indices: $R_1 = 0.0389$ ($I > 2\sigma(I)$), $wR_2 = 0.0991$ (all data); max./min. residual electron density $1006/-694 \text{ e nm}^{-3}$. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-145888 (2), CCDC-145889 (3), CCDC-145981 (4), and CCDC-145982 (5). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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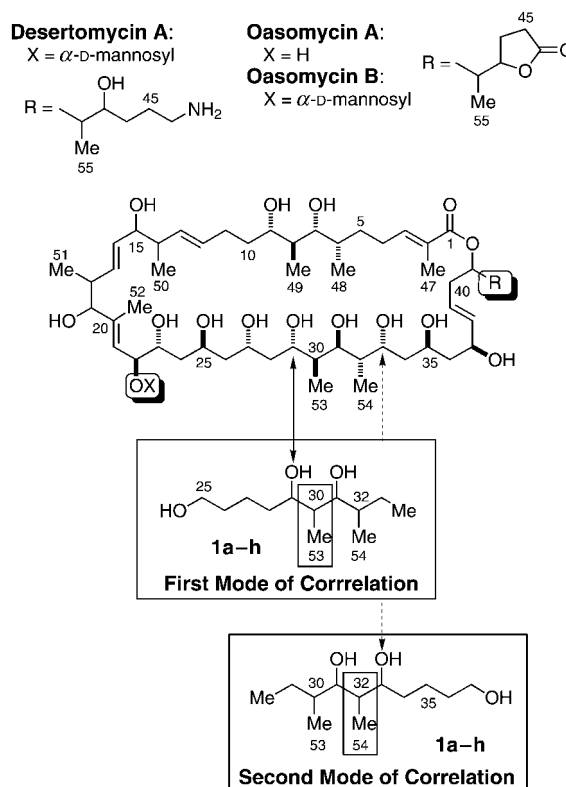
Stereochemical Assignment of the C21–C38 Portion of the Desertomycin/Oasomycin Class of Natural Products by Using Universal NMR Databases: Prediction**

Yoshihisa Kobayashi, Choon-Hong Tan, and Yoshito Kishi*

We recently reported our first step toward the creation of a universal NMR database through analysis of a typical structural motif often found in polypropionate natural products.^[1] We then demonstrated the reliability and usefulness of such an NMR database by using the C5–C10 portion of the desertomycin/oasomycin class of natural products.^[2] In this and the following paper,^[3] we report a further development of this approach by predicting, and proving, the stereochemistry of the C21–C38 portion of the desertomycin/oasomycin class of natural products.^[4]

The C29–C33 portion of desertomycins/oasomycins is viewed as a contiguous dipropionate unit so that our

propionate database^[1] could be applied to predict its stereochemistry. There are apparently two modes of correlation of the NMR database with this portion of desertomycins/oasomycins (see the lower half of Scheme 1). By following



Scheme 1. Structures of desertomycin A and oasomycins A and B, as well as two modes of structural correlation with the contiguous dipropionate NMR database **1a–h**.

the same procedure described previously,^[2] we have compared the ^{13}C NMR characteristics of the C29–C32 (first mode of correlation) and C30–C33 (second mode of correlation) portions of the natural products with those of each of the eight diastereomers.^[5] As seen from the graphs (Figures 1 and 2), none of the eight diastereomers match the corresponding structural portion of the natural products at a satisfactory level.^[6] However, this outcome is not totally surprising when one takes into account the fact that the natural products have additional hydroxyl groups at C27 and C35. As these hydroxyl groups are attached on the contiguous dipropionate unit and linked through a bridge consisting of one methylene group,^[7] a significant steric and/or stereo-electronic effect would be expected from them on the basis of NMR characteristics of the structural portion in question. Hence, we chose to focus only on the ^{13}C NMR characteristics of the C30 and C53 (see the smaller box in the first mode of correlation in Scheme 1) and of the C32 and C54 (see the smaller box in the second mode of correlation) portions of the natural products. From these comparisons (Figure 3) one (**1h** = $\alpha\beta\beta\alpha$) and three (**1a** = $\beta\beta\alpha\alpha$, **1f** = $\alpha\alpha\alpha\beta$, and **1h** = $\beta\alpha\alpha\beta$) candidates emerge from the first and second modes of correlation, respectively.

[*] Prof. Dr. Y. Kishi, Dr. Y. Kobayashi, Dr. C.-H. Tan
Department of Chemistry and Chemical Biology
Harvard University
Cambridge, MA 02138 (USA)
Fax: (+1) 617-495-5150
E-mail: kishi@chemistry.harvard.edu

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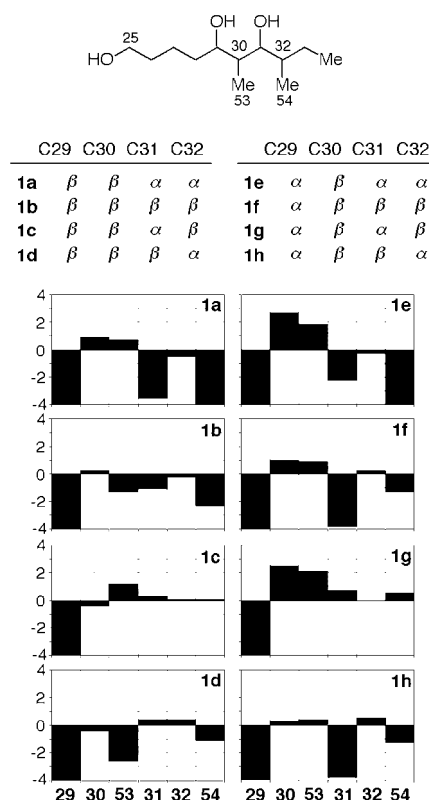


Figure 1. First mode of structural correlation. Difference between the chemical shifts of the carbon atoms of oasomycin B and those of each of **1a–h** (100 MHz, $[D_6]DMSO$). The x- and y-axes represent the carbon number and $\Delta\delta$ ($\delta\mathbf{1a-h} - \delta\text{oasomycin B}$), respectively, for all the charts in this paper.

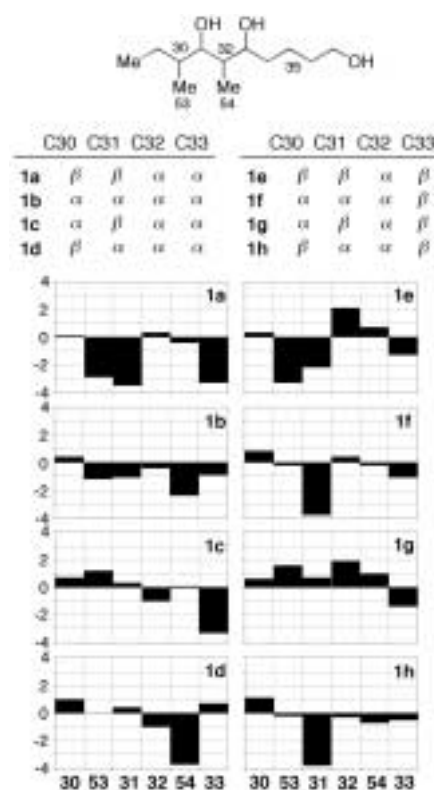


Figure 2. Second mode of structural correlation. Difference between the chemical shifts of the carbon atoms of oasomycin B and those of each of **1a–h** (100 MHz, $[D_6]DMSO$).

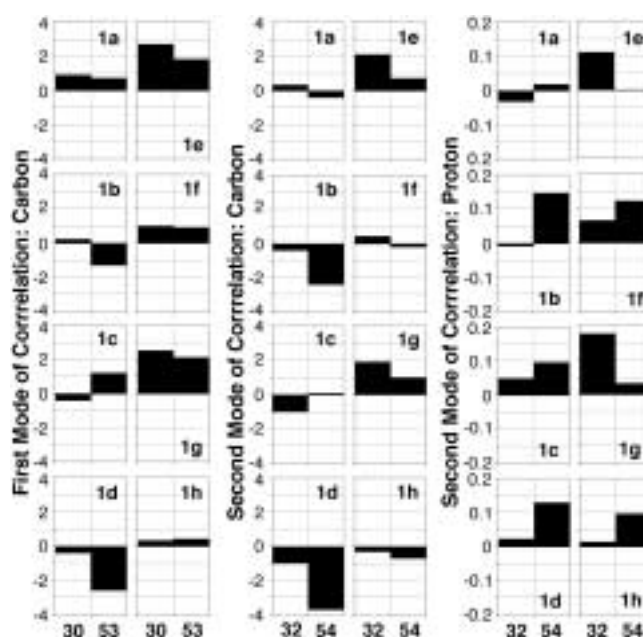
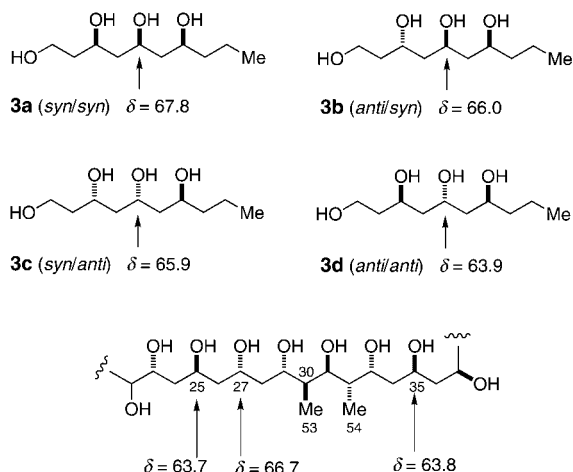


Figure 3. Structural correlation only at C30 and C53, and at C32 and C54. Difference between the chemical shifts of the carbon atoms (100 MHz, $[D_6]DMSO$) and protons (500 MHz, $[D_6]DMSO$) of oasomycin B and those of each of **1a–h**.

For the following two reasons we have reached the conclusion that, of the three candidates emerging from the second mode of correlation, **1a** ($\beta\beta\alpha\alpha$) represents the relative stereochemistry at C30, C31, C32, and C33. First, the relative stereochemistry from C30 to C32 needs to be shared by both modes of correlation, but **1f** ($\alpha\alpha\alpha\beta$) and **1h** ($\beta\alpha\alpha\beta$) do not meet with this condition. Second, as recognized in many cases previously studied,^[8] the ^{13}C and ^1H NMR characteristics are complementary for this type of comparison. Comparison of the chemical shifts of the protons attached at C32 and C54 (Figure 3)^[9] clearly demonstrates that only **1a** ($\beta\beta\alpha\alpha$) matches the corresponding structural portion of the natural products at a satisfactory level.^[6]

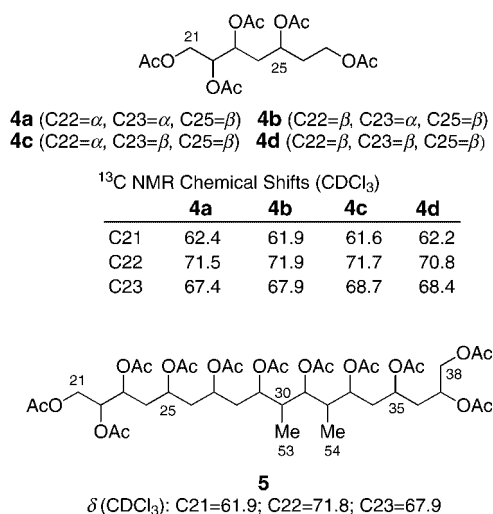
In order to extend the stereochemical information beyond the C29–C33 portion of desertomycins/oasomycins we required a new NMR database.^[10] In line with the analysis presented above, we recognized a possibility that the central carbon atom in a 1,3,5-triol such as $-\text{CH}(\text{OH})\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{CH}(\text{OH})-$ may exhibit a distinctive chemical shift which is *dependent* on the relative stereochemistry at 1/3 and 3/5, but is *independent* of the functional groups present outside of this structural motif. We have demonstrated this indeed to be the case.^[11, 12] The chemical shifts for the central carbon atom of **3a–d** are summarized in Scheme 2 as an example. In general, the chemical shifts for the central carbon atoms of 1,3,5-triols are classified into three subgroups, with those of *anti/anti* triols clustering around $\delta = 64$ ($[D_6]DMSO$), those of *anti/syn* and *syn/anti* triols around $\delta = 66$, and those of *syn/syn* triols around $\delta = 68$. Comparison of this NMR database with the chemical shifts of the carbon atoms reported for the C25 ($\delta = 63.7$ in $[D_6]DMSO$), C27 (66.7), and C35 (63.8) carbon atoms of desertomycins/oasomycins^[4b] immediately predicts the rela-



Scheme 2. Chemical shifts (100 MHz, $[D_6]DMSO$) of the central carbon atoms in 1,3,5-triols **3a–d** and the relevant portion of oasomycin A.

tive stereochemistry at C23, C25, C27, and C29 as well as at C33, C35, and C37 to be the one depicted in Scheme 2.

Finally, in order to address the relative stereochemistry of C22 and C23 we have created one additional NMR database for a 1,2,3,5-tetraol peracetate represented by **4a–d**.^[5, 13] Comparison of this database with the chemical shifts for the carbon atoms found for the C21–C38 degradation product **5**^[3] (Scheme 3) allows the relative stereochemistry at C22 and C23 to be assigned to that found in **4b**.

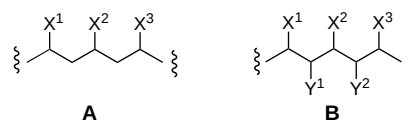


Scheme 3. Chemical shifts (100 MHz, $CDCl_3$) for 1,2,3,5-tetraol peracetates **4a–d** and the relevant portion of the C21–C38 degradation product **5**.

In conclusion, the relative stereochemistry of the C21–C38 portion of desertomycins/oasomycins is predicted to be the one shown in the structure in Scheme 1. This prediction has been made by using only three simple universal NMR databases, and we will present the evidence to prove the predicted relative stereochemistry in the following paper.^[3]

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- [5] The numbering used in this paper corresponds to the desertomycins/oasomycins numbering; see the structure in Scheme 1.
- [6] We have found that differences in ^{13}C and ^1H chemical shifts are within $\Delta\delta = 0.5$ and 0.03 , respectively, in the previous cases studied.
- [7] Steric and/or stereoelectronic interactions between the structural motifs connected either directly or through a bridge of one methylene group are significant, whereas interactions between the structural motifs connected through a bridge of two or more methylene groups are almost negligible; see the examples given in the refs. [2a, b] and refs. [3a, b] quoted in ref. [1].
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- [10] We created an NMR database for a 1,3-diol using the *syn* and *anti* diastereomers of $Me(CH_2)_2CH(OH)CH_2CH(OH)(CH_2)_4OH$.^[11] This database was then applied in a stepwise manner to the C33/C35, C35/C37, C29/C27, C27/C25, and C25/C23 1,3-diol systems, which predicted the same relative stereochemistry as the one derived from the approach outlined in this paper. We also created an NMR database for a 1,2,4-triol peracetate using the diastereomers of $AcOCH_2CH(OAc)CH_2CH(OAc)(CH_2)_2OAc$ [^{13}C NMR (100 MHz, $CDCl_3$): *syn* diastereomer: $\delta = 60.4$ (C33), 32.9 (C34), 67.8 (C35), 35.1 (C36), 68.4 (C37), 64.6 (C38); *anti* diastereomer: $\delta = 60.6$ (C33), 33.6 (C34), 66.9 (C35), 35.3 (C36), 67.4 (C37), 65.1 (C38)]. This NMR data also suggested the same relative configuration at C35 and C37 as the one given in the text.
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- [12] We recognized that 1,3,5-trisubstituted acyclic compounds, represented by **A**, might exhibit this distinctive characteristic, and chose **3a–d** as an experimental example to demonstrate this. In our view, this phenomenon can be extended to a more generalized structure such as **B**.



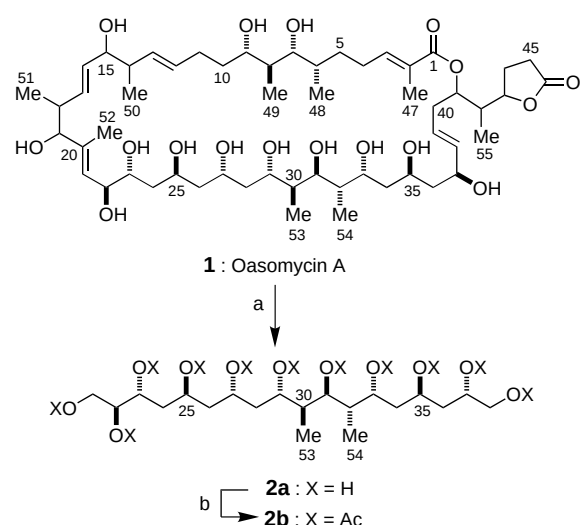
- [13] **4a–d** were prepared in nine steps: 1) (2*S*)-[2-(phenylmethoxy)ethyl]oxirane, $CH_2=CHMgBr$, $CuBr$, THF; 2) TBSCl, imidazole, CH_2Cl_2 ; 3) OsO_4 , NMO, acetone/ H_2O , followed by $Pb(OAc)_4$, benzene; 4) $CH_2=CHMgBr$, THF; 5) CSA, MeOH; 6) $PhCH(OMe)_2$, CSA, benzene, followed by chromatographic separation; 7) AD-mix- α or - β , $tBuOH/H_2O$; 8) H_2 , Pd/C, MeOH; 9) Ac_2O , Py, DMAP. CSA = 10-camphorsulfonic acid; DMAP = 4-dimethylaminopyridine; NMO = *N*-methylmorpholine *N*-oxide; Py = pyridine; TBS = *tert*-butyldimethylsilyl; THF = tetrahydrofuran. ^{13}C NMR (100 MHz, $CDCl_3$): **4a**: $\delta = 62.4$ (C21), 71.5 (C22), 67.4 (C23), 35.1 (C24), 66.8 (C25), 33.6 (C26), 60.5 (C27); **4b**: $\delta = 61.9$ (C21), 71.9 (C22), 67.9 (C23), 34.4 (C24), 66.7 (C25), 33.6 (C26), 60.5 (C27); **4c**: $\delta = 61.6$ (C21), 71.7 (C22), 68.7 (C23), 34.6 (C24), 68.0 (C25), 32.6 (C26), 60.4 (C27); **4d**: $\delta = 62.2$ (C21), 70.8 (C22), 68.4 (C23), 35.1 (C24), 67.8 (C25), 32.8 (C26), 60.3 (C27).

Stereochemical Assignment of the C21–C38 Portion of the Desertomycin/Oasomycin Class of Natural Products by Using Universal NMR Databases: Proof**

Choon-Hong Tan, Yoshihisa Kobayashi, and Yoshito Kishi*

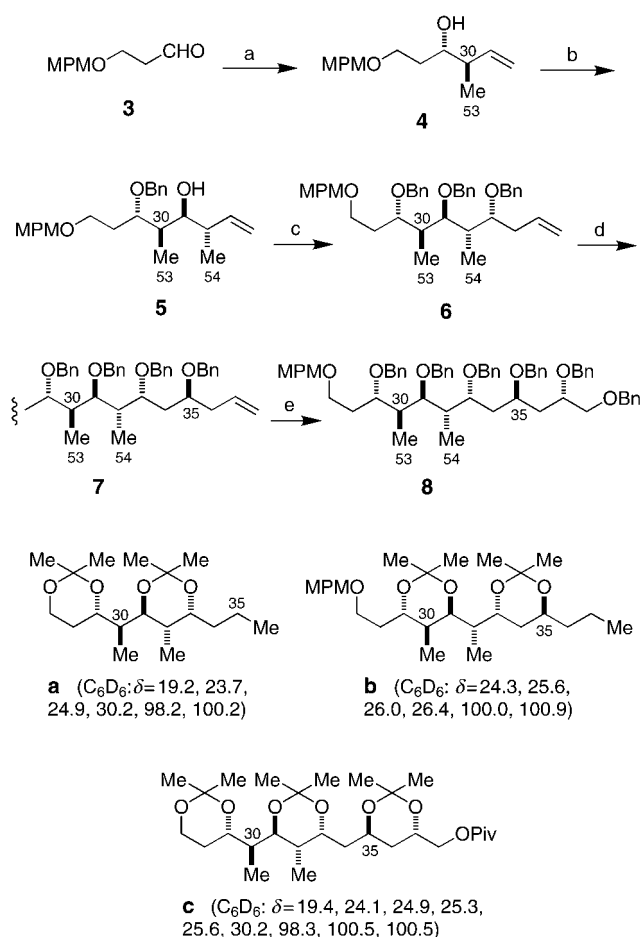
We have predicted the relative stereochemistry of the C21–C38 portion of the desertomycin/oasomycin class of natural products from three simple universal NMR databases.^[1] Herein we report a stereoselective synthesis of the C21–C38 degradation product with the predicted relative stereochemistry, thereby proving our prediction.

The C21–C38 degradation product **2a** was obtained in two steps from oasomycin A (**1**; Scheme 1). For practical reasons the degradation product **2a** was isolated and fully characterized as its peracetate **2b**.^[2, 3]



Scheme 1. Reagents and conditions: a) 1. O₃, MeOH, –78 °C; 2. NaBH₄; b) Ac₂O, Py, DMAP, 60 % (3 steps). See ref. [16] for abbreviations.

Scheme 2 outlines a stereoselective synthesis of the right half of the predicted diastereomer. The C29–C32 moiety corresponds to one of the eight diastereomers used for the creation of the contiguous dipropionate NMR database, and we have shown that this particular diastereomer can be obtained with high stereoselectivity by two consecutive Brown crotylboration.^[4, 5] Indeed, the first crotylboration gave the desired *anti* enantiomer **4** with 92 % *ee*, whereas the second crotylboration gave the desired diastereomer **5** in a 10:1 diastereoselectivity with > 98 % *ee*.^[6, 7] In these reactions the relative stereochemistry at C29/C30 and C31/C32 was set



Scheme 2. Reagents and conditions: a) *t*BuOK, (*E*)-2-butene, *n*BuLi, (+)-(Ipc)₂BOMe, BF₃·OEt₂, THF, –78 °C, 62 %; b) 1. BnBr, NaH, *n*Bu₄NI, DMF, RT; 2. OsO₄, NMO, acetone/H₂O, RT; 3. Pb(OAc)₄, benzene, RT; 4. *t*BuOK, (*E*)-2-butene, *n*BuLi, (–)-(Ipc)₂BOMe, BF₃·OEt₂, THF, –78 °C, 60 % (4 steps); c) 1. BnBr, NaH, *n*Bu₄NI, DMF, RT; 2. OsO₄, NMO, acetone/H₂O, RT; 3. Pb(OAc)₄, benzene, RT; 4. (+)-(Ipc)₂BOMe, CH₂=CHCH₂MgBr, THF, –78 °C; 5. BnBr, NaH, *n*Bu₄NI, DMF, RT, 73 % (5 steps); d) 1. OsO₄, NMO, acetone/H₂O, RT; 2. Pb(OAc)₄, benzene, RT; 3. (–)-(Ipc)₂BOMe, CH₂=CHCH₂MgBr, THF, –78 °C; 4. BnBr, NaH, *n*Bu₄NI, DMF, RT, 67 % (4 steps); e) 1. OsO₄, Corey (*S,S*)-ligand, CH₂Cl₂, –90 °C, 87 %; 2. BnBr, NaH, *n*Bu₄NI, DMF, RT, 88 %. See ref. [16] for abbreviations.

through the *E* configuration of butene, whereas the absolute configuration was set by the configuration of (Ipc)₂BOMe. The C33 and C35 stereogenic centers were then introduced through two consecutive Brown allylations with diastereoselectivities of > 15:1 and 15:1, respectively.^[4, 7] In these transformations the chirality of the (Ipc)₂BOMe used determined the absolute configuration of the newly introduced stereogenic center, and the stereochemistry of the major product in each step was assigned on the basis of literature precedents. This assignment was further confirmed through ¹³C NMR analysis^[8] of the 1,3-acetonides **a** and **b**, derived from **6** and **7**, respectively (Scheme 2, compare the chemical shifts of the carbon atoms given for the acetonide moieties).

The asymmetric dihydroxylation of **7** in the presence of the Corey (*S,S*)-ligand,^[9] followed by benzylation, furnished the right-half segment **8** as an 8:1 mixture of two diastereomers.^[7] The stereochemistry of the major product was assigned from

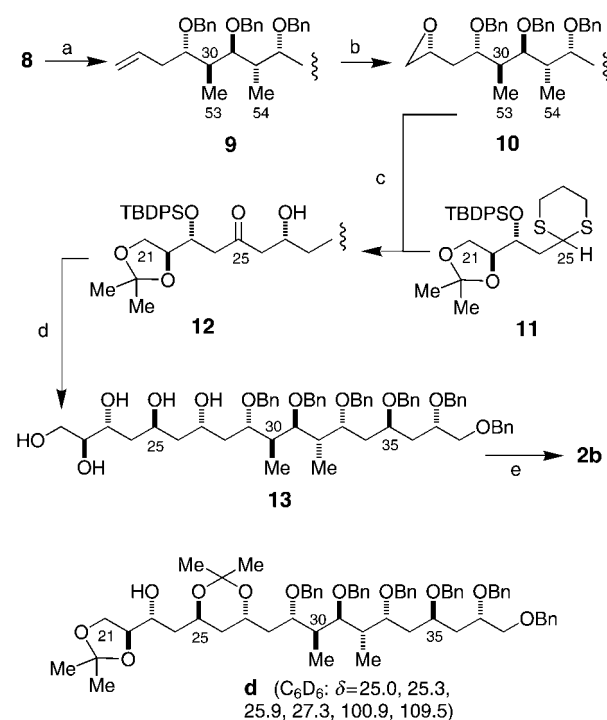
[*] Prof. Dr. Y. Kishi, Dr. C.-H. Tan, Dr. Y. Kobayashi
Department of Chemistry and Chemical Biology
Harvard University
Cambridge, MA 02138 (USA)
Fax: (+1) 617-495-5150
E-mail: kishi@chemistry.harvard.edu

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literature precedents, which was further supported by the observation that the dihydroxylation carried out with the Corey (*R,R*)-ligand yielded a 1:>15 mixture of the two diastereomers. This stereochemical assignment was confirmed through ^{13}C NMR analysis of the 1,3-acetonide **c**, derived from **8** (Scheme 2).

After completion of the synthesis of the right-hand side of the molecule, the left terminus of **8** was transformed to the olefinic group in three standard synthetic steps. Asymmetric dihydroxylation of the olefin **9** was conducted again in the presence of the Corey (*R,R*)-ligand^[9] to yield the desired diol (stereoselectivity >15:1).^[10] The diol was converted into the α -epoxide **10**, which was treated with the anion generated from the dithiane **11**^[11] to give **12** in 79% yield. After deprotection of the dithiane, **12** was subjected to reduction with $\text{Me}_4\text{NBH}(\text{OAc})_3$.^[12] As scrambling of the *tert*-butyldiphenylsilyloxy and acetonide groups was observed under the reduction condition employed, the product was isolated and characterized after the deprotection. In this way the stereoselectivity of the reduction was estimated to be approximately 10:1.^[7] The stereochemistry of the major diastereomer produced in the $\text{Me}_4\text{NBH}(\text{OAc})_3$ reduction was assigned to be an *anti*-diol on the basis of literature precedents,^[12] and this assignment was confirmed through the ^{13}C NMR analysis of acetonide **d** derived from **13** (Scheme 3).^[13]

Debenzylation and then acetylation of **13** furnished the synthetic peracetate **2b**. A comparison of the spectroscopic (^1H NMR (Figure 1), ^{13}C NMR, and MS)^[14] and chromatographic data of the synthetic peracetate with the peracetate



Scheme 3. Reagents and conditions: a) 1. DDOQ, $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, RT; 2. DMP, CH_2Cl_2 , RT; 3. $\text{CH}_3\text{PPh}_3\text{Br}$, *n*BuLi, THF, 84% (3 steps); b) 1. OsO_4 , Corey (*R,R*)-ligand, CH_2Cl_2 , -90°C , 85%; 2. Ts-imidazole, NaH, THF, 73%; c) 1. **11**, *n*BuLi, THF, -20°C , 2 h; then **10**, 14 h, 79%; 2. $\text{Hg}(\text{ClO}_4)_2 \cdot 3\text{H}_2\text{O}$, THF/ H_2O , 86%; 3. 1. $\text{Me}_4\text{NBH}(\text{OAc})_3$, AcOH, acetone, 91%; 2. aq HF, MeCN, 76%; e) 1. H_2 , Pd/C, MeOH, RT; 2. Ac_2O , Py, DMAP, 60% (2 steps). See ref. [16] for abbreviations.

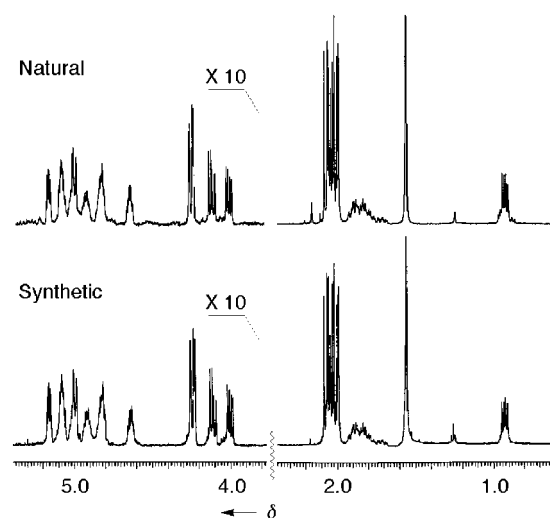
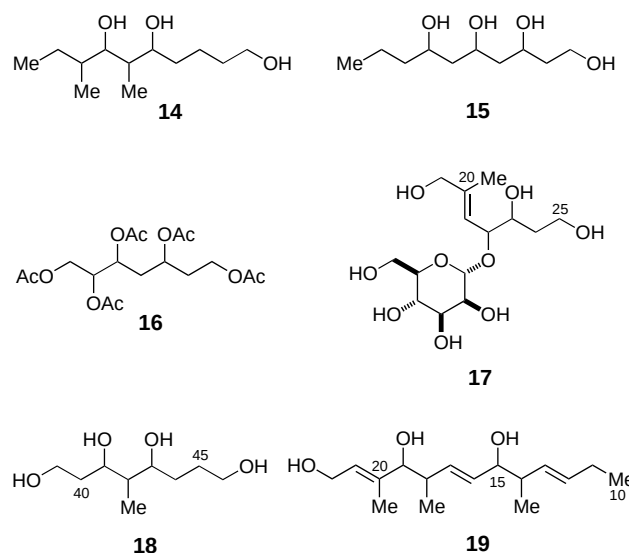


Figure 1. Proton NMR spectra (500 MHz, CDCl_3) of the C21–C38 degradation product **2b** of oasomycins. The signal intensity in the $\delta = 4$ –5 region is ten times that of the $\delta = 1$ –2 region.

derived from oasomycin A has unambiguously demonstrated their identity, thereby proving the predicted relative stereochemistry for the C21–C38 portion of the desertomycin/oasomycin class of natural products. In addition, its absolute configuration was concluded to be the one shown in structure **1** (Scheme 1) on the basis of the sign of its $[\alpha]_D^{20}$ value.^[14] Related to this, it is worthwhile mentioning a possibility that the assignment of the absolute configuration could be achieved through an NMR database such as **17**^[15] (Scheme 4).



Scheme 4. Chemical structures of **14**–**19**.

In conclusion, using only three simple universal NMR databases **14**–**16** (Scheme 4), we have predicted the stereochemistry of the C21–C38 portion of the desertomycin/oasomycin class of natural products and have proven the predicted stereochemistry by a stereoselective synthesis of the C21–C38 degradation product. We are currently creating two

additional databases **18** and **19** (Scheme 4) to establish the complete structure of the desertomycin/oasomycin class of natural products.

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- [3] We thank Dr. Gerhard Kretzschmar for samples of oasomycins A and B.
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- [10] Dihydroxylation of **9** in the presence of the Corey (*S,S*)-ligand gave a 1:7 mixture of the two diols.
- [11] The dithiane **11** was prepared from L-(+)-arabinose. By using Gray's procedure (M. Y. H. Wong, G. R. Gray, *J. Am. Chem. Soc.* **1978**, *100*, 3548), L-(+)-arabinose was transformed to the diethylthioacetal, corresponding to structure **4** in Gray's paper. This diethylthioacetal was then subjected to the following three steps: 1) TBDPSCl, imidazole, DMF, RT; 2) HgO, HgCl₂, acetone/H₂O (10/1), RT; 3) 1,3-propanedithiol, BF₃·OEt₂, CH₂Cl₂, 0°C (26% overall yield from L-(+)-arabinose).
- [12] D. A. Evans, K. T. Chapman, E. M. Carreira, *J. Am. Chem. Soc.* **1988**, *110*, 3560.
- [13] Acetonization (MeC(OMe)₂Me, acetone, CSA) of **13** yielded an approximately 1:1:1 mixture of the acetonides. The acetonide **d** was isolated in a pure form by chromatography on silica gel.
- [14] **2b** (synthetic): ¹H NMR (500 MHz, CDCl₃): see Figure 1; ¹³C NMR (100 MHz, CDCl₃): δ = 9.4, 10.7, 20.8–21.2 (overlapped), 34.8, 35.5, 36.6, 37.0, 37.3, 37.6, 39.1, 61.9, 65.1, 65.6, 67.1, 67.2, 67.6, 67.9, 71.5, 71.6, 71.8, 77.2, 169.9–170.5 (overlapped); [α]_D²⁰ = +5.7 (*c* = 0.23 in MeOH). **2b** (natural): ¹H NMR (500 MHz, CDCl₃): see Figure 1; ¹³C NMR (100 MHz, CDCl₃): identical to synthetic **2b**; [α]_D²⁰ = +7.8 (*c* = 0.25 in MeOH).
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- [16] Abbreviations: Ac = acetyl; Bu = butyl; Bn = benzyl; CSA = 10-camphorsulfonic acid; DMAP = 4-dimethylaminopyridine; DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DMF = *N,N*-dimethylformamide; DMP = Dess–Martin periodinane; DMSO = dimethylsulfoxide; Ipc = isopinocampheyl; MPM = 4-methoxyphenylmethyl; NMO = *N*-methylmorpholine *N*-oxide; Piv = pivaloyl = trimethylacetyl; Py = pyridine; TBS = *tert*-butyldimethylsilyl; TBDPS = *tert*-butyldiphenylsilyl; THF = tetrahydrofuran; Ts = toluene-4-sulfonyl.

Tuning the Regiospecificity of Cleavage in Fe^{III} Catecholate Complexes: Tridentate Facial versus Meridional Ligands**

Du-Hwan Jo and Lawrence Que, Jr.*

Bacterial catechol dioxygenases are a component of nature's strategy for degrading aromatic molecules to aliphatic products in the environment.^[1] These enzymes catalyze the oxidative cleavage of catechols, which leads to the scission of the C1–C2 (intradiol) or C2–C3 (extradiol) bond. Intradiol-cleaving enzymes have an iron(III) active site, while extradiol-cleaving enzymes require Fe^{II} or Mn^{II}.^[1, 2] To date the factors that determine the regiospecificity of cleavage are not well understood. Most biomimetic studies have focused on iron(III) catecholate complexes with tetradentate ligands, all of which performed only intradiol cleavage.^[3] However, the few examples of iron(III) catecholate complexes having tridentate ligands result in at least some extradiol cleavage products.^[4] To further investigate the factors that determine the cleavage site, we characterized a series of mononuclear iron(III) catecholate complexes [(L)Fe(DBC)Cl] [L = Me₃-TACN (**1**), TPY (**2**), BnBPA (**3**)],^[5] containing tridentate ligands that can coordinate the metal center in a facial or meridional fashion. Their reactivity towards O₂ provides insight into the factors that tune the regiospecificity of cleavage.

The reactions of [(L)FeCl₃], DBCH₂, and NaOCH₃ in a 1:1:2 ratio in CH₂Cl₂ under N₂ afforded complexes **1–3** as purple-black solids, which were recrystallized from THF/hexane, acetone, and DMF/Et₂O, respectively. All of these complexes have high-spin iron(III) centers and exhibit two intense catecholate-to-iron(III) charge transfer bands in the spectral region of 400–1000 nm, similar to [(TPA)-Fe^{III}(DBC)]BPh₄ (**4**).^[3c] The crystal structures of **1** and **2** (Figure 1) reveal a distorted octahedral geometry with a facial (**1**) or meridional (**2**) tridentate ligand, a catecholate dianion, and a chloride ligand at the sixth coordination site.^[6] The Fe–N and Fe–O bond lengths in both complexes are typical of high-spin iron(III) complexes. The fairly long Fe–Cl bond lengths (average 2.385(2) Å for **1** and 2.325(1) Å for **2**) suggest that the chloride ligand should be highly labile in solution.

Complexes **1–3** react with O₂ in the presence of one equivalent of AgOTf to afford oxidative cleavage products of DBC. The addition of silver salt removes the chloride ligand to generate an empty coordination site on the metal center and enhances the reactivity of the complex toward O₂.^[7] Complex **1**, with the *fac*-Me₃TACN ligand, affords only

[*] Prof. Dr. L. Que, Jr., Dr. D.-H. Jo

Department of Chemistry and Center for Metals in Biocatalysis
University of Minnesota, Minneapolis, MN 55455 (USA)
Fax: (+1) 612-624-7029
E-mail: que@chem.umn.edu

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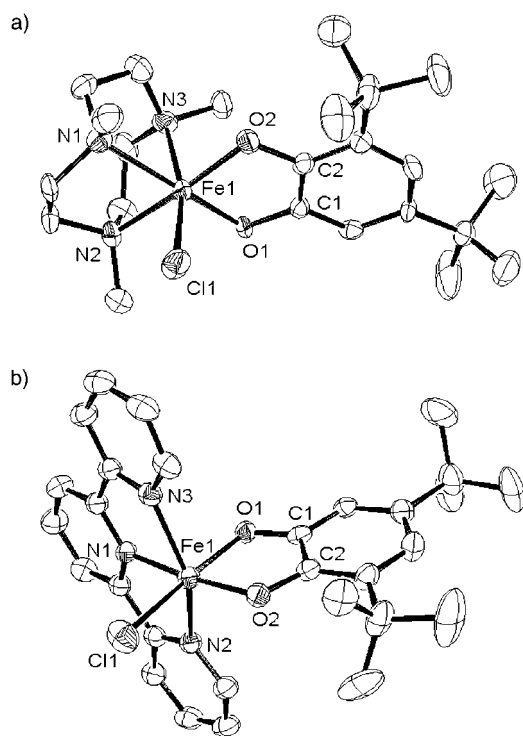


Figure 1. ORTEP plots for **1A** (a) and **2** (b) with hydrogen atoms omitted for clarity. The structure of **1B**, the other molecule in the asymmetric unit of **1**, is not shown. Selected bond lengths [Å] for **1A** and corresponding values for **1B** in brackets: Fe1–N1 [Fe2–N4] 2.226(6) [2.232(6)], Fe1–N2 [Fe2–N5] 2.202(6) [2.195(6)], Fe1–N3 [Fe2–N6] 2.276(6) [2.278(6)], Fe1–O1 [Fe2–O3] 1.941(4) [1.954(4)], Fe1–O2 [Fe2–O4] 1.920(5) [1.914(4)], Fe1–Cl1 [Fe2–Cl2] 2.381(2) [2.388(2)], O1–C1 [O3–C24] 1.361(8) [1.369(8)], O2–C2 [O4–C25] 1.366(8) [1.359(8)]. Selected bond lengths [Å] for **2**: Fe1–N1 2.137(2), Fe1–N2 2.143(2), Fe1–N3 2.173(3), Fe1–O1 1.990(2), Fe1–O2 1.913(2), Fe1–Cl1 2.325(1), O1–C1 1.329(3), O2–C2 1.343(9).

extradiol cleavage products (3,5- and 4,6-di-*tert*-butyl-2-pyrone) in nearly quantitative yield (97%). Previously, we reported that the TACN analogue of **1** also afforded extradiol cleavage products in nearly quantitative yield, but the addition of pyridine was needed to direct the reaction from simple auto-oxidation to form quinone to the desired cleavage.^[4b, 8] Complex **2**, with the *mer*-TPY ligand, gives 3,5-di-*tert*-butyl-benzoquinone (78%) and the intradiol cleavage product 3,5-di-*tert*-butyl-1-oxacyclohepta-3,5-diene-2,7-dione (20%). Interestingly, **3** yields both extradiol (72%) and intradiol (25%) cleavage products. These results must be viewed in light of previously published papers which showed

that [(L)Fe^{III}(DBC)] complexes with tetradentate ligands gave high yields of intradiol cleavage products (e.g., 98% for **4**).^[3]

Table 1 summarizes the results for [(L)Fe^{III}(DBC)] complexes reported thus far. The accumulated results show that the coordination geometry imposed by the ancillary ligand controls the regioselectivity of the oxidative cleavage reaction. Figure 2 shows a mechanistic scheme we propose to

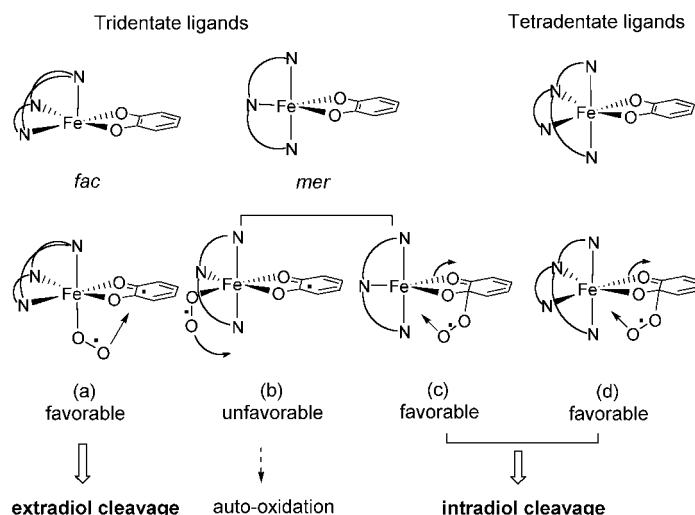


Figure 2. Proposed mechanism for the reaction of O₂ with [(L)Fe(DBC)] complexes.

rationalize the results. As discussed previously, the low energy of the catecholate-to-Fe^{III} charge-transfer transitions confers an enhanced iron(II) semiquinonate character on the complexes, which is reflected in their spectroscopic properties.^[3b, 3c] Thus two potential sites exist for interaction with O₂: the metal center and the bound catecholate, and the nature of the products is determined by the initial site of O₂ attack. For [(L)Fe^{III}(DBC)] complexes with tetradentate ligands, the metal center is coordinatively saturated, so O₂ attacks the catecholate to give rise to a bidentate peroxo intermediate that leads to intradiol cleavage, consistent with the mechanism of substrate activation proposed for intradiol-cleaving dioxygenases (Figure 2d).^[1b,c] For complexes with tridentate ligands, the metal center has an available site for O₂ attack. For **1**, with the *fac*-Me₃TACN ligand, only extradiol cleavage is observed. We propose that O₂ exclusively binds to the metal center and becomes activated to attack the bound

Table 1. Cleavage products obtained upon reaction of [(L)Fe^{III}(DBC)] with O₂.

L	Solvent	Intradiol [%]	Extradiol ^[a] [%]	Quinone [%]	Ref.
TACN	CH ₂ Cl ₂	–	3	82	[4b]
TACN	CH ₃ CN	–	35 (30/5)	65	[4a]
TACN + 20 equiv 4-picoline	CH ₂ Cl ₂	–	98 (78/20)	trace	[4b]
Me ₃ TACN	CH ₂ Cl ₂	–	97 (83/14)	–	this work
TPY	CH ₂ Cl ₂	20	–	78	this work
BnBPA	CH ₂ Cl ₂	25	72 (62/10)	–	this work
Tp ^{Pr2}	toluene	33	67 (39/28)	–	[4c]
tetradentate ligands	DMF, CH ₃ CN	84–98	–	–	[3a–e, h]

[a] Ratio in parentheses is that of 3,5- to 4,6-di-*tert*-butyl-2-pyrone.

catecholate. The facial nature of the ancillary ligand allows the two reactants to occupy the opposite face and form a tridentate peroxo intermediate that leads to extradiol cleavage (Figure 2a). This ideal geometry cannot be attained for **2**, a complex of the tridentate *mer*-TPY, which does not afford extradiol cleavage. Although O₂ can bind to the metal center in this complex, it is constrained to be in the same plane as the catecholate dianion, which it hence cannot attack (Figure 2b). Instead, the metal center acts as a conduit for electrons from substrate to O₂, and this generates quinone (78%) as the major product. The minor amount of intradiol cleavage (20%) observed is due to the attack of O₂ on the bound substrate (Figure 2c). In the case of **3**, in which the tridentate BnBPA has the flexibility to act as a facial or meridional ligand, both intradiol and extradiol cleavage products are observed.^[4c] The major extradiol cleavage product is formed by a mechanism analogous to that proposed for **1**, while the minor intradiol cleavage product results from attack of O₂ on bound substrate, as for the complexes with tetradentate ligands.

The above model studies show that a facial tridentate ancillary ligand is critical to elicit extradiol cleavage. In our proposed mechanism, the facial ligand allows O₂ and substrate to occupy the opposite face and form an intermediate that leads to the desired extradiol products. Such an intermediate may resemble the crystallographically characterized tridentate peroxo species derived from the reaction of catecholate complexes of iridium and rhodium with O₂.^[9] The facial tridentate ligand would then correspond to the common 2-His-1-carboxylate facial triad^[10] found in the active sites of several extradiol dioxygenases, despite their having differing tertiary structures.^[11] Our current model complexes are imperfect since they contain iron(III), not iron(II), centers; however, they do elicit the desired extradiol cleavage. We also do not yet understand how extradiol cleavage occurs once the key peroxo intermediate is formed. Attempts are in progress to trap this intermediate to gain further insight into the mechanism of this novel class of dioxygenase enzymes.

Experimental Section

Syntheses of complexes: All Fe^{III} catecholate complexes were prepared under nitrogen atmosphere. [(L)FeCl₃] (1.0 mmol)^[12] was dissolved in CH₂Cl₂ (30 mL), and a solution of DBCH₂ (1.0 mmol) and NaOCH₃ (2.2 mmol) in CH₂Cl₂ was slowly added. The mixture was stirred for 2 h, dried by evaporation in vacuo, then recrystallized from an appropriate solvent to give purple-black crystals. **1**: recrystallized from THF/hexane (yield 90%); elemental analysis calcd for **1**·0.5 THF (C₂₅H₄₅ClFeN₃O_{2.5}): C 57.86, H 8.74, N 8.10, Cl 6.83; found: C 57.96, H 8.65, N 8.20, Cl 7.03; electronic spectrum (CH₃CN): λ_{max} (ε) = 496 (1700), 820 nm (2100). **2**: recrystallized from CH₂Cl₂/Et₂O and then DMF/Et₂O (yield 60%); elemental analysis calcd for **2**·DMF (C₃₂H₃₈ClFeN₄O₃): C 62.19, H 6.20, N 9.07, Cl 5.90; found: C 61.85, H 6.23, N 8.98, Cl 5.44; electronic spectrum (CH₃CN): λ_{max} (ε) = 524 (1500), 830 nm (2100). **3**: recrystallized from acetone (yield 80%); elemental analysis calcd for **3** (C₃₃H₃₉ClFeN₃O₂): C 65.95, H 6.54, N 6.99, Cl 5.90; found: C 65.88, H 6.44, N 6.84, Cl 5.78; electronic spectrum (CH₃CN): λ_{max} (ε) = 520 (1200), 850 nm (2300).

Reactions with O₂: 0.05 mmol of complex and AgOTf were dissolved in CH₂Cl₂ (10 mL) and exposed to O₂ for 3 h. The solution was concentrated, and the organic products were extracted with diethyl ether (3 × 5 mL). The solid residue was then acidified with 3 N HCl to pH 3 to decompose the

metal complexes and extracted with diethyl ether (3 × 5 mL). The combined diethyl ether extracts were dried over anhydrous Na₂SO₄, concentrated, subjected to GC analysis (Hewlett-Packard 6890 Series gas chromatograph with a DB-5 column), and quantified by using 1,1'-biphenyl as internal standard.

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- [5] Abbreviations: BnBPA = *N*-benzyl *N,N*-bis(2-pyridylmethyl)amine; DBCH₂ = 3,5-di-*tert*-butylcatechol; Me₃TACN = 1,4,7-trimethyl-1,4,7-triazacyclononane; TACN = 1,4,7-triazacyclononane; TPA = tris(2-pyridylmethyl)amine; Tp^{tr2} = hydrotris(3,5-diisopropyl-1-pyrazolyl)-borate; TPY = 2,2':6',2''-terpyridine.
- [6] Crystal structure analysis: The data were collected on a Siemens SMART platform diffractometer equipped with a CCD detector (MoK_α radiation; λ = 0.71073 Å). The structures were solved by direct methods and refined by full-matrix least-squares methods on F² by using the SHELXTL Plus program package (Version 5.1, Bruker Analytical X-Ray Systems, Madison, WI). All non-hydrogen atoms were refined with anisotropic thermal parameters, and all hydrogen atoms were placed in ideal positions and refined as riding atoms with individual isotropic displacement parameters. Crystal data for **1** at 173(2) K: C₂₅H₄₅ClFeN₃O_{2.5}, M_r = 518.94, purple-black crystal of dimensions 0.6 × 0.38 × 0.08 mm, triclinic, space group P $\bar{1}$, a = 13.0395(2), b = 15.5233(2), c = 16.7486(1) Å, α = 113.703(1), β = 111.192(1), γ = 92.132(1)°, V = 2827.35(6) Å³, Z = 4, ρ_{calcd} = 1.219 g cm⁻³. On the basis of 9513 unique reflections (R_{int} = 0.0572) and 633 variable parameters, final R values (I > 2σ(I)): R1 = 0.0787, wR2 = 0.1821. Crystal data for **2** at 173(2) K: C₃₂H₃₈ClFeN₄O₃, M_r = 617.96, purple crystal of dimensions 0.40 × 0.12 × 0.05 mm, monoclinic, space group P2₁/c, a = 12.3347(9), b = 13.552(11), c = 19.179(1) Å, α = 90, β = 97.426(2), γ = 90°, V = 3197.1(4) Å³, Z = 4, ρ_{calcd} = 1.291 g cm⁻³. On the basis of 7190 unique reflections (R_{int} = 0.0393) and 409 variable parameters, final R values (I > 2σ(I)): R1 = 0.0512, wR2 = 0.1450. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-145996 (**1**) and CCDC-145997 (**2**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [7] Without AgOTf, the reactions require at least 24 h for completion but afford similar cleavage products.
- [8] Given that **1** affords nearly quantitative extradiol cleavage without the addition of pyridine, we do not currently understand the role that pyridine plays in the cleavage reaction with the TACN complex.

Clearly more work needs to be done on the apparently more complex TACN system.

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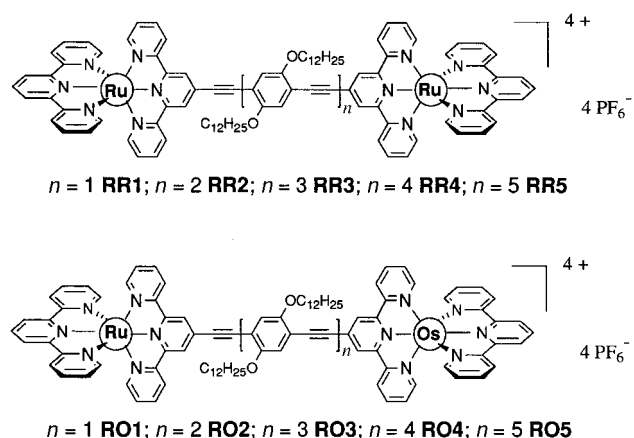
An Unusually Shallow Distance-Dependence for Triplet-Energy Transfer**

Anthony Harriman,* Abderrahim Khatyr, Raymond Ziessel,* and Andrew C. Benniston

There have been several reports of how the rate of intramolecular electron transfer^[1] or triplet-energy transfer by electron exchange^[2] decreases with increased separation of the reactants and, in many cases, an attenuation factor (β) describing an exponential drop-off has been noted. The attenuation factor, which is often better expressed in terms of the number of interspersed repeat units rather than distance,^[3] has values ranging from around 0.05 to 1.7 Å⁻¹ according to the nature of the connecting framework and the process under investigation.^[4] It is recognized that the concept of an exponential-type attenuation factor applies only to those reactions that occur by way of superexchange^[3] and, in other cases, the distance dependence might take on a different form.^[5] With regard to triplet-energy transfer, this latter requisite demands that the transfer process involves electron exchange rather than dipole–dipole interactions. It is also important to ensure that the triplet energy of the interspersed connector unit does not fall below that of the donor triplet.

This realization presents a particular problem with regard to the construction of long bridges from conjugated subunits where, as happens for polyacetylenes,^[6] the triplet energy of the connector decreases with increasing length. In such cases, long-range triplet energy transfer will be replaced by sequential short-range steps that give a deceptively small β value.^[7] Herein we report that superexchange-type behavior is retained over more than 50 Å when the polyacetylene is doped with phenylene groups. This system provides an unusually small attenuation factor for triplet energy transfer.^[8, 9]

The required set of heterodinuclear complexes (Scheme 1), comprising ruthenium(II) and osmium(II) bis(2,2':6',2''-terpyridine) terminals separated by a 1,4-diethynylene-2,5-dialkoxybenzene connector, was prepared in a two-step procedure.



Scheme 1. The dinuclear Ru^{II}-based complexes and the corresponding mixed-metal Ru^{II}/Os^{II} complexes.

First, the soluble polytopic ligands were treated with [Ru(terpy)(dmsO)Cl₂]^[10] (terpy = 2,2':6',2''-terpyridine), dehalogenated with silver, in a mixture of dichloromethane and methanol to give the corresponding mono-Ru^{II} complexes in 54 to 81% yield. These complexes, which contain an uncoordinated terpy fragment, were subsequently treated with [Os(terpy)(O)₂(OH)](NO₃)·2H₂O in aqueous tetrahydrofuran using excess hydrazine hydrate as reducing agent.^[4] The resultant mixed-metal Ru/Os hybrids were precipitated as PF₆⁻ salts and purified by column chromatography on alumina prior to recrystallization from dichloromethane/hexane. Characterization was made by full spectroscopic and elemental analyses, including FAB⁺ mass spectra which exhibited a molecular ion peak with the expected isotopic distributions and no significant peaks at higher mass. The corresponding dinuclear ruthenium(II) complexes were prepared according to a similar protocol using a slight excess of the metal precursor. Synthesis of the rationally designed polytopic ligands requires an iterative strategy based on palladium-promoted cross-coupling reactions to extend the central spacer framework outwards from the 1,4-diethynylene-2,5-diiododecyloxybenzene core.^[11]

Photophysical properties for the dinuclear ruthenium(II) complexes were recorded in deoxygenated acetonitrile at 20 °C following excitation at 532 nm. In particular, the luminescence maxima (λ_{MAX}), quantum yields (Φ_{LUM}), and lifetimes (τ_{LUM}) were as expected for an alkynylene-substi-

[*] Prof. A. Harriman
Department of Chemistry
University of Newcastle
Newcastle-upon-Tyne, NE1 7RU (UK)
Fax: (+44)191-222-8664
E-mail: Anthony.Harriman@newcastle.ac.uk

Dr. R. Ziessel, A. Khatyr
Laboratoire de Chimie
d'Electronique et de Photonique Moléculaires, Ecole Chimie
Polymères, Matériaux (ECPM)
UPRES-A 7008 au CNRS 25 rue Becquerel
67087 Strasbourg Cedex (France)
Fax: (+33)388-13-68-95
E-mail: ziessel@chimie.u-strasbg.fr

Dr. A. C. Benniston
Department of Chemistry, University of Glasgow, Glasgow G12 8QQ (UK)

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tuted ruthenium(II) bis(terpyridine) complex;^[12] such compounds are notably more emissive than the unsubstituted parent compounds. More importantly, the photophysical properties are quite insensitive to the length of the connecting unit (Table 1). This observation, taken together with the characteristic emission profile,^[12] strongly supports the idea that the triplet state is localized on one of the terminal metal

Table 1. Photophysical properties recorded for the various mixed-metal Ru/Os and dinuclear Ru^{II}-based complexes recorded in deoxygenated acetonitrile at 20 °C. Luminescence is from the Ru^{II}-based terminal ($\lambda_{\text{EXCT}} = 532$ nm) in the **RR** series but from the Os^{II}-based fragment ($\lambda_{\text{EXCT}} = 648$ nm) in the **RO** series.

<i>n</i>	Compound	λ_{LUM} [nm]	Φ_{LUM}	τ_{LUM} [ns]	Compound	λ_{LUM} [nm]	Φ_{LUM}	τ_{LUM} [ns]
1	RR1	693	0.0028	135	RO1	730	0.0086	165
2	RR2	695	0.0025	125	RO2	735	0.0075	140
3	RR3	692	0.0030	140	RO3	735	0.0085	160
4	RR4	694	0.0027	130	RO4	730	0.0088	160
5	RR5	695	0.0028	130	RO5	740	0.0080	155

complexes. Even for the longest connector (**RR5**) there is no indication that the triplet state associated with the polytopic ligand is populated to any reasonable extent. Laser flash photolysis studies confirmed the impression that the lowest-energy triplet state involves the terminal units, not the connector, since the transient absorption spectra show clear bleaching of the metal-to-ligand, charge-transfer absorption band associated with the metal complex. As such, the triplet state of the connector must lie above that of the terminal metal complex ($E_{\text{T}} = 15230 \text{ cm}^{-1}$)^[12] despite the apparent extended conjugation running along the molecular axis.

Similar photophysical studies were made for the mixed-metal Ru/Os complexes in deoxygenated acetonitrile at 20 °C. Initially, the excitation wavelength was 648 nm, where only the Os^{II}-(terpy) chromophore absorbs incident photons. This allows the photophysical properties of the Os^{II}-based terminal to be determined (Table 1) and, again there is no obvious dependence on the nature of the connector. Excitation at 465 nm populates both Ru^{II}- and Os^{II}-based terminals, with the molar absorption coefficients slightly favoring the Ru^{II}-(terpy) complexes. Under these conditions, the luminescence spectra appear as mixtures of the two individual components that can be resolved by reference to the corresponding “pure” spectra recorded for the dinuclear Ru^{II} complexes and for the Os^{II} complexes using 648 nm excitation. This procedure allows estimation of the relative luminescence quantum yields for both terminals (Table 2) and it appears that emission from the Ru^{II}-based fragment becomes significantly less pronounced as the connector is shortened. Under identical conditions, emission from the corresponding Os^{II}-based unit increases, as would be expected if intramolecular triplet energy transfer occurs from the Ru^{II}-based unit to its Os^{II}-based counterpart.^[13] The triplet energy gap, measured from the luminescence spectra,^[12] is about 730 cm^{-1} and remains independent of separation distance.

These studies also indicate that luminescence around 650 nm is essentially that of the Ru^{II}-based terminal with only a minor contribution from the Os^{II}-based fragment.^[12]

Table 2. Parameters associated with intramolecular triplet-energy transfer in the mixed-metal Ru/Os dyads.

<i>n</i>	Compound	$\langle \text{Ru} \rangle^{\text{[a]}}$	$\langle \text{Os} \rangle^{\text{[b]}}$	k_{ET} [10 ⁷ s ⁻¹]	<i>R</i> [Å]	<i>V</i> _{DA} [cm ⁻¹]
1	RO1	0.01	1.92	11.6	19.8	0.90
2	RO2	0.26	1.78	3.2	27.9	0.46
3	RO3	0.40	1.55	1.3	36.0	0.29
4	RO4	0.52	1.40	0.77	44.1	0.22
5	RO5	0.79	1.15	0.22	52.3	0.12

[a] Relative luminescence yield for the Ru^{II}-based fragment calculated with respect to the corresponding dinuclear Ru^{II} complex and allowing for competitive light absorption by the appended Os^{II}-based unit. [b] Relative luminescence yield for the Os^{II}-based fragment calculated with respect to the measurement made with excitation at 648 nm and allowing for differences in the molar absorption coefficient at the respective excitation wavelengths.

Consequently, triplet lifetimes could be determined for the Ru^{II}-based terminals and compared with those recorded earlier for the dinuclear Ru^{II} complexes in order to estimate rate constants (k_{ET}) for intramolecular triplet-energy transfer in the mixed-metal systems (Table 2). The derived values are shown in Figure 1 in the form of their distance dependence

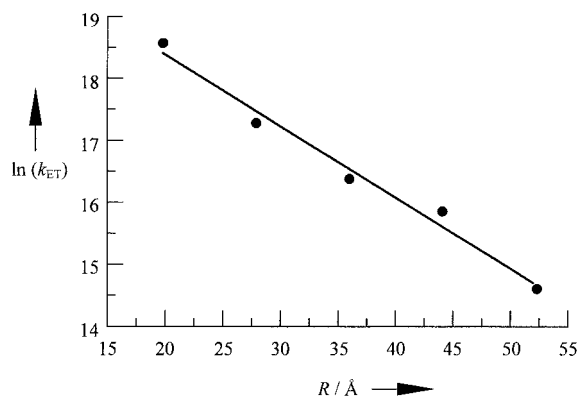


Figure 1. Effect of the metal–metal separation distance (*R*) on the rate constant (k_{ET}) for intramolecular electron exchange in the mixed-metal Ru/Os complexes.

(*R*), where distance refers to the computed average separation of the metal centers as determined by MM3 computer simulations. That intramolecular triplet-energy transfer occurs in these systems is confirmed by the observation that part of the emission associated with the Os^{II}-based terminal grows-in after the excitation pulse and on a time scale closely comparable to the decay of the Ru^{II}-based fragment. Furthermore, calculation of the rate of Förster-type dipole–dipole energy transfer^[14] indicates that this process is unlikely to contribute more than a few percent to the overall reaction. Triplet-energy transfer, therefore, can be attributed to Dexter-type electron exchange^[15] and, because of the constrained conformational mobility of these molecules, must involve long-range interactions.

The form of Figure 1 shows that the rate of intramolecular electron exchange decreases exponentially with increasing separation distance, at least up to around 52 Å, in accordance with [Eq. (1)]. Here, *R* refers to the metal–metal separation

$$k_{\text{ET}} = A \exp(-\beta R) \quad (1)$$

estimated from the energy-minimized conformation and provides an attenuation factor β of $0.11 \pm 0.01 \text{ \AA}^{-1}$. This is a remarkably small value^[8,9] that must arise from exceptionally good blending of molecular orbitals on adjacent units in the bridge, perhaps assisted by the radial distribution of the acetylenic groups. This β value implies that triplet energy transfer could be effected over a very long distance (i.e. $>100 \text{ \AA}$). The high solubility of the polytopic ligands and of the resultant metal complexes suggests that the synthesis of such long supermolecules is feasible. It is noteworthy that without the alkynylene substituent the triplet lifetime of the parent complex is too short to sustain energy transfer.^[12] Each repeating unit in the connector adds about $8.1 \text{ \AA}$ to the metal–metal separation and causes a threefold drop in the rate of electron exchange. Regardless of how β is expressed it is clear that the extrapolated rate constant for electron exchange at orbital contact ($A \approx 10^9 \text{ s}^{-1}$) is unusually small.^[16] This suggests that the first bridging unit introduces a significant barrier to electron exchange but additional units have only a modest effect on the barrier height.

Indeed, the rate of electron exchange can be considered in terms of the Fermi golden rule^[17] to estimate the magnitude of the electronic coupling matrix element (V_{DA}) for the process [Eq. (2)]. In this expression, FC is the Franck–Condon

$$k_{\text{ET}} = \frac{2\pi}{h} (V_{\text{DA}})^2 FC \quad (2)$$

weighted density of states and can be calculated from luminescence spectra recorded for the two terminals.^[18] The average value is $FC = 1.3 \times 10^{-4} \text{ cm}$. Using this value it becomes possible to estimate V_{DA} for each member of the series (Table 2). This analysis highlights the rather small V_{DA} (0.9 cm^{-1}) found for the shortest bridge and confirms the restricted attenuation with increasing number of repeat units.

It is also surprising, at least at first sight, that the triplet state remains localized on either terminal without obvious population of the connector triplet. This must mean that the latter triplet lies at too high energy to be formed as a real intermediate but is able to mediate triplet-energy transfer by way of superexchange interactions. We suppose that the interspersed aromatic units disrupt electronic coupling along the molecular axis^[19] and serve to raise the triplet energy of the connector. The alkoxy chains might further decouple the subunits because of local steric crowding and/or by forcing the phenylene rings into an unfavorable orientation. It is also important to note that, although the conformation is constrained, these molecules are far from rigid and might be expected to undergo substantial out-of-plane bending.^[20] There has been some recent commentary^[21] on how large-scale nuclear motion might be coupled to charge migration in DNA and it is possible that a similar process happens in these simple dyads.

Experimental Section

All new complexes were prepared by complexation of the back-to-back terpyridine ligands with the appropriate metal precursors and synthetic details will be reported elsewhere. Selected data are: **RO1** (52 %); FT-IR

(KBr pellets): $\tilde{\nu} = 3428 \text{ (m)}$, 2922 (s) , 2853 (s) , 1730 (w) , 1462 (m) , 1260 (m) , 1108 (m) , $843 \text{ cm}^{-1} \text{ (s)}$; UV/Vis (CH_2Cl_2): $\lambda_{\text{max}} [\text{nm}]$ ($\epsilon [\text{M}^{-1} \text{ cm}^{-1}]$); 273 (38,200), 281 (38,400), 309 (41,200), 487 (12,100); $\text{FAB}^+ m/z$ 2151 ($[M - \text{PF}_6]^+$, 100); elemental analysis calcd (%) for $\text{C}_{94}\text{H}_{104}\text{N}_{12}\text{O}_2\text{RuOsP}_4\text{F}_{24}$: C 49.20, H 4.13, N 7.32; found: C 49.53, H 4.32, N 7.62. **RO2** (48 %); FT-IR (KBr pellets): $\tilde{\nu} = 3434 \text{ (m)}$, 2922 (s) , 2851 (m) , 1960 (m) , 1636 (s) , 1460 (m) , 1258 (m) , 1022 (m) , $842 \text{ cm}^{-1} \text{ (s)}$; UV/Vis (CH_2Cl_2): $\lambda_{\text{max}} [\text{nm}]$ ($\epsilon [\text{M}^{-1} \text{ cm}^{-1}]$); 275 (45000), 310 (40600), 490 (12900); $\text{FAB}^+ 2619$ ($[M - \text{PF}_6]^+$, 100); elemental analysis calcd (%) for $\text{C}_{126}\text{H}_{146}\text{N}_{12}\text{O}_4\text{RuOsP}_4\text{F}_{24}$: C 54.76, H 5.32, N 6.08; found: C 55.04, H 5.58, N 5.89. **RO3** (58 %); FT-IR (KBr pellets): $\tilde{\nu} = 3070 \text{ (w)}$, 2925 (s) , 2853 (s) , $2205 \text{ (w, C}\equiv\text{C)}$, 1604 (s) , 1504 (m) , 1466 (s) , 1430 (m) , 1207 (s) , 1030 (m) , $843 \text{ cm}^{-1} \text{ (s)}$; UV/Vis (CH_2Cl_2): $\lambda_{\text{max}} [\text{nm}]$ ($\epsilon [\text{M}^{-1} \text{ cm}^{-1}]$); 273 (27900), 309 (33200), 498 (13500); $\text{FAB}^+ 3087$ ($[M - \text{PF}_6]^+$, 100); elemental analysis calcd (%) for $\text{C}_{158}\text{H}_{198}\text{N}_{12}\text{O}_6\text{RuOsP}_4\text{F}_{24}$: C 58.71, H 6.17, N 5.20; found: C 58.43, H 5.92, N 5.01. **RO4** (65 %); FT-IR (KBr pellets): $\tilde{\nu} = 3429 \text{ (s)}$, 2927 (m) , 2066 (m) , 2001 (m) , 1958 (s) , 1748 (w) , 1186 (s) , 923 (s) , $843 \text{ cm}^{-1} \text{ (m)}$; UV/Vis (CH_2Cl_2): $\lambda_{\text{max}} [\text{nm}]$ ($\epsilon [\text{M}^{-1} \text{ cm}^{-1}]$); 274 (53200), 308 (55400), 485 (16100); $\text{FAB}^+ 2410$ ($[M - 2\text{PF}_6]^+$, 100); elemental analysis calcd (%) for $\text{C}_{190}\text{H}_{250}\text{N}_{12}\text{O}_8\text{RuOsP}_4\text{F}_{24}$: C 61.66, H 6.81, N 4.54; found: C 61.51, H 6.43, N 4.28. **RO5** (66 %); FT-IR (KBr pellets): $\tilde{\nu} = 3434 \text{ (s)}$, 2920 (s) , 2851 (m) , $2199 \text{ (w, C}\equiv\text{C)}$, 1959 (m) , 1633 (s) , 1465 (s) , 1443 (m) , 841 (s) , $765 \text{ cm}^{-1} \text{ (s)}$; UV/Vis (CH_2Cl_2): $\lambda_{\text{max}} [\text{nm}]$ ($\epsilon [\text{M}^{-1} \text{ cm}^{-1}]$); 272 (17400), 309 (21800), 430 (14600), 498 (12900); elemental analysis calcd (%) for $\text{C}_{222}\text{H}_{302}\text{N}_{12}\text{O}_{10}\text{RuOsP}_4\text{F}_{24}$: C 63.94, H 7.30, N 4.03; found: C 63.62, H 7.12, N 3.93.

Photophysical measurements were made in deoxygenated acetonitrile at 20°C using conventional methods. Luminescence lifetimes were measured after excitation with a 25 ps laser pulse delivered from a frequency-doubled Nd-YAG laser equipped with a Raman shifter. The temporal resolution of this instrument is approximately 200 ps. Separation distances were calculated from energy-minimized conformations using the TITAN software.

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The Distinct Effect of the *o*-Carboranyl Fragment: Its Influence on the I–I Distance in R_3PI_2 Complexes*

Francesc Teixidor, Rosario Núñez, Clara Viñas,*
Reijo Sillanpää, and Raikko Kivekäs

Although compounds of stoichiometry R_3PX_2 (R = organic substituent, X = halogen) have been known for over 100 years, their solid-state structure remains largely unexplored. This is surprising considering that some of these species have found significant use as reagents in synthetic organic and inorganic chemistry and are commercially available.^[1] Interest in their solid-state structure has increased in the last decade since the structure of $t\text{Bu}_3\text{PI}_2$ was disclosed.^[2] The existence of significant iodine–iodine interaction in this compound and the four-coordinate molecular structure, novel in phosphane chemistry, stimulated the interest of researchers in the area.^[3, 4] Du Mont et al.^[2] upon the observation that the I–I bond in $t\text{Bu}_3\text{PI}_2$ (3.326(1) Å) was longer than in Ph_3AsI_2 (3.005(1) Å),^[5] suggested that it could be interpreted either as an iodophosphonium salt or as an iodine charge-transfer complex. With the incorporation of new data it was later considered as a charge-transfer complex.^[6] The interpretation of the I–I interaction in R_3PI_2 molecules is, however, still a point of controversy as has been stated recently.^[7] We shall consider the $\text{I} \cdots \text{I}$ Van der Waals distance to be 4.3 Å, thus any distance smaller than this suggests an I–I bond interaction.

[*] Dr. C. Viñas, Prof. F. Teixidor, Dr. R. Núñez
Institut de Ciència de Materials de Barcelona (CSIC)
Campus de la U.A.B.
08193 Bellaterra (Spain)
Fax: (+34)935805729
E-mail: clara@icmab.es

Dr. R. Sillanpää
Department of Chemistry, University of Turku, 20014 (Finland)

Dr. R. Kivekäs
Department of Chemistry, University of Helsinki, 00014 (Finland)

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The structures of halophosphoranes in the solid-state depend largely on the starting phosphane $\text{R}_n\text{PX}_{3-n}$, the solvent used, the halogen, and the phosphane:halogen ratio.^[8] In the solid-state structures of R_3PX_2 three basic structural motifs are found: trigonal bipyramidal in Ph_3PF_2 ,^[9] $(\text{C}_6\text{F}_5)_3\text{PCl}_2$, $\text{Ph}_2(\text{C}_6\text{F}_5)\text{PCl}_2$,^[10] and Ph_3PCl_2 ,^[11] the molecular charge-transfer “spoke” structure for $t\text{Bu}_3\text{PI}_2$,^[2] Ph_3PI_2 ,^[3a] PhMe_2PI_2 ,^[3b] Ph_3PBr_2 ,^[12] $i\text{Pr}_3\text{PI}_2$,^[13] and Ph_3PIBr ,^[14] and the ionic form, for example, in $[n\text{Pr}_3\text{PCl}]\text{Cl}$,^[10] and $[\text{Ph}_3\text{PCl}]\text{Cl}$.^[4a] In the last two types of structure the P center is tetracoordinate. For this coordination, the distinction between molecular charge-transfer “spoke” structure and the ionic form is very tenuous, and there seems to be a continuum in the X–X bond strength. If attention is focused solely on R_3PI_2 compounds, a few examples exist which bring information on the I–I and P–I distances: in PhMe_2PI_2 ,^[3b] $i\text{Pr}_3\text{PI}_2$,^[13] $t\text{Bu}_3\text{PI}_2$,^[2] and Ph_3PI_2 ,^[3a] the observed I–I bond lengths [Å], P–I bond lengths are in parenthesis, are 3.408(2) [2.410(2)], 3.383(1) [2.409(2)] and 3.372(1) [2.420(2)], 3.326(2) [2.461(2)], and 3.161(2) [2.481(4)], respectively. The shortening of the I–I bond implies that elongation of the P–I bond takes place. In no case has an I–I bond been found that is as short as that in Ph_3AsI_2 (3.005(1) Å).^[5] For comparison, the I–I bond in I_2 is 2.660 Å.^[15] The elongation of the I–I bond is expected since electron density is transferred into the σ^* orbital of the diiodine by the electron donor. As a consequence the more basic the phosphanes are the longer the I–I bond will be and thus the shorter the P–I bond.

The *o*-carborane, $1,2\text{-C}_2\text{B}_{10}\text{H}_{12}$, is an icosahedral cluster in which the two carbon atoms are in adjacent positions. One way to build the orbital set of *o*-carborane is to consider that each participating atom has as its valence orbital set two sp, and two p orbitals. This situation is very similar to the molecular orbitals in acetylene. Consequently, the C–H group of *o*-carborane is acidic and may be removed by strong bases. Moreover, the *o*-carborane cage through substitution at carbon is extremely electron-withdrawing. During our research on *o*-carboranylmonophosphane derivatives, $1\text{-PR}_2\text{-}2\text{-R}'\text{-}1,2\text{-C}_2\text{B}_{10}\text{H}_{10}$ ^[16] (Figure 1), we considered the possibility that a molecular charge-transfer “spoke” structure could be obtained. This would provide data on the electron-withdrawing capacity of the *o*-carboranyl cluster through the influence on the I–I distance, and also allow new data concerning the $\text{R}_3\text{P-I-I}$ continuum to be obtained. There are several *o*-carboranylmonophosphanes of the type (*o*-carboranyl) R_2P available, but we decided to look for one with the highest basicity. The R' radical in $1\text{-PR}_2\text{-}2\text{-R}'\text{-}1,2\text{-C}_2\text{B}_{10}\text{H}_{10}$ can be varied and although its influence on the basicity is not considerable it is adequate to improve crystallization; the R radicals influence on the phosphorous center is important. Thus, a basic carboranylphosphane was chosen to compensate for the –I (inductive effect) influence of the *o*-carboranyl cluster on the phosphorus center.

First assays with $1\text{-PiPr}_2\text{-}2\text{-Me-}1,2\text{-C}_2\text{B}_{10}\text{H}_{10}$ (**1**) were titrations with I_2 in dry CHCl_3 . This has been a standard experiment in the development of this R_3PI_2 chemistry, and

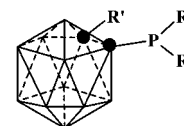


Figure 1. *closo*-Carboranylmonophosphane (● = C, other vertices are BH).

provides information on the complex equilibrium established. The ^{31}P NMR results are shown in Table 1. A nice point is the continuous upfield shift from the plain *closo*-carboranylmonophosphane up to the 1:1 stoichiometry ($\delta 33.0$ to 15.2), from

Table 1. Titration study of **1** with I_2 in CDCl_3 .

I: I_2 ratio	^{31}P NMR (δ)
1:0	33.0
1:0.25	24.7
1:0.50	15.4
1:1	15.2
1:1.25	21.1
1:1.50	28.8
1:2	30.7

that point on there is a continuous downfield shift ($\delta 15.2$ to 30.7 ppm), after the addition of further amounts of iodine. This behavior is not abnormal for triorganophosphanes, and can be interpreted considering the formation of $\text{R}_2\text{R}'\text{PI-I}$ followed by the formation of $[\text{R}_2\text{R}'\text{PI}]_3$.^[17] The formation of the latter is corroborated by UV/Vis spectra in which the electronic absorption bands at 241, 302 and 363 nm are indicative of I_3^- ions.^[3a, 6] The I_3^- ion is less nucleophilic than I^- ions, thus facilitating the formation of the cationic $[\text{R}_2\text{R}'\text{PI}]^+$ species. However, contrary to previous studies, no formation of solid phases was observed during the titration. These results suggested that *o*-carboranylphosphanes could produce the sought after $\text{R}_2\text{R}'\text{PI}_2$ molecular “spoke” charge-transfer complexes, and that the *o*-carboranylphosphanes do not behave like phosphorus triiodide (PI_3) or alkyl-diiodophosphanes (RPI_2) which cannot compete in solution with iodide anions for coordination to the I^+ unit of the $[\text{R}_2\text{R}'\text{PI}]^+$ ion.^[17a]

The compound $(1\text{-PiPr}_2\text{-2-Me-1,2-C}_2\text{B}_{10}\text{H}_{10}) \cdot \text{I}_2$ (**2**) is isolated as a red brown crystalline solid from the reaction of stoichiometric quantities of **1** and I_2 in toluene at $+4^\circ\text{C}$. The spectroscopic data indicated a minor discrepancy between the $^{31}\text{P}\{^1\text{H}\}$ NMR of the CDCl_3 solution of **2** and the 1:1 ratio I_2 titration of **1** in CDCl_3 ($\delta = 14.0$ versus 15.2). At present we do not have further data to see if this variation has structural consequences or if it is because of a kinetic effect. The $^{11}\text{B}\{^1\text{H}\}$ NMR spectrum of **2** in CDCl_3 demonstrated that changes had taken place at the P atom that affected the ^{11}B NMR resonance signals of the cluster. This was to be expected since increasing or decreasing the electron density of the P atom alters the electron density distribution of the cluster, a result of the good cluster–phosphorous communication that we have described.^[16a]

Crystals suitable for X-ray analysis were obtained directly from the toluene solution used for the synthesis of **2**. The crystal structure of **2** confirmed our expectations^[18] (Figure 2) and has a structure similar to that of the charge-transfer “spoke” R_3PI_2 and Ph_3AsI_2 complexes. However, it has a particular characteristic that makes it notable: its I–I bond (3.021(1) Å) is practically equal to that found in Ph_3AsI_2 (3.005(1) Å) and represents the shortest reported I–I interaction in organophosphane–diiodine chemistry. This short bond length also implies that the As atom of the Ph_3As group behaves electronically like the P atom of 1- PiPr_2 -2-Me-1,2- $\text{C}_2\text{B}_{10}\text{H}_{10}$.

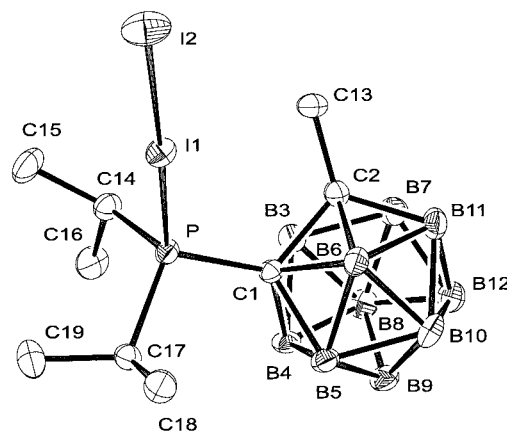


Figure 2. Molecular structure of **2** showing the atom labeling scheme. The thermal ellipsoids are set at the 30% probability level and the hydrogen atoms are omitted for clarity. Selected interatomic distances [Å] and angles [$^\circ$]: I1–P 2.5978(14), I1–I2 3.021(1), P–C14 1.851(4), P–C17 1.855(4), P–C1 1.871(4), C1–C2 1.690(6); P–I1–I2 177.49(3), C14–P–C17 112.6(2), C14–P–C1 107.3(2), C17–P–C1 105.0(2), C14–P–I1 109.75(15), C17–P–I1 107.17(15), C1–P–I1 115.11(14).

This was one of our objectives, to show that the $\text{R}_3\text{P-I-I}$ bond could be modulated and that there is a continuum in the I–I bond; compound **2** has provided one of the limits. Research is underway to find out if this I–I bond can be made even shorter by adequately combining carboranyl/alkyl or aryl substituents in phosphane chemistry. The second objective was to show the distinct properties of the *o*-carborane cage, the electron-pulling capacity, lipophilicity, B–H bonds, bulkiness, and the capacity for substitution on boron atoms should make this species a distinct fragment that can provide a new chemistry.^[19]

Experimental Section

2: I_2 (13.9 mg, 5.5×10^{-2} mmol) was added to a solution of **1** (15 mg, 5.5×10^{-2} mmol) in toluene (5 mL). The solution was stirred at room temperature for 1 h and left for two months at $+4^\circ\text{C}$. After this time red brown crystals were obtained, yield: 91%; $^1\text{H}\{^{11}\text{B}\}$ NMR (300.13 MHz, CDCl_3 , 25°C , TMS): $\delta = 1.48$ (dd, $^3J(\text{H,H}) = 7.0$ Hz, $^3J(\text{P,H}) = 17.4$ Hz, 6H; CH_3), 1.54 (dd, $^3J(\text{H,H}) = 6.8$ Hz, $^3J(\text{P,H}) = 18.8$ Hz, 6H; CH_3), 2.33 (s, 3H; CH_3), 2.52 (ddq, $^2J(\text{P,H}) = 2.3$ Hz, $^3J(\text{H,H}) = 6.8$ Hz, $^3J(\text{P,H}) = 7.0$ Hz, 2H; CH); ^{11}B NMR (96.29 MHz, CDCl_3 , 25°C , $\text{Et}_2\text{O} \cdot \text{BF}_3$): $\delta = 2.6$ (d, $^1J(\text{B,H}) = 144$ Hz; 1B), -3.9 (d, $^1J(\text{B,H}) = 154$ Hz, 1B), -7.7 (d, $^1J(\text{B,H}) = 154$ Hz, 5B), -9.5 (d, $^1J(\text{B,H}) = 176$ Hz, 3B); $^{31}\text{P}\{^1\text{H}\}$ NMR (121.48 MHz, CDCl_3 , 25°C , 85% H_3PO_4): $\delta = 14.0$ (s); $^{13}\text{C}\{^1\text{H}\}$ NMR (75.47 MHz, CDCl_3 , 25°C , TMS): $\delta = 18.9$ (s), 21.9 (s), 26.3 (s), 30.6 (s), 67.7 (d, $^1J(\text{P,C}) = 48.1$ Hz), 79.2 (s); elemental analysis calcd (%) for $\text{C}_9\text{H}_{27}\text{B}_{10}\text{I}_2\text{P}$: C 20.46, H 5.12; found: C 20.35, H 5.19.

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- [18] The X-ray diffraction data were collected at 294 K with a Rigaku AFC-5S four-circle diffractometer using graphite-monochromated MoK α radiation ($\lambda = 0.71069 \text{ \AA}$). Crystal data for **2**: Formula C₉H₂₇B₁₀I₂P, $M_r = 528.18$, crystal size: $0.36 \times 0.34 \times 0.30 \text{ mm}$, monoclinic, space group $P2_1/a$ (No. 14), $a = 14.087(4)$, $b = 10.358(2)$, $c = 15.230(3) \text{ \AA}$, $\beta = 110.33(2)^\circ$, $V = 2083.9(8) \text{ \AA}^3$, $Z = 4$, $\rho_{\text{calcd}} = 1.684 \text{ g cm}^{-3}$, $\mu = 3.082 \text{ mm}^{-1}$, $F(000) = 1008$, reflections collected 3835, unique 3673 [$R_{\text{int}} = 0.0187$]. An empirical absorption correction with ψ -scan data was applied. The structure was solved using SIR92 and was refined against $|F^2|$ using program SHELXL-97 (230 parameters). R indices were (all data): $R_1 = 0.0524$, $wR_2 = 0.0727$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-144823. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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Remote Enantioselection Transmitted by an Achiral Peptide Nucleic Acid Backbone**

Igor A. Kozlov, Leslie E. Orgel,* and Peter E. Nielsen*

Peptide nucleic acid (PNA; Figure 1 b) is an achiral DNA (or RNA) mimic with an amide (pseudopeptide) backbone. PNA oligomers form double helices with complementary DNA or RNA through Watson–Crick base pairing.^[1] Complementary strands of PNA form DNA-like double helices but the left-handed and right-handed duplexes have equal stability in the absence of chiral influences.^[2]

The RNA-world hypothesis which states that our biological life was preceded by a prebiotic system in which RNA oligomers functioned both as genetic materials and as enzyme-like catalysts is widely accepted.^[3] This hypothesis raises the problem of the origin of RNA and, in particular, emphasizes the difficulty of forming and replicating a homochiral nucleic acid in a solution of racemic nucleotides.^[4] One possible solution to this problem involves a gradual transition from an achiral genetic material (for example, one resembling PNA) to RNA.

Although there are now claims that PNA could have been a prebiotic molecule,^[5] this is highly speculative. We have used PNA extensively as a model for the type of achiral polymer that could have preceded RNA.^[6] The influence of chiral substituents on the distribution of left- and right-handed PNA helices has been reported at some length.^[2, 7] Here we continue to explore the possibility of a transition from an achiral nucleic acid analogue to RNA, and show that as few as two D-deoxynucleotides incorporated at one end of a decameric PNA double helix can control the handedness of the helix. Furthermore, we show that this can result in enantioselective chemistry both remote from and at residues not covalently connected to the inducing chiral dinucleotide. Remote enantioselection of this kind could overcome the problem of enantiomeric cross-inhibition, and thus greatly simplify a stepwise evolutionary transition from a prebiotic achiral to a biological homochiral genetic material.

We prepared a series of PNA-containing duplexes (Figure 1 a): DS is an achiral PNA double helix, while D1 is the corresponding duplex in which the N-terminus cytosine

[*] Prof. Dr. L. E. Orgel, Dr. I. A. Kozlov
The Salk Institute for Biological Studies
Post Office Box 85800
San Diego, CA 92186 (USA)
Fax: (+1) 619-558-7359
E-mail: orgel@salk.edu

Prof. Dr. P. E. Nielsen
Center for Biomolecular Recognition
IMBG, The Panum Institute
Blegdamsvej 3c, 2200 N, Copenhagen (Denmark)
Fax: (+45) 35396042
E-mail: pen@imb.gu.dk

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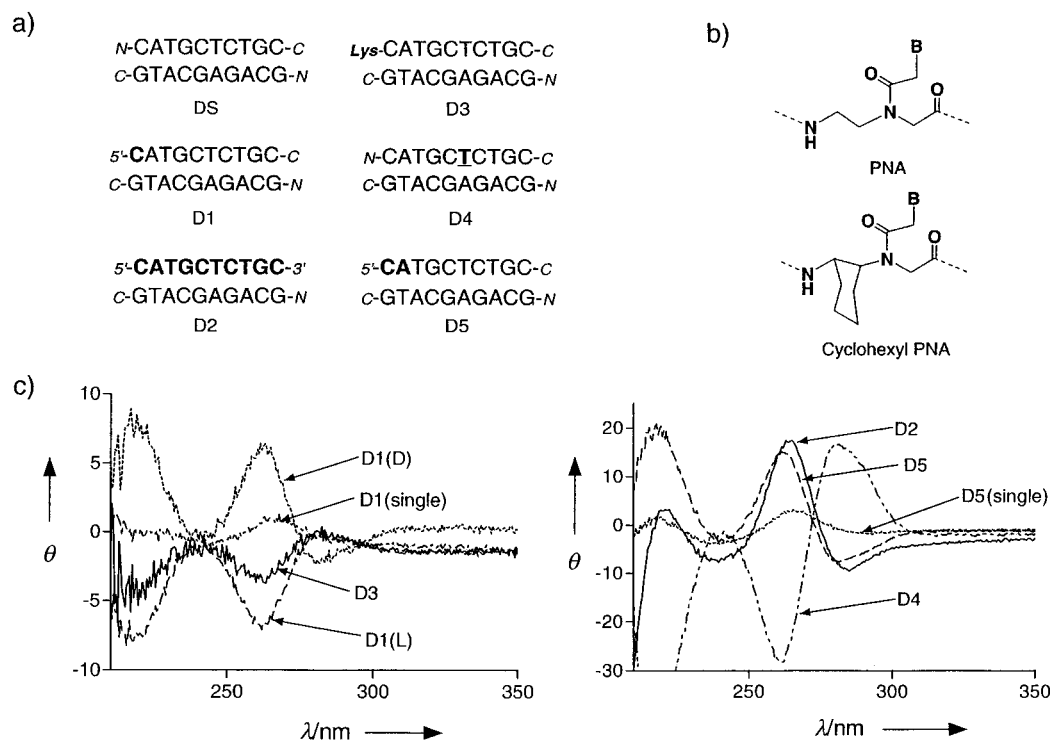


Figure 1. a) Sequences of the basic PNA-PNA duplex DS and the modified duplexes D1-D5. The N and C termini of the PNAs are indicated by -N and -C, respectively. Deoxyribonucleotides are shown in bold. Lys- denotes an N-terminus L-lysine, and **T** is a backbone PNA thymine residue modified with an (*R,R*)-cyclohexyl group. b) The structures of a PNA residue and a cyclohexyl PNA residue. c) CD spectra of duplexes D1-D5. The D1(D) duplex contains a D-dC residue, and the D1(L) duplex contains an L-dC residue. D1(single) denotes the chimeric strand of D1 (5'-CATGCTCTGC-C). D5(single) denotes the chimeric strand of D5 (5'-CATGCTCTGC-C). All spectra were recorded at identical oligomer concentrations ($OD_{260} = 1.0$ (OD = optical density)) in 10 mM sodium phosphate at pH 7.0 containing 100 mM NaCl.

residue has been replaced by a D-deoxynucleotide (D-dC). D2 is a helix formed from one DNA and one PNA strand, D3 is a PNA double helix in which a single L-lysine was attached to the N-terminus of one of the strands, and D4 is a PNA double helix with an internal thymine residue replaced by a conformationally constrained PNA residue modified with a chiral cyclohexyl group^[8] (Figure 1 b).

The CD spectra of these double-helical molecules at equal concentrations are presented in Figure 1 c. The results show that the D-dC nucleotide does, indeed, induce a right-handed helicity in the D1 duplex. A comparison of the CD spectra of D1 and D3 suggests that a single D-dC residue is about twice as effective as an attached chiral lysinamide in inducing chirality. A comparison of the spectrum of D1 with those of D2 and D4, which we assume to be locked in right- and left-handed helices, respectively, allows us to estimate that D1 is about 75 % right-handed and 25 % left-handed, since D1 gives rise to approximately half the CD intensity of the two locked helices. The results also confirm that the N-terminal L-lysine residue induces a left-handed PNA helix,^[7] and that about 62 % of the helices are left-handed.

Since one chiral deoxyribonucleotide unit at the N-terminus of a 10-mer PNA is not sufficient to generate a homochiral population of PNA duplexes, we next prepared a DNA-PNA chimera (5'-d(CA)TGCTCTGC-C) in which the two N-terminal nucleobase units were D-deoxyribonucleotides. We then recorded the CD spectrum of a duplex formed between this

chimera and the antiparallel oligomer of complementary PNA (Figure 1, D5). Judging from the intensity of the band at 260 nm in the CD spectrum this duplex deviates only slightly from homochirality. Thus the chirality of the N-terminal dinucleotide has been transmitted efficiently through the covalently linked first PNA strand to the hydrogen-bonded and base-stacked second PNA strand.

The above results suggest that the chirality induced by two nucleotides in a template strand could be transmitted through normally achiral PNA and result in a chirally selective template-directed remote elongation of an achiral primer strand. We, therefore, studied ligation in the system illustrated in Figure 2.

The structure of the duplex that we used for the ligation experiments resembles the structure of the duplex D5 (Figure 1 a). The upper strand (template strand) contains two D-deoxynucleotides at its N terminus, while the primer strand which lacks two residues at its N terminus is made up entirely of PNA monomers. We extended the C terminus of this strand by three thymine residues, solely to simplify the HPLC analysis of the ligation products. The substrate was either the DD or the LL enantiomer of a dinucleotide activated as a phosphorimidazolid (5'-d(GC)pIm-3'). In control experiments we used a duplex in which both the template and primer were composed exclusively of PNA.

In the absence of a template no detectable product was formed (Figure 2 a, d). When the template strand was entirely PNA both DD and LL substrates gave product yields of about 15 % (Figure 2 b, e). However, when the template strand included two D nucleotides the yield of the reaction with the DD dimer doubled to about 30 % while the yield of the reaction with the LL isomer decreased to less than 5 % (compare Figures 2 c and 2 f). When we used the racemic dimer as the substrate we found that the presence of the LL isomer did not inhibit the reaction of the DD isomer significantly (compare Figures 2 c and 2 g). Thus the reaction is more than 90 % stereoselective for the DD enantiomer.

Our results can be interpreted as only right-handed helices react with the DD dimer and left-handed helices with the LL dimer. As far as we are aware these experiments demon-

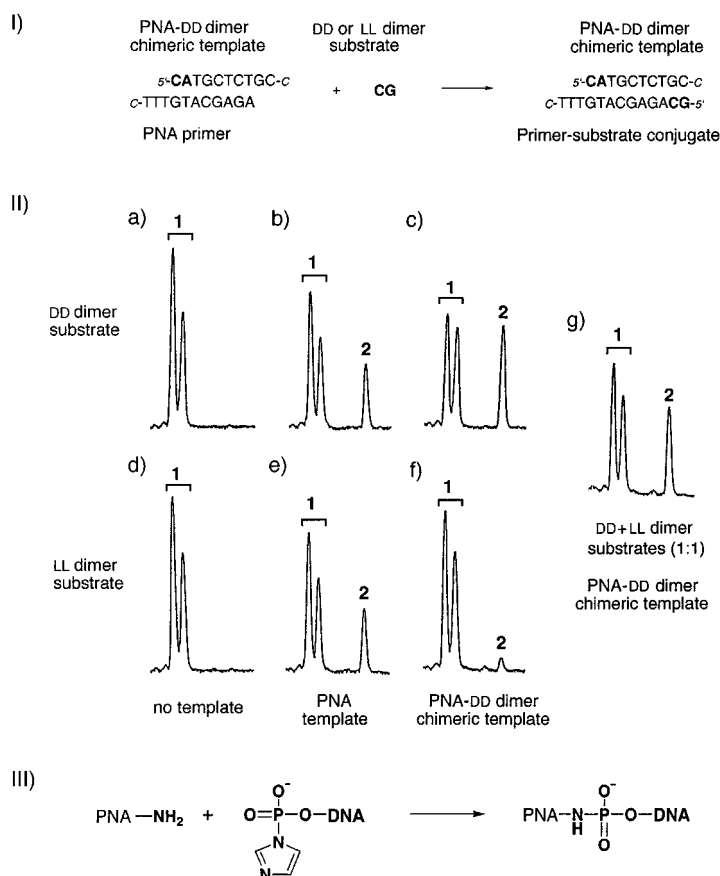


Figure 2. Remote enantioselection in PNA-directed PNA-DNA ligation. The design of the experiment is illustrated in (I) and the nature of the ligation step in (III). Diagrams a–g in (II) present HPLC elution profiles of the products formed by ligation of the PNA primer to the DD and LL enantiomers of 5'-d(GC)pIm-3'. The reaction mixtures contain the DD dimer (a–c), the LL dimer (d–f), or a 1:1 mixture of DD and LL dimers (g). The template is omitted in a and d; the PNA template is present in b and e; and the chimeric PNA-DD-d(CA) template (PNA-DNA chimeric template) is present in c, f, and g. The numbers above the peaks indicate 1: the starting PNA primer, C-TTTGTACGAGA-N, 2: the product, a primer-dimer chimera, C-TTTGTACGAGACG-5'. The double peak representing the starting PNA primer arises from an N-terminal rearrangement of the PNA at pH 12 before and during HPLC.^[17] The rearranged product does not undergo template-directed elongation (data not shown).

strate for the first time that chiral influences can be transmitted over long distances through both covalent and non-covalent interactions in nonbiological systems, and result in remote stereoselective chemistry. Previously, Green et al. and others showed that certain synthetic polymers are very sensitive to chiral induction,^[9] but these systems cannot carry genetic information and no enantiomeric chemical selection was demonstrated.

The present results also have implications for possible scenarios for the development of homochirality in a prebiotic genetic material. We have previously proposed PNA as a model for an achiral prebiotic genetic material, and have provided evidence that a transition from a “PNA-like” genetic world to an RNA (or DNA) genetic world is in principle possible either through a “one-step” information transfer by PNA-directed RNA oligomerization,^[6a,b] or through a multi-step process involving PNA-directed PNA-DNA ligation.^[6c]

However, these experiments do not solve the problem of chirality in the transition from achiral PNA to homochiral DNA (or RNA). On the contrary, it was found that enantiomeric cross-inhibition in PNA-directed RNA polymerization was as profound as seen previously for RNA-directed RNA polymerization.^[10]

The results presented here show that the introduction of a short homochiral segment of DNA into a PNA helix could have guaranteed that the next short segment of DNA to be incorporated would have the same handedness as the first. Once two segments of the same handedness were present, the probability that a third segment would have the same handedness would increase, and so on. Evolution could then slowly “dilute out” the PNA part. This scenario would ultimately allow the formation of a chiral oligonucleotide by processes that are largely resistant to enantiomeric cross-inhibition.

It is important to note that the ligation of homochiral dinucleotides on a nucleic acid template would probably be at least as enantiospecific as the reaction that we have studied. The disadvantage of using chiral monomers as components of a replicating system arises from the difficulty of generating a first long homochiral template from a racemic mixture of monomers, although results of experiments designed to overcome this difficulty by employing homochiral tetramers have been reported.^[4a] The probability of obtaining a homochiral *n*-mer from achiral substrates is approximately $1/2^{n-1}$ if the nontemplate-directed extension of the primer is not enantioselective. Hence, it would be very hard to get started with a homochiral 40-mer, for example. No such difficulty exists in a scenario that originates with an achiral genetic material and in which the incorporation of very few chiral monomers in this achiral background gradually progresses towards homochirality.

It seems possible that some PNA sequences could act as catalysts, analogous to ribozymes, even though PNA lacks clear metal binding sites. Although such catalysts could not be enantioselective, the incorporation of as few as two chiral nucleotides could then impose chiral specificity on the system. Furthermore, such patch chimeras could help to bridge the gap in catalytic potential between PNA and RNA, while guaranteeing enantioselectivity.

Experimental Section

Unless otherwise noted all chemicals were purchased from commercial sources and were used without further purification. L-dCp was synthesised as described.^[11] PNA-dC conjugates (D or L) were obtained as described.^[12] Unless otherwise stated, the PNA oligomers were prepared as C-terminal carboxamides. The dimers DD-d(CA)p, DD-d(CG)p, and LL-d(CG)p were synthesised on a 10-μm scale on a 391A automatic DNA synthesizer (Applied Biosystems) using appropriate D- or L- phosphoramidites and 3'-phosphate CPG (Chemgenes). DD-d(CA)pIm was synthesized by using the experimental procedure described for mononucleotides.^[13, 14] Reactions between PNA and DD-d(CA)pIm and the subsequent purification of the product were performed under conditions previously reported.^[12] These procedures led to PNA-DNA chimeras joined by a favorable DNA-3'-phosphoramidate linkage directly to the PNA amino terminus.^[6c, 15] The yield of the PNA-dimer conjugate was 60%. The product showed the correct mass as characterized by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (found: 2751, calcd: 2753).

Ligation reactions were run in total volumes of 10 μ L of 0.25 M imidazole (Im) buffer, pH 7.7, for 3 days at 0 °C. The concentration of the PNA primer was 8 μ M, of the template 12 μ M, and of the activated dimer 0.8 mM. When we used a mixture of DD and LL dimers, the concentration of each enantiomer was 0.8 mM. The duplexes between the primer and templates were preformed by preincubation at 20 °C for 2 h in 5 μ L of 10 mM sodium phosphate buffer, pH 7.0, containing 100 mM NaCl and 0.1 mM ethylenediaminetetraacetate (EDTA). The dimers were activated in 0.1 M Im buffer at pH 6.0 containing 0.1 M 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide (EDC) by incubating the solution for 3 h at 20 °C and then diluting with an equal volume of 1 M Im buffer at pH 7.7. Aliquots of the resulting solution (5 μ L) were added to reaction tubes containing the preformed duplexes. HPLC analyses of the reaction mixtures were performed on an RPC5 column as previously described.^[16] Reaction products were eluted with a linear gradient of NaClO₄ (pH 12, 0–0.06 M over 60 min) and the UV absorption at 254 nm monitored.

Circular dichroism spectra were obtained using equimolar concentrations (10 μ M) of the two complementary strands hybridized in 10 mM sodium phosphate at pH 7.0 containing 100 mM NaCl.

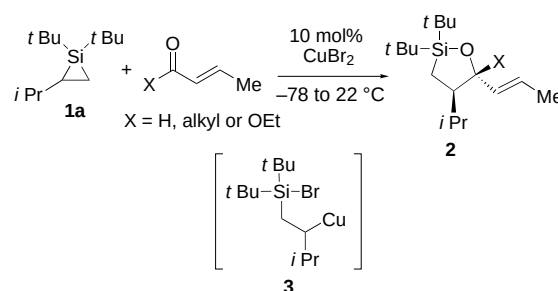
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ZnBr₂-Catalyzed Insertions of Carbonyl Compounds into Silacyclopropanes: Regiochemical Reversal Dependent on Metal Salt**

Annaliese K. Franz and K. A. Woerpel*

We recently reported that copper salts catalyze the insertion of various carbonyl compounds into the C–Si bond of silacyclopropane **1a** in a stereospecific and highly stereo-, regio-, and chemoselective fashion (see Scheme 1).^[1] While formamides, formate esters, and α,β -unsaturated carbonyl compounds inserted under these conditions, saturated aldehydes such as butyraldehyde could not be coaxed to react. Ring-opening of the unsymmetrically substituted silacyclopropane **1a** occurred exclusively with cleavage at the more substituted C–Si bond to afford insertion products such as **2**. The observed regiochemistry and carbonyl reactivity was rationalized by consideration of organometallic species **3** (Scheme 1) which is believed to form upon transmetalation of the more substituted C–Si bond to copper. We have obtained selectively products with this 1,2-regiochemistry for all insertion reactions of silacyclopropanes thus far.^[2–4]



Scheme 1. Copper-catalyzed insertions into silacyclopropane **1a** proceed with up to 98:2 diastereoselectivity and >99:1 regioselectivity.

To complement our previous results, we desired a method to functionalize the less substituted C–Si bond and insert saturated carbonyl compounds. Insertion into the less substituted C–Si bond would provide access to the 1,3-regioisomer **4** which can be oxidized^[5] to obtain the 1,3-diol motif **5** (Scheme 2).^[6,7] Herein, we report that metal salts such as ZnBr₂ can be employed to access previously unavailable

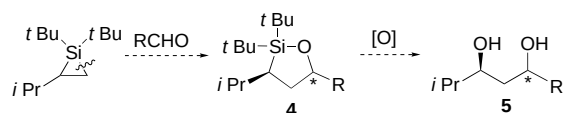
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[*] Prof. K. A. Woerpel, A. K. Franz
Department of Chemistry
University of California
Irvine, CA 92697-2025 (USA)
Fax: (+1) 949-824-2210
E-mail: kwoerpel@uci.edu

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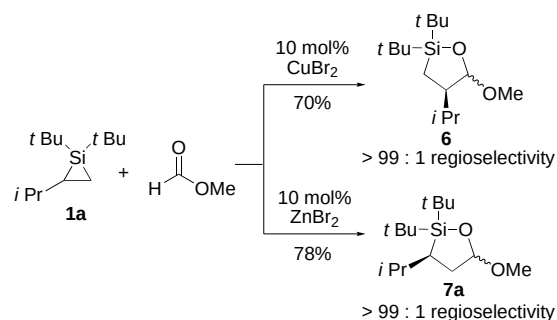
Scheme 2. Stereoselective synthesis of 1,3-diols by an insertion and oxidation sequence.

insertion products with a complete reversal of the regiochemistry compared to that of the copper-catalyzed conditions. By the appropriate choice of metal salt, precise control of the silacyclopentane ring-opening can be achieved to obtain either the 1,2- or the 1,3-regioisomeric insertion product with excellent regioselectivity (> 99:1).

While attempting to elucidate the role of the metal salt in the ring-opening of silacyclopentanes, we discovered that zinc and copper salts exhibit complementary regioselectivities for the insertion of formate esters. Methyl formate inserted into the more substituted C–Si bond of silacyclopentane **1a** to afford 1,2-regioisomer **6** (Scheme 3) when copper salts were used, but a complete reversal of regiochemistry to give 1,3-regioisomer **7a** was observed when ZnBr₂ was employed.^[8, 9] We have shown that oxasilacyclopentane acetals such as **6** and **7a** serve as precursors to oxocarbenium ions that undergo Lewis acid mediated nucleophilic substitution with excellent diastereoselectivities.^[10, 11]

The regiochemical preference of the ZnBr₂-catalyzed formate insertion was investigated for a series of monosubstituted silacyclopentanes with increasing steric size (Table 1). Unsymmetrical silacyclopentanes **1a** and **1b** (entries 1 and 2) reacted to afford exclusively 1,3-products **7a** and **7b**, respectively. The presence of an electron-withdrawing group on the β -carbon in silacyclopentane **1c** did not affect the regioselectivity of the insertion (entry 3). When the size of the substituent on the strained ring was increased to *tert*-butyl (**1d**), however, an erosion of regioselectivity (83:17) was observed (entry 4). The selective functionalization of tri-*tert*-butyl substituted **1d** indicates that steric interactions between the substituent on carbon and the *tert*-butyl groups on silicon exert only a minor influence on the regioselectivity of insertion.

Catalysis by ZnBr₂ was determined to be general for the insertion of both aliphatic and aromatic aldehydes and ketones to provide oxasilacyclopentanes **8a–j** (Table 2). Employing ZnBr₂ as the catalyst allowed the insertion of saturated carbonyl compounds, such as butyraldehyde,



Scheme 3. Catalyst-based regiocontrol of methyl formate insertion to obtain either the 1,2- or 1,3-regioisomeric product.

Table 1. Regioselectivity of ZnBr₂-catalyzed insertion of methyl formate into silacyclopentanes **1a–d**.

Entry	R	Product	Yield [%]	Regioselectivity ^[a]
1	<i>i</i> Pr	7a	78	> 99:1
2	<i>n</i> Bu	7b	79	> 99:1
3	–CH ₂ CH ₂ OTIPS	7c	72 ^[b]	> 99:1
4	<i>t</i> Bu	7d	77	83:17

[a] As determined on the basis of GC analysis. [b] This yield includes the synthesis of silacyclopentane **1c** from *t*Bu₂SiCl₂. TIPS = Triisopropylsilyl.

Table 2. ZnBr₂-catalyzed insertion of aliphatic and aromatic aldehydes and ketones into silacyclopentane **1a**.

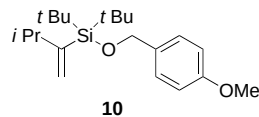
Entry	Carbonyl compound	Major product	Yield [%] ^[a]	Diastereoselectivity ^[b]	Regioselectivity ^[b]
1		8a	70	55:45	> 99:1
2		8b	73	57:43	> 99:1
3		8c	71	57:43	96:4 ^[c]
4		8d	72	79:21	> 99:1
5		8e	74	66:34	> 99:1
6	X = H	8f	63	60:40	> 99:1
7	X = CF ₃	8g	78	93:7	61:39 ^[c]
8	X = CH ₃	8h	68	55:45 ^[d]	72:28
9		8i	58	–	> 99:1
10		8j	70	66:34	> 99:1

[a] Yield of purified materials. [b] As determined on the basis of GC and GC/MS analysis. [c] Reaction was run in the presence of 4-Å activated molecular sieves. [d] When the *p*-anisaldehyde insertion product was resubmitted to reaction conditions, isomerization occurred to afford a 94:6 diastereoselectivity for the 1,3-regioisomer.

which do not react under any other conditions.^[12] The 1,3-regioisomer resulting from cleavage of the less substituted C–Si bond was obtained predominantly ($\geq 96:4$) for all substrates, with the exception of the electron-rich aryl aldehydes *p*-tolualdehyde and *p*-anisaldehyde (entries 7 and 8), which afforded **8g** and **8h** with reduced regioselectivities. Products **8a–j** have complementary regioselectivity compared to the insertion of carbonyl compounds under all conditions reported previously.^[2–4]

An extensive screening of catalysts demonstrated that ZnBr_2 is the optimal catalyst for the insertion of aldehydes into silacyclopropane **1a** with excellent regiocontrol and yield. Metal salts such as $\text{Zn}(\text{OTf})_2$ (OTf = trifluoromethanesulfonate), InCl_3 , InBr_3 , MnBr_2 , and SnBr_2 were also effective catalysts for the aldehyde insertion pathway. Sources of bromide ion (LiBr , MgBr_2 , and Bu_4NBr) did not catalyze the insertion of aldehydes into **1a**. Other Lewis acids (AlCl_3 , $\text{Sc}(\text{OTf})_3$, TiCl_4 , $\text{BF}_3 \cdot \text{OEt}_2$, SnBr_4 , and $\text{MgBr}_2 \cdot \text{OEt}_2$) proved to be too reactive in the presence of the silacyclopropane, affording only products of decomposition.

In an effort to optimize the regioselectivity for insertions of electron-rich aldehydes (Table 2, entries 7 and 8), we evaluated the influence of reaction conditions and reagents on the insertion of *p*-anisaldehyde. None of the factors investigated initially (reaction temperature, catalyst loading, equivalents of aldehyde, concentration, and order of addition) affected the regiochemistry of insertion. When the heterogeneous reaction mixture was treated with a drop of water, however, the unexpected 1,2-regioisomer **9** was observed with $>99:1$ regioselectivity (entries 1 and 2, Table 3). The hydride transfer product **10** (5%) was also formed under these conditions.^[3, 13] When di-*tert*-butylpyridine was added to rule out the possibility of protic acid catalysis, excellent regioselectivity ($>99:1$) was still observed (en-



try 3). A control experiment with only di-*tert*-butylpyridine also afforded 1,2-regioisomer **9** with high regioselectivity (93:7; entry 4). Additional experiments indicated that this effect also extends to reagents such as THF and menthol (entries 5 and 6) while treatment with *N*-benzyl-*N*-methylformamide or pyridine leads to recovery of the silacyclopropane starting material (entries 7 and 8).^[14] We believe that additives containing a less nucleophilic oxygen or of increased steric size (entries 2–6) serve as ligands to increase the solubility of the ZnBr_2 in CH_2Cl_2 and enhance the activity of the catalyst.^[15] In the presence of pyridine or formamide, the ZnBr_2 most likely forms a stable complex which is catalytically inactive.^[16]

An unexpected reversal of regiochemistry was also observed for the insertion of α,β -unsaturated carbonyl compounds relative to their saturated analogues, butyraldehyde and 3-pentanone (Table 2, entries 1 and 9). Under ZnBr_2 -catalyzed conditions, the insertion of crotonaldehyde and cyclohexenone into silacyclopropane **1a** proceeded with excellent regioselectivity to afford 1,2-regioisomers **11** and **12**, identical products to those obtained under copper-catalyzed conditions [Eq. (1) and (2)].^[17] These results

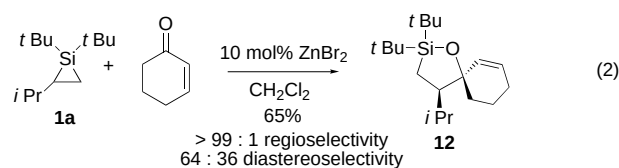
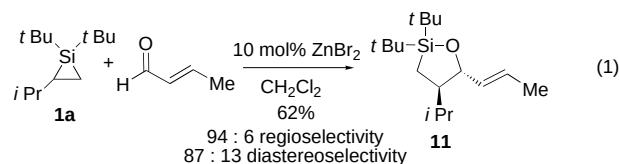


Table 3. Effect of various additives on the regioselectivity of the ZnBr_2 -catalyzed insertion of *p*-anisaldehyde into silacyclopropane **1a**.

Entry	Additive ^[a]	Yield [%]	Diastereoselectivity ^[b]	Regioselectivity ^[b]
1	none	68	55:45 ^[c]	28:72
2	H_2O	66	$>99:1$	$>99:1$
3	$\text{H}_2\text{O}/\text{di-}t\text{-butylpyridine}$	67	$>99:1$	$>99:1$
4	di- <i>tert</i> -butylpyridine	72	$>99:1$	93:7
5	THF	76	$>99:1$	94:6
6	menthol	39	93:7	90:10
7	<i>N</i> -benzyl- <i>N</i> -methylformamide	n.r.	–	–
8	pyridine	n.r.	–	–

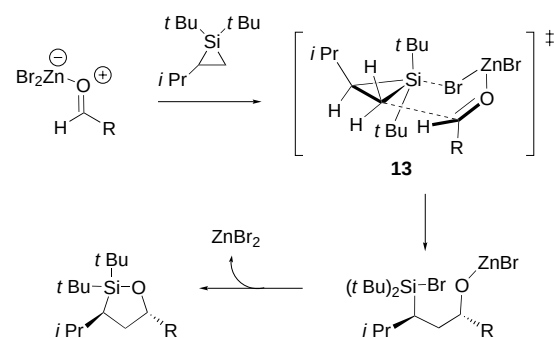
[a] Two equivalents of each additive were employed with the exception of THF which was employed as the solvent for convenience. All reagents were rigorously purified to exclude H_2O . [b] As determined on the basis of GC analysis. [c] This diastereoselectivity was determined for the 1,3-regioisomer; the 1,2-regioisomer was produced exclusively ($>99:1$) as one isomer as determined on the basis of GC analysis. n.r. = no reaction.

prompted us to investigate the relative reactivity of saturated and α,β -unsaturated aldehydes under ZnBr_2 conditions. When silacyclopropane **1a** was treated with an excess of both crotonaldehyde and butyraldehyde in the presence of ZnBr_2 , only the crotonaldehyde insertion product was observed with ^1H NMR spectroscopy. Although the effect of the conjugated alkene on reactivity and regiochemistry is unclear, it appears that an alternate reaction pathway is available for α,β -unsaturated aldehydes and ketones.

Three possible roles can be envisioned for ZnBr_2 in this insertion pathway: 1) a source of bromide ion that activates the silane, 2) a metal salt that undergoes a direct interaction with the strained σ bond of the silacyclopropane, or 3) a Lewis acid that complexes to the carbonyl compound. It is unlikely that the ZnBr_2 in this system is acting solely as a bromide source since alternate bromide sources did not catalyze this reaction. We also do not believe that there is evidence to support a direct interaction between the metal and the strained σ bond^[18] of silacyclopropane. In contrast to copper salts, no evidence of an interaction was observed between silacyclopropane **1a** and ZnBr_2 in either the ^1H or ^{13}C NMR spectra. In addition, the transmetalation reaction proposed

for the related copper-catalyzed insertion into silacyclopropane **1a** exhibits different carbonyl reactivity and affords products of opposite regiochemistry (Scheme 1).^[1] A radical pathway has been discounted based on the isolation of cyclopropane-containing products for the insertion of cyclopropane carboxaldehyde (Table 2, entry 3).^[19]

The initial results presented herein support our hypothesis that ZnBr_2 serves as a Lewis acid to activate the carbonyl moiety through complexation.^[20–22] We envision that a ligand on the ZnBr_2 catalyst can coordinate to the silicon to form a penta- or hexacoordinate silane,^[23] thus enhancing the nucleophilicity of the silacyclopropane.^[24, 25] This mechanism is similar to those proposed for the Lewis acid-catalyzed cyanosilation or hydrosilation of aldehydes and ketones (Scheme 4).^[26–29] We believe that formation of the 1,3-



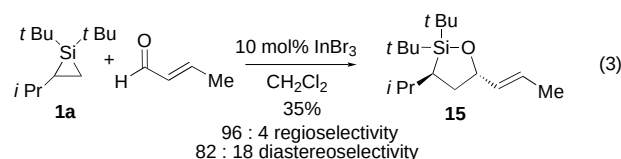
Scheme 4. Proposed mechanism for the ZnBr_2 -catalyzed insertion of carbonyl compounds into silacyclopropane **1a**.

regioisomer is favored because the substituent on the silacyclopropane encounters less steric interaction with the *tert*-butyl groups on silicon (transition structure **13**; Scheme 4) than with the carbonyl substrate (transition structure **14**). The effect of additives such as H_2O , di-*tert*-butylpyridine, and menthol on the regiochemistry of insertion is unclear. These reagents could either prevent formation of the silicate intermediate or increase the steric congestion around silicon to provide the 1,2-regioisomer as the major product.

The most intriguing mechanistic question is the divergent regioselectivity and enhanced reactivity of crotonaldehyde as compared to the saturated or alkynal analogues. We believe that a simple coordination between the metal salt and the C–C π bond can be precluded because the α,β -unsaturated alkyne and the unconjugated alkene^[30] do not exhibit the same effect on regiochemistry; reactions of these substrates afford solely 1,3-regioisomeric products (Table 2, entries 4 and 10). Based on our investigations with copper-catalyzed insertions into silacyclopropanes, it is possible that the α,β -unsaturated alkene moiety facilitates a silicon to zinc transmetalation that cannot occur for the saturated or alkynal substrates. Further investigations are underway to determine the factors that dictate the regiochemistry of this process.

Preliminary results indicate that the regioisomeric products not observed using ZnBr_2 - or copper-catalyzed conditions can

be obtained by employing other metal salts as catalysts. When silacyclopropane **1a** and crotonaldehyde were treated with 10 mol % InBr_3 , 1,3-regioisomer **15** was obtained as the major insertion product with 96:4 regioselectivity [Eq. (3)] Conditions will be optimized to increase the efficiency of this insertion reaction.



In conclusion, we have shown that the regiochemistry of silacyclopropane ring-opening is dependent on the choice of metal salt catalyst. Employing ZnBr_2 as a catalyst provides a method of complementary regiocontrol for insertion reactions of silacyclopropanes as compared to copper-catalyzed conditions. We have also demonstrated that the regioselectivity of the insertion process is influenced by the electronic nature of the carbonyl substrate and the presence of an α,β -unsaturated alkene moiety. The importance of ligands such as THF, menthol, and di-*tert*-butylpyridine as factors which can affect the regioselectivity and reactivity of this process will be further examined.

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A Linker Scaffold to Present Dimers of Pharmacophores Prepared by Solid-Phase Syntheses**

Mookda Pattarawarapan and Kevin Burgess*

Protein–protein interactions generally feature contact points (“hot-spots”) situated on discontinuous sites on the protein surfaces.^[1] It is therefore difficult to find small monomeric compounds that mimic or disrupt protein–protein interactions. This situation is unfortunate because this type of target is important, and is likely to become even more prevalent as data from The Human Genome Project are processed.

[*] Prof. Dr. K. Burgess, M. Pattarawarapan
Department of Chemistry
Texas A & M University
PO Box 30012, College Station, TX 77842-3012 (USA)
Fax: (+1) 979-845-8839
E-mail: burgess@tamu.edu

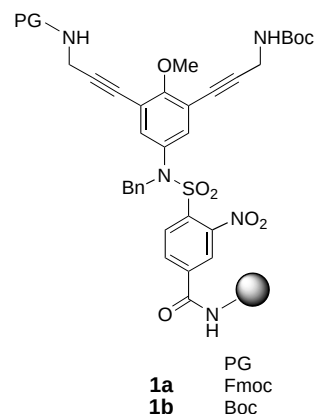
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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

One apparent approach to mimick discontinuous hot-spots is to link potential pharmacophores on a scaffold that holds them apart. An early example of this is the coupling of FK506 ligands to form FK1012 and its analogues.^[2] This approach featured rational design rather than high-throughput or combinatorial methods. More recently, NMR techniques have been used to design combinations of small pharmacophores wherein the monomers used are usually found by random screening.^[3] Others have designed solution-phase approaches involving mixtures of compounds.^[4,5] However, none of the existing approaches capitalize on the advantages of separating the products from excess reagents in solid-phase syntheses. Moreover, the scaffolds featured in this solution-phase work were relatively flexible ones.

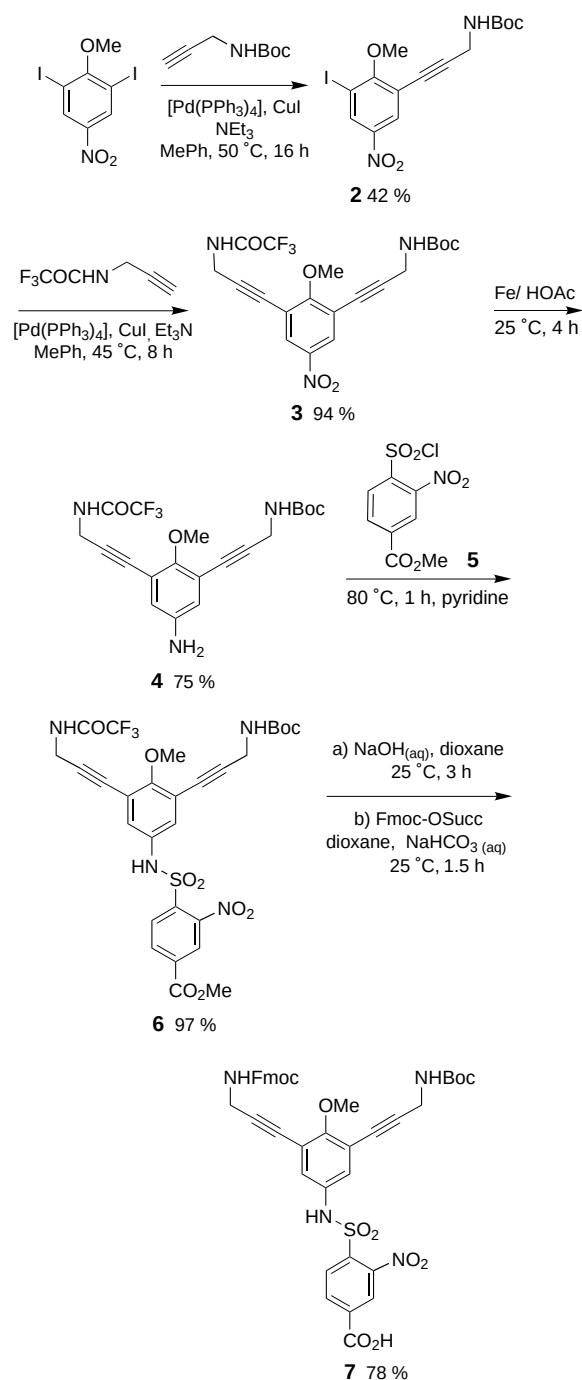
Solid-phase syntheses of combinations of pharmacophores from libraries of monomers can also provide a numerical advantage for the rapid generation of large libraries. A library of n components could, for instance, be attached in a one-compound-per-well format to one arm of a scaffold supported on a solid phase. If the samples of the resin were then distributed appropriately and the monomers coupled to the second arm of the scaffold it would be possible to form $n(n+1)/2$ dimers, one per well. If nonoverlapping libraries of n and m components were used then the number of combinations would be $n \times m$.

Herein we describe the synthesis of new “linker scaffolds” **1** for the solid-phase syntheses of dimeric combinations of pharmacophores. Linker scaffold **1a** has two orthogonally



protected reactive groups on scaffold arms that can support pharmacophores at a relatively rigid separation of approximately 10 Å. The scaffold is attached to the resin through a cleavable linker that is stable to the coupling steps and to the acidic conditions typically used to remove side-chain protecting groups (for example, trifluoroacetic acid). Moreover, cleavage of the scaffold from the resin involves a reagent that gives only volatile or innocuous by-products, so the crude materials could be taken directly from the resin-cleavage step into biological assays.

Scheme 1 delineates a synthesis of linker scaffold **1a**. Sequential Sonogashira couplings were used to introduce the two scaffold arms in reactions that are promoted by the electron-withdrawing nitro group. Reduction of that nitro functionality gives an aryl amine **4** for coupling to a known^[6]



Scheme 1. Synthesis of the linker scaffold unit **7**. Boc = *tert*-butoxycarbonyl; Fmoc = 9-fluorenylmethoxycarbonyl; Succ = succinimide.

sulfonyl chloride **5**. Ester hydrolysis and replacement of the trifluoroacetate **6** with a fluorenylmethoxycarbonyl protecting group gave the acid **7** (Table 1) for coupling to the resin. Full experimental details are provided in the Supporting Information.

The development of appropriate linker modifications and cleavage conditions took a significant amount of time to achieve. The linker scaffolds **7** and **8** were coupled to aminomethyl polystyrene resin (Scheme 2) and the loadings of the resin were checked (Fmoc assay after coupling with Fmoc-protected glycines). Cleavage of the 2-nitrosulfonamide

Table 1. Spectroscopic data for key compounds.

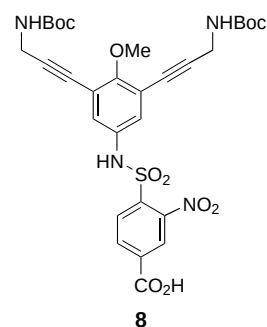
7: yellow oil (78 %): ^1H NMR (300 MHz, $[\text{D}_6]\text{acetone}$): δ = 8.40 (brs, 1H), 8.28 (d, J = 7.8 Hz, 1H), 8.01 (m, 1H), 7.82 (d, J = 7.5 Hz, 2H), 7.67 (d, J = 7.5 Hz, 2H), 7.37 (m, 3H), 7.30 (m, 4H), 7.05 (brt, 1H), 6.47 (brt, 1H), 4.35 (d, J = 7.0 Hz, 2H), 4.24 (m, 3H), 4.09 (d, J = 5.7 Hz, 2H), 3.87 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (75 MHz, $[\text{D}_6]\text{acetone}$): δ = 172.7, 167.2, 161.8, 155.9, 151.8, 143.5, 139.2, 136.8, 131.2, 131.1, 129.2, 127.6, 125.9, 124.0, 123.2, 122.6, 122.1, 120.5, 115.5, 113.4, 113.3, 88.4, 75.9, 73.4, 62.7, 62.5, 56.9, 56.1, 42.5, 27.2, 26.8, 23.8; HR-MS (matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)): calcd for $[\text{C}_{40}\text{H}_{36}\text{N}_4\text{O}_{11}\text{SNa}]^+$: 803.1999; found: 803.2030

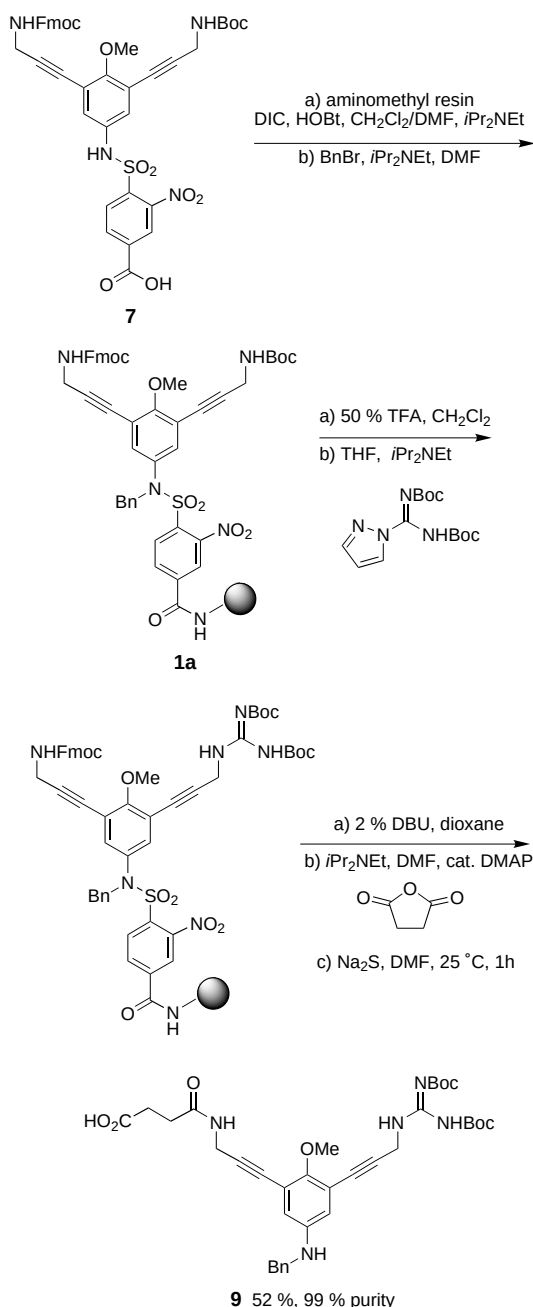
8: yellow oil (95 %): ^1H NMR (300 MHz, $[\text{D}_6]\text{acetone}$): δ = 8.45 (d, J = 1.5 Hz, 1H), 8.34 (dd, J = 8.4 Hz, 1.5 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.32 (s, 2H), 6.53 (brt, 2H), 4.14 (brs, 4H), 3.94 (s, 3H), 1.45 (s, 18H); ^{13}C NMR (75 MHz, $[\text{D}_6]\text{acetone}$): δ = 166.8, 160.4, 156.5, 148.4, 141.1, 133.5, 133.1, 131.7, 131.4, 127.8, 125.97, 118.34, 92.9, 78.4, 76.8, 60.9, 30.6, 27.9; HR-MS (MALDI-MS): calcd for $[\text{C}_{30}\text{H}_{34}\text{N}_4\text{O}_{11}\text{SNa}]^+$: 681.1842; found: 681.1844

9: yellow oil (52 %, 99 % purity): ^1H NMR (300 MHz, CDCl_3): δ = 11.46 (brs, 1H), 8.56 (brt, 1H), 7.32 (brs, 5H), 6.59 (brs, 2H), 4.44 (brd, 2H), 4.23 (brs, 4H), 3.85 (s, 3H), 3.67 (brs, 4H), 2.65 (m, 2H), 1.51 (s, 18H); ^{13}C NMR (75 MHz, CDCl_3): δ = 177.4, 169.1, 163.2, 159.3, 155.6, 154.1, 153.0, 143.9, 138.7, 128.7, 127.4, 117.6, 117.2, 114.6, 88.2, 83.4, 79.7, 79.6, 61.6, 48.4, 36.4, 31.6, 28.2, 28.0, 24.5, 24.5. Analytical HPLC: homogeneous single peak, retention time = 19.0 min (5–90 % MeCN in H_2O over 30 min). HR-MS (MALDI-MS): calcd $[\text{C}_{35}\text{H}_{43}\text{N}_5\text{O}_8 + \text{H}]^+$: 662.31; found: 462.20 $[\text{M} - 2t\text{BuOCO}]^+$

linkage is only facile after N-alkylation. It was shown that N-benylation slightly attenuated the reactivity of the linkage relative to that in the *N*-methyl derivative. N-Benylation of the linker scaffold was conveniently achieved by alkylation under mild conditions. Nosyl groups are normally cleaved using sodium thiophenylate or amines such as propylamine.^[7] Sodium thiophenylate, however, would leave noxious residues that would likely interfere with biological assays. Cleavage of linker scaffold **1b** was investigated to optimize the rate and reaction efficiency. HPLC assays showed that while *n*-propylamine was insufficiently reactive for solid-phase work, treatment of **1b** with sodium sulfide/DMF caused clean and efficient cleavage in less than 1 h. The hydrolysis of excess sodium sulfide gives only hydrogen sulfide and sodium hydroxide. Though toxic, hydrogen sulfide is easily and completely removed by evaporation and/or lyophilization of the solvent media. The aqueous buffers typically used in biological assays will conveniently neutralize small amounts of sodium hydroxide; consequently both the by-products formed from the sodium sulfide mediated cleavage are relatively innocuous.

Scheme 2 illustrates how the linker-scaffold can be used to attach two different pharmacophores to the scaffold arms. The Boc-protecting group was removed first, the liberated amine was then coupled with the first component, and the Fmoc-protected amine was unmasked and coupled with the second component. It is important that a non-nucleophilic base be used in the Fmoc-deprotection step to avoid unwanted





Scheme 2. Attachment of **7** to the resin, N-alkylation, and functionalization of the linker scaffold **1a**. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; DIC = diisopropylcarbodiimide; DMAP = 4-dimethylaminopyridine; HOBT = 1-hydroxy-1*H*-benzotriazole; TFA = trifluoroacetic acid.

cleavage of the linker fragment. Only one compound **9** (Table 1) is shown in Scheme 2, but a small library of homo- and heterodimeric ureas, pyridinium salts, carboxylic acids, and guanidines has been prepared. Full details of this library synthesis will be submitted shortly in the full account of this work.

The specific design of the linker scaffold **1** is special because it is the first to be produced to address the problem of making libraries of pharmacophores to mimic or disrupt protein–protein interactions. The most relevant prior research in this area involves scaffolds that do not have custom-made cleavable linkers, but have either flexible amines^[8–10] or

carbohydrate scaffolds.^[11, 12] Many other linker scaffold systems using other skeletons attached to orthogonally cleavable linking groups could be envisaged. Indeed, slight modifications of the approaches presented here could be used to produce systems with longer or shorter arms, and more or less rigidity. It is likely that this type of work will evolve into an important area of solid-phase synthesis.

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First Generation Light-Harvesting Dendrimers with a [Ru(bpy)₃]²⁺ Core and Aryl Ether Ligands Functionalized with Coumarin 450

Xiaoli Zhou, Daniel S. Tyson, and Felix N. Castellano*

Dendritic macromolecules are well defined, treelike structures that are typically prepared by a series of repetitive synthetic steps.^[1–3] Dendrimer chemistry continues to rapidly develop as a field, since dendritic molecules are both fundamentally interesting and have many potential applications. Of particular interest are dendrimers that can be fabricated to control patterns of energy migration throughout the structure, enabling efficient light harvesting.^[4, 5] Dendritic molecules constructed around Ru^{II} polypyridyl complexes that display metal-to-ligand charge transfer (MLCT) excited

[*] Prof. F. N. Castellano, X. Zhou, D. S. Tyson
Department of Chemistry
and Center for Photochemical Sciences
Bowling Green State University
Bowling Green, OH 43403 (USA)
Fax: (+1) 419-372-9809
E-mail: castell@bgsu.edu

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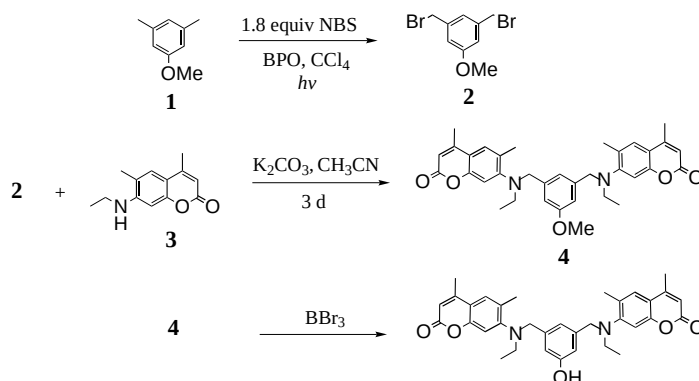
states are especially intriguing because of their outstanding photochemical, photophysical, and electrochemical properties.^[6] In this regard, many large polynuclear dendritic arrays of MLCT complexes have been synthesized and their photophysical and electrochemical properties generally display novel characteristics.^[7] Very recently, it has been reported that a single $[\text{Ru}(\text{bpy})_3]^{2+}$ core (bpy = 2,2'-bipyridine) can be tethered to a well defined array of benzyl, 4'-*tert*-butylphenoxy, or naphthyl peripheral units.^[8, 9] The dendritic wedges employed in these studies have been shown to improve the luminescence properties of the metal complex and can modulate the rate of intermolecular electron transfer reactions from the $[\text{Ru}(\text{bpy})_3]^{2+}$ core.^[8, 9]

In addition to metal-containing photoactive dendrimers, there have been marked advances in the development of purely organic light-harvesting dendrimers.^[4, 5, 10] In the latter, light is primarily absorbed by the peripheral antenna units (energy transfer donors) and is transferred rapidly and efficiently to a single fluorescent moiety, whose emission is sensitized. A recent strategy using long-range Förster-type resonance energy transfer^[11] has been shown to funnel the photonic energy to the final acceptor, independent of the dendritic architecture.^[10] Molecules based on dendritic architectures permit large cross-sections for photon collection, a feature particularly suited to light-harvesting purposes.

Continuing our efforts in the field of hybrid organic/inorganic light-harvesting systems based on long-range resonance energy transfer,^[12] we report the synthesis and photophysical characterization of a new bipyridine ligand, bpy-(C-450)₄, which carries a first-generation poly(aryl ether) dendritic wedge containing coumarin 450 (C-450) units in the 4,4'-positions, and two associated Ru^{II} complexes, $[\text{Ru}(\text{bpy})_2\{\text{bpy}(\text{C-450})_4\}]^{2+}$ and $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$, which possess four and twelve C-450 peripheral units, respectively. The singlet C-450 fluorescence substantially overlaps the absorption spectrum of the $[\text{Ru}(\text{bpy})_3]^{2+}$ core (Förster distance $R_0 = 41.2 \text{ \AA}$ in CH_3CN , assuming an orientation factor $\kappa^2 = 2/3$); such conditions are favorable for Förster-type singlet-singlet resonance energy transfer. Importantly, the C-450 fluorescence displays a large Stokes shift which lowers the probability for self-quenching by energy transfer between the donors.^[13] Excitation of the C-450 antennae initiates nonradiative energy transfer to the central $[\text{Ru}(\text{bpy})_3]^{2+}$ complex, which then displays its characteristic long lifetime, sensitized photoluminescence. This hybrid architecture harvests near-visible light and converts the blue nanosecond C-450 fluorescence to an orange, long lifetime MLCT-based emission.

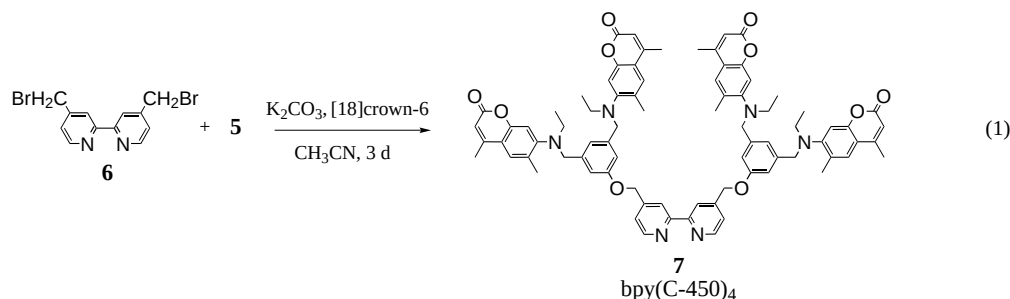
The synthesis of the dendritic wedge was modeled after the "reversed" convergent growth of laser dye-labeled poly(aryl ether) dendrimers developed by Fréchet and co-workers, Scheme 1.^[10] Compound **5** represents a first generation

dendritic wedge and contains two peripheral C-450 chromophores. Equation 1 presents the one-pot synthesis of bpy-(C-450)₄ prepared from the dye-labeled phenol **5** and 4,4'-bis(bromomethyl)-2,2'-bipyridine. This step doubles the number of peripheral C-450 chromophores to four. The dendritic metal-organic light harvesting complexes, $[\text{Ru}(\text{bpy})_2\{\text{bpy}(\text{C-450})_4\}]^{2+}$ and $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$, are shown in Scheme 2. Both metal complexes were prepared according to standard procedures and purified by gel filtration chroma-



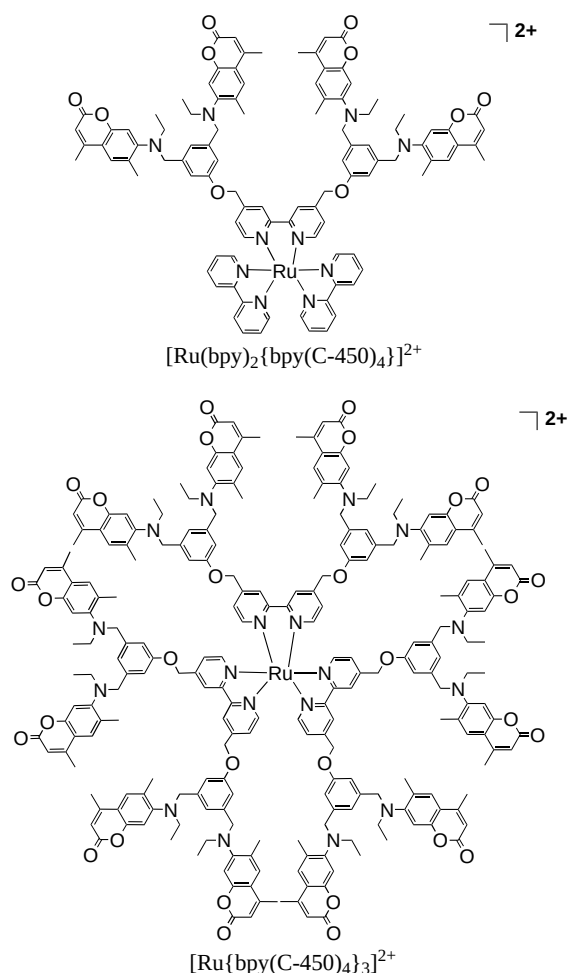
Scheme 1. Synthesis of the C-450 dye-labeled dendritic wedge. **5**

tography on Sephadex LH-20. Analytically pure samples were obtained using reversed-phase HPLC. Since two monomer wedges are covalently attached to a single diimine ligand, and three such diimine ligands are converged onto a single Ru^{II} metal center in $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$, the number of peripheral organic chromophores using the above hybrid design



obey the growth model 6×2^n , where n is the generation number of the wedge. Molecular modeling of $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$ using a Merck force field (MMFF 94, Spartan 5.0) shows that the donor-acceptor distances range from 7.7 to 16.4 $\text{\AA}$, well within the Förster distance.

Figure 1 displays the absorption and uncorrected emission spectra of $[\text{Ru}(\text{bpy})_2\{\text{bpy}(\text{C-450})_4\}]^{2+}$ and $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$ in CH_3CN . The electronic spectra for each complex contains typical $[\text{Ru}(\text{bpy})_3]^{2+}$ visible absorption bands at 460 nm with extinction coefficients of 14200 and $15100 \text{ M}^{-1} \text{ cm}^{-1}$ for $[\text{Ru}(\text{bpy})_2\{\text{bpy}(\text{C-450})_4\}]^{2+}$ and $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$, respectively. In both complexes, the C-450 units are largely responsible for the broad absorption band centered at 343 nm. The molar extinction coefficients at 343 nm increase from $\sim 8000 \text{ M}^{-1} \text{ cm}^{-1}$ for $[\text{Ru}(\text{bpy})_3]^{2+}$,



Scheme 2. Structures of the first generation metal-organic light-harvesting dendrimers.

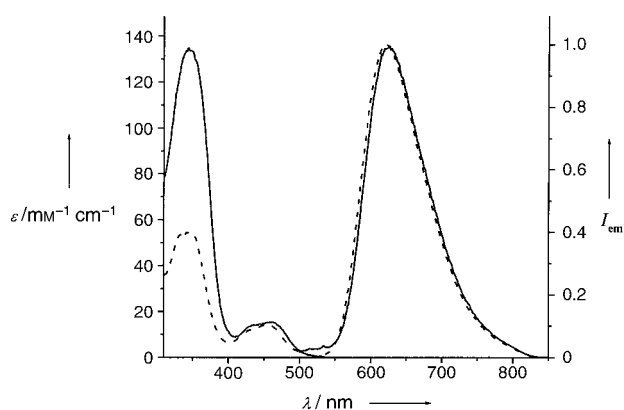


Figure 1. Absorption (left side) and uncorrected emission (right side) spectra of $[\text{Ru}(\text{bpy})_2\{\text{bpy}(\text{C-450})_4\}]^{2+}$ (---) and $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$ (—) in CH_3CN at 20°C .

to $53\,200\text{ M}^{-1}\text{ cm}^{-1}$ for $[\text{Ru}(\text{bpy})_2\{\text{bpy}(\text{C-450})_4\}]^{2+}$, and $136\,000\text{ M}^{-1}\text{ cm}^{-1}$ for $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$. The C-450 chromophores account for 85 % and 94 % of the light absorbed at 343 nm in $[\text{Ru}(\text{bpy})_2\{\text{bpy}(\text{C-450})_4\}]^{2+}$ and $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$, respectively. Since the lowest energy C-450 absorption bands are well removed from the $[\text{Ru}(\text{bpy})_3]^{2+}$ transitions, good

photoselectivity in this wavelength region can be achieved. The enhanced absorption cross-sections between 300 and 380 nm from the C-450 chromophores provide an antenna system for the efficient harvesting of near-visible light. Excitation at wavelengths between 300 and 550 nm results in the observation of $[\text{Ru}(\text{bpy})_3]^{2+}$ MLCT-based emission centered near 620 nm in both light-harvesting complexes (Figure 1).

The absorption and corrected excitation spectra of $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$ in CH_3CN are presented in Figure 2. For brevity, $[\text{Ru}(\text{bpy})_2\{\text{bpy}(\text{C-450})_4\}]^{2+}$ is omitted from this discussion,

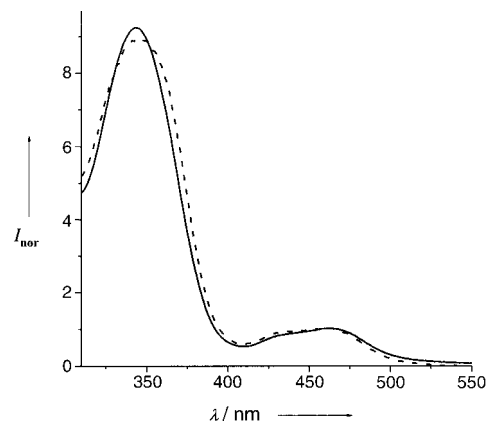


Figure 2. Absorption (—) and corrected excitation (---) spectra of $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$ in CH_3CN normalized at 460 nm. The excitation spectrum was detected at 610 nm.

however, the experimental results are similar to that observed with $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$. The absorption and corrected excitation spectra of $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$ are nearly superimposable at all wavelengths between 310 and 550 nm, which suggests an energy transfer efficiency close to unity. By directly comparing the magnitude of the absorption and corrected excitation spectra,^[5, 10, 12] the energy transfer efficiency of $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$ was determined to be $\sim 95 \pm 10\%$. We realize that this is a crude estimate of the energy transfer efficiency and further experimentation is required to obtain a more reliable value. In addition, there is always a minor amount of residual C-450 fluorescence (Figure 3) which is present in all analytically pure samples, suggesting that the transfer efficiency is less than unity.

The luminescence quantum yields of $[\text{Ru}(\text{bpy})_2\{\text{bpy}(\text{C-450})_4\}]^{2+}$ and $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$, measured in deaerated CH_3CN , are very similar to that observed for the “naked” $[\text{Ru}(\text{bpy})_3]^{2+}$ complex, within experimental error (Table 1). The presence of four or twelve C-450 units tethered to the $[\text{Ru}(\text{bpy})_3]^{2+}$ core through poly(aryl ether) spacers does not appear to provide any additional nonradiative decay pathways which could potentially shorten the MLCT excited states.

Complete spectroscopic and photophysical data for the compounds in this study are presented in Table 1. Using our N_2 pumped dye laser apparatus (instrument response function (IRF) = 15 ns), the excited state lifetimes of $[\text{Ru}(\text{bpy})_2\{\text{bpy}(\text{C-450})_4\}]^{2+}$ and $[\text{Ru}\{\text{bpy}(\text{C-450})_4\}_3]^{2+}$ measured at 610 nm in deaerated CH_3CN are 1.00 ± 0.05 and $1.04 \pm 0.05\ \mu\text{s}$, respectively, and follow single exponential decays, regardless

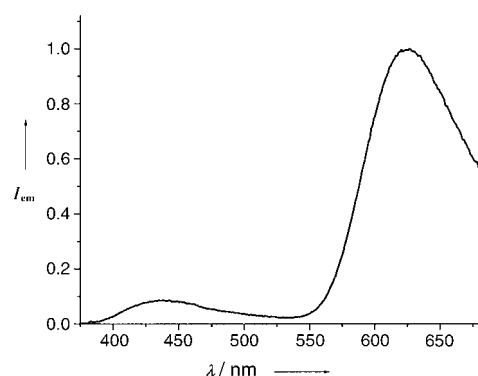


Figure 3. Uncorrected emission spectrum of $[\text{Ru}(\text{bpy}(\text{C-450})_4)_3]^{2+}$ in CH_3CN , excited at 343 nm.

of excitation wavelength (350–480 nm). These values resemble that observed for $[\text{Ru}(\text{bpy})_3]^{2+}$ in deaerated CH_3CN as measured in our laboratory ($\tau = 920 \pm 50$ ns). However, air-equilibrated solutions of the same complexes are quite different. For example, $[\text{Ru}(\text{bpy}(\text{C-450})_4)_3]^{2+}$ displays the longest excited state lifetime, which can be most likely attributed to shielding of the $[\text{Ru}(\text{bpy})_3]^{2+}$ core by the dendrimer branches, hindering the access of dioxygen. This effect has been previously observed in related dendritic metal complexes and it should be noted that the extent of shielding generally increases with increasing dendrimer generation.^[8, 9] $[\text{Ru}(\text{bpy}(\text{C-450})_4)_3]^{2+}$, a first generation dendrimer, displays similar sensitivity to dioxygen as a second generation Ru^{II} core dendrimer that contains 24 peripheral benzyl units, suggesting that higher generations of the C-450 labeled molecules may provide more complete shielding of the metal core in fewer generations. We would like to note that $[\text{Ru}(\text{bpy})_3]^{2+}$ is not necessarily the perfect model of the dendrimer core, therefore the lifetimes contrasted in aerated solution should be viewed with caution.

In an attempt to estimate the rate of singlet energy transfer, time-correlated single-photon counting measurements were employed. With 343 nm pulsed excitation from a H_2 lamp, no measurable rise time was observed for the MLCT-based emission at 620 nm, indicating that the rate is rapid, $> 10^9 \text{ s}^{-1}$. However, we do observe some residual coumarin emission with complex kinetic behavior. At present, we are unable to resolve these components due to the time resolution limits of our single photon counting experiment.

Light-harvesting systems based on hybrid organic/inorganic designs may be applicable to single molecule spectroscopy.^[14] The MLCT-based orange emission of nanomolar concentrations of $[\text{Ru}(\text{bpy}(\text{C-450})_4)_3]^{2+}$ (in CH_3CN) can be detected with the naked eye in a darkened room with 4 Watt long-wave black-light excitation. For this first generation light-harvesting system, the sensitized $[\text{Ru}(\text{bpy})_3]^{2+}$ -based emission can be detected to a 250 femtomolar concentration using a conventional single photon counting fluorimeter. Lower detection limits may be achieved by utilizing higher generation (2nd and 3rd) laser-dye labeled dendrimers. Alternatively, detection limits in the present system could be markedly improved by using a pulsed laser excitation source in conjunction with gated detection, as routinely employed in time-resolved luminescence-based immunoassays.^[15]

The present dendritic hybrid light-harvesting system exhibits a high quantum yield sensitized emission with a $\sim 275 \text{ nm}$ ($\sim 13025 \text{ cm}^{-1}$) Stokes shift (relative to C-450 excitation) that has a single exponential decay and a long lifetime. Due to the increased absorption coefficients and high efficiency of energy transfer, excitation of the C-450 units yielded substantially more MLCT-based emission than was observed from direct excitation of the inorganic core. The well defined, highly soluble structures permitted exact analytical characterization of the macromolecules. This hybrid design permits the attachment of more peripheral chromophores in fewer generations than is possible with a pure organic structure. In the current system, the Ru^{II} MLCT core is somewhat protected from dioxygen quenching in the first generation, suggesting that higher generations will provide a larger degree of shielding and allow their use under ambient conditions. The long lifetimes associated with these new structures permit bimolecular solution chemistry commonly associated with $[\text{Ru}(\text{bpy})_3]^{2+}$, such as electron transfer reactions.^[6, 9] The current approach could potentially be extended to other donor–acceptor organic–inorganic pairs to harvest visible light and exhibit sensitized long lifetime emission in the red to near IR range.

The development of photochemically stable light-harvesting complexes that possess long excited state lifetimes is important for the analytical applications of fluorescence spectroscopy, such as lifetime-based chemical sensing.^[16] In addition, long lifetime extrinsic luminescence probes with large absorption cross-sections have potential applications in biological systems where low analyte concentrations require

Table 1. Spectroscopic and photophysical data at room temperature in CH_3CN .

Complex	λ_{max} (ε) [nm] ([$\text{M}^{-1}\text{cm}^{-1}$])	$\lambda_{\text{em max}}$ [nm]	$\tau_{\text{em}}^{\text{[a]}}$ (deaerated) [μs]	$\tau_{\text{em}}^{\text{[a]}}$ (air equil.) [ns]	$\Phi_{\text{f}}^{\text{[b]}}$	Energy transfer efficiency [%]
$[\text{Ru}(\text{bpy})_3]^{2+}$	452 (13 000)	615	0.920 ± 0.05	165 ± 15	0.062 ± 0.006	NA
$[\text{Ru}(\text{bpy})_2(\text{bpy}(\text{C-450})_4)]^{2+}$	343 (53 200), 455 (14 200)	621	1.00 ± 0.05	277 ± 15	0.071 ± 0.007	96 ± 10
$[\text{Ru}(\text{bpy}(\text{C-450})_4)_3]^{2+}$	343 (136 000), 462 (15 100)	625	1.04 ± 0.05	306 ± 15	0.073 ± 0.007	95 ± 10
$\text{bpy}(\text{C-450})_4$	343 (39 000)	450	NA	$3.9 \pm 0.2^{\text{[c]}}$ (75 %) 1.6 ± 0.1 (25 %)	NA	NA

[a] Lifetimes represent an average of at least six measurements with an uncertainty of less than 5%. Here, the data were obtained using excitation at $458 \pm 2 \text{ nm}$. [b] Errors represent reproducibility within 2σ , including the uncertainty in the measurement of the standard. [c] Measured by time-correlated single photon counting (TCSPC) with excitation at 343 nm. Decays were complex and two lifetimes were observed. Numbers in parenthesis indicate relative amplitudes of each decay component.

detection. Such long-lived luminophores are amenable to situations where off gating can be used to suppress auto-fluorescence in biological systems or scattered light in opaque samples.

Experimental Section

All manipulations were performed under an inert and dry argon atmosphere using standard techniques. 4,6-Dimethyl-7-ethylaminocoumarin (Coumarin 2 or Coumarin 450), 3,5-dimethylanisole, 4,4'-dimethyl-2,2'-bipyridine, anhydrous THF, and anhydrous CH_2Cl_2 were purchased and used as received. Potassium carbonate was ground and dried in an oven (140 °C) at least 12 h before use. Acetonitrile was dried over CaH_2 and 4 Å molecular sieves and distilled from CaH_2 immediately before use. Diisopropylamine was also distilled from CaH_2 immediately before use. Water was deionized with a Barnstead E-Pure system. $[\text{Ru}(\text{bpy})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$ ^[17] and $[\text{Ru}(\text{DMSO})_4]\text{Cl}_2$ ^[18] were prepared according to published procedures. 3,5-Bis(*N*-(4,6-dimethyl-7-ethylaminocoumarin)methyl)phenol, **5**, was synthesized as described.^[10] Commercially available $[\text{Ru}(\text{bpy})_3]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ (Aldrich) was converted into the corresponding PF_6^- salt by metathesis with NH_4PF_6 (Aldrich) in water. Reversed-phase HPLC (C18, semi-preparative) was performed with a Waters HPLC system equipped with a diode array absorption detector.

¹H NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer. All chemical shifts are referenced to residual solvent signals previously referenced to TMS. Direct insertion probe (DIP) mass spectra were obtained in-house using a Shimadzu GC-MS QP5050A equipped with a QP5000 direct sample inlet device. Fast atom bombardment (FAB) mass spectra were measured at the mass spectrometry facilities of either the University of Maryland (College Park) or at the University of Illinois at Urbana-Champaign. All photophysical measurements were performed on optically dilute samples (optical density ~0.1) in Optima Grade CH_3CN (Fisher), as described previously.^[19]

Full details of the syntheses and characterization of the materials are given in the Supporting Information.

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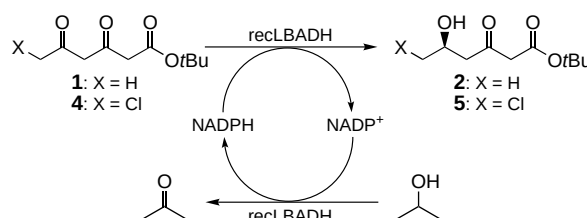
Highly Regio- and Enantioselective Reduction of 3,5-Dioxocarboxylates**

Michael Wolberg, Werner Hummel, Christian Wandrey, and Michael Müller*

3,5-Dioxocarboxylates (β,δ -diketo esters) unify the substructures of β -diketones and β -keto esters in an overlapping manner within a single molecule. While β -diketones and β -keto esters can be reduced with excellent enantioselectivity by metal-catalyzed hydrogenation^[1] and microbial methods,^[2] this does not likewise hold for 3,5-dioxocarboxylates. The question of regioselectivity must additionally be addressed if only one keto group of a 3,5-dioxocarboxylate is to be reduced. Chiral ruthenium catalysts were applied for this purpose and gave respectable, albeit improvable, results.^[3] Clearly, 3,5-dioxocarboxylates represent a class of exceptionally demanding substrates with respect to regio- and enantioselective reduction and hence require particularly sophisticated catalysts. Alcohol dehydrogenases are such catalysts,^[4] and indeed two publications related to biocatalytic reduction of 3,5-dioxocarboxylates report on enhanced enantioselectivities, albeit without simultaneous improvement of regioselectivity in the case of single-site reduction.^[5] Nevertheless, we reasoned that the application of isolated alcohol dehydrogenases is highly promising when both high enantioselectivity and high regioselectivity are desired.

Herein we present a hitherto unprecedented highly enantioselective reduction of 3,5-dioxocarboxylates exclusively at position C-5 (δ). The resulting 5-hydroxy-3-oxocarboxylates are valuable intermediates in the synthesis of chiral building blocks such as 3,5-dihydroxycarboxylates^[6] and β -keto δ -lactones. These compounds, in turn, have found frequent application in the stereoselective synthesis of pharmaceuticals and natural products.^[7]

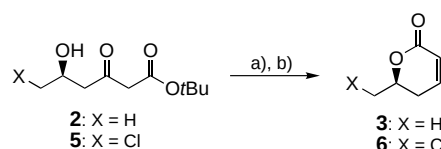
On photometrically screening the NAD(P)H consumption of several reductases in the presence of alkyl 3,5-dioxocarboxylates we found an NADP-dependent alcohol dehydrogenase of *Lactobacillus brevis* (LBADH) that accepts these compounds as substrates. From the viewpoint of synthetic organic chemistry, it is noteworthy that LBADH can be efficiently overexpressed in a recombinant *Escherichia coli* strain and used in the form of a crude cell extract (recLBADH).^[8] Preparative-scale reduction of *tert*-butyl 3,5-dioxohexanoate (**1**)^[9] with recLBADH gave the known



Scheme 1. Enzymatic reduction of 3,5-dioxocarboxylates and substrate-coupled recycling of the cofactor. **2**: 99.4 % *ee*, 77 %; **5**: >99.5 % *ee*, 72 %.

hydroxy keto ester **2**^[10] with 99.4 % *ee* and complete regioselectivity (Scheme 1). Neither the regioisomeric 3-hydroxy-5-oxohexanoate was detected by GC-MS and NMR spectroscopy nor were 3,5-dihydroxyhexanoates. The product **2**, which we used in the synthesis of (*R*)-*semi*-vioxanthin,^[11] is not a substrate for LBADH with regard to reduction of the second keto group, as was determined by photometry.

For determination of the enantiomeric excess, hydroxy keto ester **2** was transformed into (*R*)-parasorbic acid **3**, the two enantiomers of which are easily separable by analytical HPLC on a chiral stationary phase (Scheme 2).^[12]



Scheme 2. Synthesis of α,β -unsaturated δ -lactones **3** and **6**. a) NaBH_4 , EtOH, 0 °C (quant.); b) cat. TsOH, toluene, Δ (60–70 %).

To further increase the usefulness of this new enzymatic reduction as a synthetic method, a functional group was introduced at C-6 of the substrate (Scheme 1). We chose a chloro substituent because it is a generally exploitable functional group. Compared to its unchlorinated analogue **1**, the chloro derivative **4**^[13] is accepted by LBADH with equally good activity. The product of the enzymatic reduction was identified as the expected *tert*-butyl (*S*)-6-chloro-5-hydroxy-3-oxohexanoate (**5**), and regio- and enantioselectivity were again outstanding (>99.5 % *ee*, 72 % yield of isolated product). The absolute configuration of the hydroxy keto ester **5** was established by independent synthesis of an authentic sample and comparison of the signs of the specific rotations.^[14] The enantiomeric excess was determined after conversion to the chlorinated δ -lactone **6** as described above (Scheme 2).^[12]

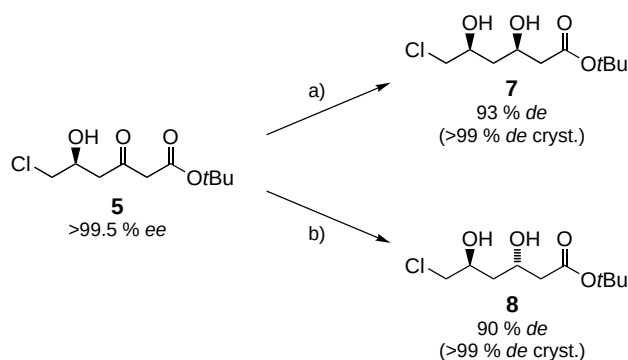
Hydroxy keto ester **5** is an advanced building block in the synthesis of a mevinic acid type HMG-CoA reductase inhibitor.^[7g] Prasad's *syn*-selective reduction method^[6a] was applied in that work. In our hands, this method gave quantitatively *syn*-configured (3*R*,5*S*)-3,5-dihydroxyhexanoate (**7**) with 93 % *de* (Scheme 3). Furthermore, we found that Evans' *anti*-selective method^[6b,c] resulted in the diastereomer (3*S*,5*S*)-3,5-dihydroxyhexanoate (**8**) with 90 % *de* and quantitative yield. The diastereomeric excess of both diols can be readily increased to greater than 99 % by a single recrystallization step (70 % yield).^[15]

[*] Dr. M. Müller, Dipl.-Chem. M. Wolberg, Prof. Dr. C. Wandrey

Institut für Biotechnologie 2
Forschungszentrum Jülich GmbH
52425 Jülich (Germany)
Fax (+49) 2461-61-3870
E-mail: mi.mueller@fz-juelich.de

Dr. W. Hummel
Institut für Enzymtechnologie
Heinrich-Heine-Universität Düsseldorf
40225 Düsseldorf (Germany)

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Scheme 3. Diastereoselective reduction of hydroxy keto ester **5**. a) 1. B(OMe)₃, THF/MeOH (80/20 v/v), –70 °C, 20 min; 2. NaBH₄, –70 °C, 3 h (quant.); b) Me₄N[B(OAc)₃H], MeCN/AcOH (50/50 v/v), –25 °C, 5 h (quant.).

The hydroxy keto esters **2** and **5** were prepared in 77 and 72 % yield by using 0.3–1.0 mol % of NADP⁺ (not optimized) and simple batch techniques. Advantageously, LBADH itself can recycle its cofactor by oxidation of 2-propanol, which is used as an additive (Scheme 1). The excess of 2-propanol is assumed to be the driving force of the reaction, and conversions of greater than 90 % were attained in each case, as was determined by GC-MS and NMR spectroscopy. The enzymatic reduction of the chlorinated diketo ester **4** can be easily scaled up and is routinely performed on a 75-g scale (8 L fed batch) in our laboratory.

In conclusion we have established the first highly regio- and enantioselective method to efficiently reduce polyketide-like compounds by applying isolated enzymes. At present, we are screening for biocatalysts with the opposite sense of enantioselectivity. Investigations concerning the scope of this new method, for example its applicability to dynamic kinetic resolution of C-4-substituted 3,5-dioxocarboxylates, are in progress.

Experimental Section

A solution of recLBADH was prepared by mechanically disrupting wet cells of recombinant *E. coli* strain recADH-HB101 + .^[8] One unit (U) of enzyme activity is defined as the amount of recLBADH that catalyzes the oxidation of 1 μmol NADPH per minute when incubated with acetophenone (10 mM) and NADPH (0.25 mM) at 25 °C and pH 6.5 (100 mM phosphate buffer, 1 mM MgCl₂).

2: In a round-bottom flask, a solution of **1** (1.98 g, 9.9 mmol) in 2-propanol (5.1 mL, 66 mmol) was added to triethanolamine/HCl buffer (330 mL, 250 mM, pH 7.0) containing MgCl₂ (1 mM), and the mixture was vigorously stirred for 10 min. The stirring speed was adjusted to 60 rpm, and NADP⁺ (28 mg, 33 μmol; FLUKA Nr. 93210, 90 %) and recLBADH (660 U) were added. After stirring for 24 h at room temperature, the solution was saturated with NaCl and extracted with ethyl acetate three times. The combined organic phases were dried over MgSO₄, the solvent was evaporated, and the crude product was purified by flash chromatography (silica, ethyl acetate/isohexane 40/60, v/v), to give hydroxy keto ester **2** (1.54 g; 77 %). The ¹H NMR spectrum was in agreement with published data.^[10] ¹³C NMR (75.5 MHz, CDCl₃, 20 °C, only signals of the keto form are given): δ = 22.6 (C-6), 28.1 (C(CH₃)₃), 51.2, 51.3 (C-2, C-4), 63.9 (C-5), 82.4 (C(CH₃)₃), 166.4 (C-1), 204.4 (C-3); [α]_D²⁵ = –40.1 (c = 1.9, CHCl₃), 99.4 % ee;^[10] [α]_D²⁶ = –39.6 (c = 2, CHCl₃), 99 % ee.

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- [13] Prepared by acylation of the lithium bis-enolate of *tert*-butyl acetoacetate with methyl chloroacetate (THF, –60 °C, 70 % yield of isolated product after column chromatography).
- [14] Prepared according to ref. [7g], starting from commercially available ethyl (S)-4-chloro-3-hydroxybutyrate (Aldrich, 97 % ee). [α]_D²⁵ = –23.0 (c = 1.5, CHCl₃), 97 % ee; enzymatic product **5**: [α]_D²⁵ = –24.9 (c = 1.4, CHCl₃), > 99.5 % ee; ¹H NMR (300 MHz, CDCl₃, 20 °C, only

signals of the keto form are given): δ = 4.31 (m, 1H; *CHOH*), 3.62 (dd, J = 11.2, 5.1 Hz, 1H; H-6), 3.57 (dd, J = 11.2, 5.0 Hz, 1H; H-6), 3.41 (s, 2H; H-2), 3.10 (brs, 1H; OH), 2.90 (dd, J = 17.5, 5.0 Hz, 1H; H-4), 2.83 (dd, J = 17.5, 7.3 Hz, 1H; H-4), 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C, only signals of the keto form are given): δ = 28.1 ($C(CH_3)_3$), 46.6, 48.4, 51.3 (C-2, C-4, C-6), 67.6 (C-5), 82.7 ($C(CH_3)_3$), 166.2 (C-1), 202.9 (C-3).

[15] The diastereomeric excess was determined by GC after formation of the corresponding acetonides (2,2-dimethoxypropane, cat. camphorsulfonic acid).

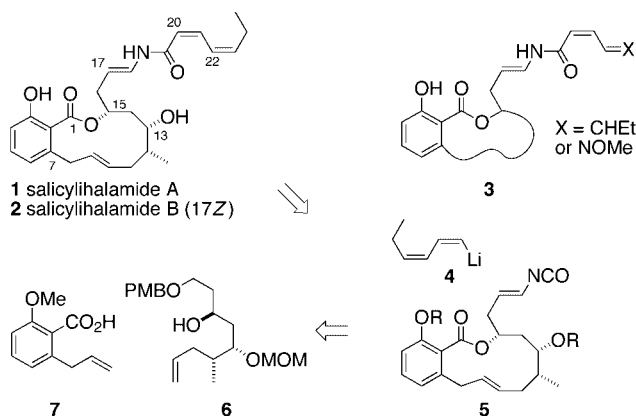
Revision of the Absolute Configuration of Salicylihalamide A through Asymmetric Total Synthesis**

Yusheng Wu, Lothar Esser, and Jef K. De Brabander*

Natural products that elicit a specific and unique biological response in mammalian cells represent valuable tools to identify, study, and target possible new gene products. In this context, the recent isolation of salicylihalamides A and B (**1** and **2**, Scheme 1) from the marine sponge *Haliclona* sp. is noteworthy.^[1] Pattern-recognition analysis of their unique differential 60-cell mean-graph screening profiles (National Cancer Institute) suggests that the salicylihalamides belong to a potentially new mechanistic class of antitumor compounds.^[1] Since their discovery in 1997, an emerging class of novel bioactive metabolites have been isolated that structurally relate to the salicylihalamides by virtue of an unprecedented highly unsaturated enamide attached to a macrocyclic salicylate (generalized structure **3**, Scheme 1). These include the mechanistically related lobatamides,^[2] the potent cytostatic apicularens,^[3] and selective inhibitors of oncogene-transformed cells (oximidines),^[4] as well as compounds that induce low density lipoprotein (LDL) receptor gene expression.^[5] The opportunity to develop chemistry in this area—none of these compounds have been synthesized previously—as well as to access variants for mode of action studies, prompted us to initiate a synthetic program towards this intriguing class of natural products.^[6] Herein, we disclose the total synthesis of **1** and demonstrate unequivocally that the absolute configuration of natural (–)-salicylihalamide A, formulated as **1** through Mosher's ester ^1H NMR spectro-

scopic experiments by the group who isolated it,^[1] was misassigned.

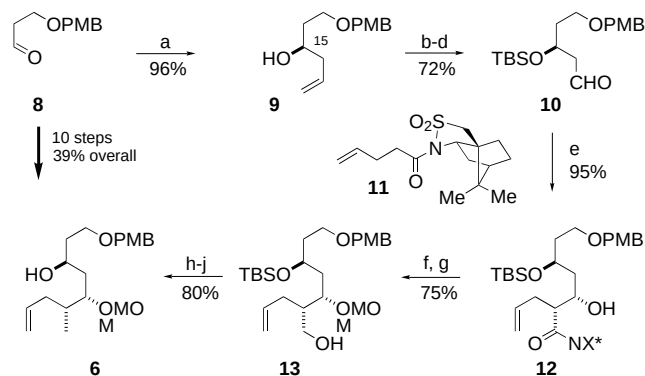
From the onset, we deemed it crucial to introduce the sensitive *N*-(alkenyl)heptadienamide side chain at a late stage in the synthesis (Scheme 1). Considering the options, we felt



Scheme 1. Synthetic strategy for salicylihalamide A. PMB = *para*-methoxybenzyl, MOM = methoxymethyl.

that the addition of 1-lithio-1,3-hexadiene (**4**) to isocyanate **5** would offer the distinct advantage of mild reaction conditions and control of stereochemistry.^[7] Isocyanate **5** was to be derived from the corresponding *E*- α,β -unsaturated carboxylic acid (acyl azide formation/Curtius rearrangement), in turn accessible from a C17 aldehyde by Horner–Wadsworth–Emmons homologation. A Mitsunobu esterification/olefin ring-closing metathesis (RCM) tactic would ultimately unravel the 12-membered benzolactone ring into its primary components, polyol fragment **6** and benzoic acid derivative **7**.

A fully optimized procedure, delivering gram quantities of alcohol **6**, is presented in Scheme 2. We opted for an



Scheme 2. Reagents and conditions: a) 2-4-iodobenzyl allyl ether,^[8] Et_2O , –78 °C, then $NaOOH$, 96%; b) TBSCl, imidazole, DMAP, DMF, 94%; c) cat. OsO_4 , NMO, acetone/ H_2O ; d) $Pb(OAc)_4$, pyridine, PhH, 77% (steps c, d); e) **11**, $TiCl_4$, iPr_2NEt , CH_2Cl_2 , –78 °C, then **10**, –78 °C, 95%; f) MOMCl, NaI, iPr_2NEt , CH_2Cl_2 , 91%; g) $LiEt_3BH$, THF, –78 °C → RT, 82%; h) TsCl, Et_3N , DMAP, CH_2Cl_2 , 91% (5% recovery of **13**); i) $LiEt_3BH$, THF, –78 °C → RT, 90%; j) TBAF, THF, 98%. Icr = isocaranyl, TBS = *tert*-butyldimethylsilyl, DMAP = 4-dimethylaminopyridine, DMF = dimethylformamide, NMO = 4-methylmorpholine *N*-oxide, RT = room temperature, Ts = tosyl = toluene-4-sulfonyl, TBAF = tetrabutylammonium fluoride, X* = boranesultam.

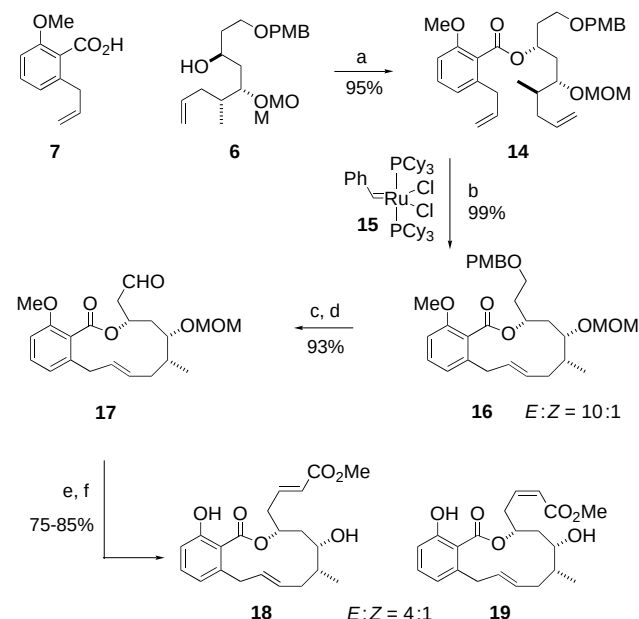
[*] Prof. Dr. J. K. De Brabander, Dr. Y. Wu, Dr. L. Esser^[+]
Department of Biochemistry
University of Texas Southwestern Medical Center at Dallas
5323 Harry Hines Boulevard, Dallas, TX 75390-9038 (USA)
Fax: (+1) 214-648-6455
E-mail: jdebra@biochem.swmed.edu

[+] Author to whom correspondence regarding X-ray crystallographic analysis should be addressed. E-mail: esser@chop.swmed.edu

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enantioselective allylation of aldehyde **8** to set the absolute stereochemistry at C15.^[8] The corresponding homoallyl alcohol **9**, obtained in 96 % yield, was silylated followed by oxidative double-bond cleavage (72 % yield, 3 steps). Treatment of the corresponding aldehyde **10** with the in situ prepared *Z*-(*O*)-titanium enolate^[9] derived from (2*R*)-*N*-(4-pentenyl)bornanesultam (**11**)^[10] produced exclusively one diastereomeric aldol product **12** in 95 % yield.^[11] Reduction of the corresponding MOM ether delivered primary alcohol **13** (75 %, 2 steps), which was further converted into **6** by tosylate formation, reduction, and fluoride-assisted liberation of the C15 alcohol (80 %, 3 steps).

Setting the stage for the RCM,^[12] fragment **6** was joined with carboxylic acid **7**^[13] through a Mitsunobu inversion (Scheme 3).^[14] Exposure of **14** to a catalytic amount of Grubbs' ruthenium carbene complex **15**^[15] preferentially

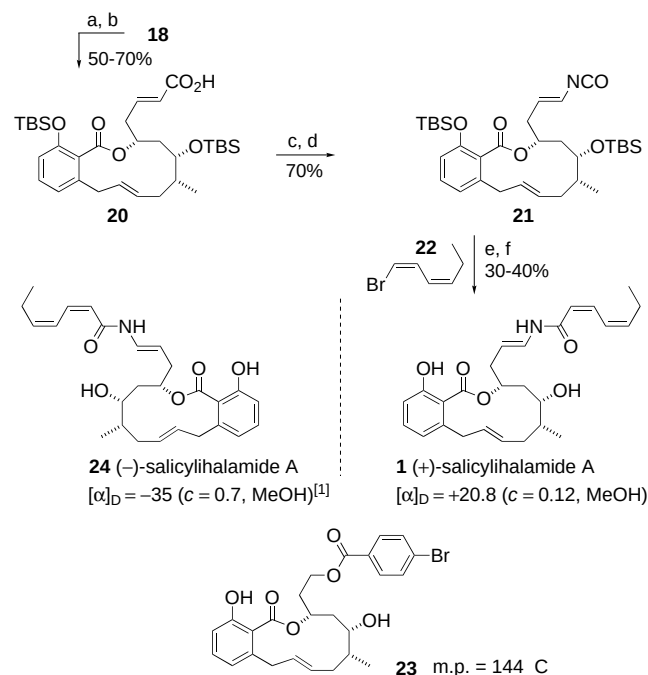


Scheme 3. Reagents and conditions: a) DEAD, PPh_3 , Et_2O , 95 %; b) 10 mol % $[(\text{Cy}_3\text{P})_2\text{Cl}_2\text{Ru}=\text{CHPh}]$ (**15**), CH_2Cl_2 , 99 %; c) DDQ, $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, 96 %; d) Dess–Martin periodinane, CH_2Cl_2 , 97 %; e) trimethyl phosphonoacetate, NaH, THF, 0 °C, 90 %; f) BBr_3 , CH_2Cl_2 , –78 °C, 90 % (**18**:**19** = 4:1). DEAD = diethylazodicarboxylate, Cy = cyclohexyl, DDQ = 2,3-dichloro-4,6-dicyano-1,4-benzoquinone.

produced the desired *E* isomer **16** with an impressive (and reproducible!) selectivity of 10–11:1 (*E*:*Z*).^[16] Moving forward, **16** was oxidatively deprotected (DDQ) and further oxidized to aldehyde **17** by exposure to Dess–Martin periodinane (93 %, 2 steps).^[17] At this stage, we had achieved an extremely efficient synthesis of the lactone core of the salicylihalamides, delivering gram quantities of **17** in 34 % overall yield from aldehyde **8** (14 steps).

All that remained to complete the total synthesis was the introduction of the side chain followed by final deprotection. Thus, following homologation of aldehyde **17** with trimethyl phosphonoacetate (NaH, THF), treatment with BBr_3 led to the corresponding *E*- and *Z*-methyl esters **18** and **19** (4:1), which were separated by flash column chromatography (silica, EtOAc /hexanes (35/65)).^[18]

Hydrolysis ($\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$) of the major *E*-methyl ester **18** was followed by complete silylation with excess TBSCl (Scheme 4). Upon workup, silyl ester hydrolysis had occurred,



Scheme 4. Reagents and conditions: a) $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, MeOH; b) TBSCl, imidazole, DMF, 50–70 % (from **18**); c) $(\text{PhO})_2\text{P}(\text{O})\text{N}_3$, Et_3N , PhH; d) PhH, 80 °C, 70 % (from **20**); e) **22**, *t*BuLi, Et_2O , –78 °C, then add **21**, –78 °C → 0 °C, 55–65 %; f) HF·pyridine, pyridine/THF, 40–60 %.

allowing the corresponding acid **20** to be converted into isocyanate **21** as shown. We were now in a position to explore the crucial installation of the dienamide side chain. Incorporation proceeded smoothly through the addition of hexadienyllithium, prepared in situ from bromide **22**^[19] by metal–halogen exchange (*t*BuLi), to a –78 °C solution of isocyanate **21** (pure compound according to chromatographic analysis).^[7b] The total synthesis was completed by deprotection of the silyl ether protecting groups with HF·pyridine (1:1) in THF, to afford synthetic salicylihalamide A (**1**).^[20] This material was found to be identical to natural salicylihalamide A according to NMR ($[\text{D}_6]\text{benzene}$ and $[\text{D}_4]\text{methanol}$), IR, and UV spectroscopy, as well as HPLC, and thin layer chromatography (3 different solvent systems).^[21]

We were completely surprised, however, that the optical rotation of synthetic salicylihalamide A (**1**) was of opposite sign ($[\alpha]_D^{25} = +20.8$ ($c = 0.12$, MeOH)) to the optical rotation recorded for natural salicylihalamide A ($[\alpha]_D^{25} = -35$ ($c = 0.7$, MeOH)).^[1] Moreover, synthetic **1** was completely ineffective (up to 20 μM) in arresting the growth of SK-MEL-28, a human melanoma cancer cell-line reported to be sensitive to natural salicylihalamides with a GI_{50} of 100 nM (GI_{50} = the concentration at which growth of 50 % of the cells is inhibited).^[1] At this point we had the fortune that *para*-bromobenzoate derivative **23**^[22] provided crystals suitable for X-ray diffraction studies, confirming the absolute configuration of our synthetic lactones.^[23] Based on all the available evidence, the absolute configuration of natural (–)-salicyli-

halamide A was revised unambiguously to the one represented by structure **24** (*ent*-1).

In summary, we have accomplished the first synthesis of (+)-salicylhalamide A and revised the absolute configuration of the natural product to 12*S*,13*R*,15*S*. Our approach features a highly efficient, *trans*-selective ring-closing olefin metathesis for the assembly of the 12-membered salicylate skeleton and can be readily adapted to obtain the natural enantiomer.

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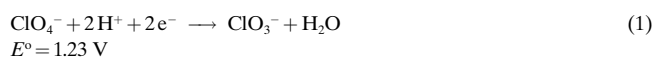
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- [18] Note that **19** is a useful intermediate for the synthesis of *ent*-salicylhalamide B (**2**).
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- [20] The 22*E* isomer (≈ 20%) was removed by preparative HPLC (normal phase, acetone/hexanes (3/7)).^[19]
- [21] A sample and the NMR spectra of natural (–)-salicylhalamide A were kindly provided by Dr. M. R. Boyd, National Cancer Institute (Frederick, MD, USA).
- [22] This derivative was prepared from **16**: 1) DDQ, CH₂Cl₂/H₂O, RT; 2) *para*-bromobenzoic acid, PPh₃, DEAD, Et₂O, RT; 3) BBr₃ (3 equiv), CH₂Cl₂, –78 °C.
- [23] Crystallographic data (excluding structure factors) for the structure reported in this paper (**23**) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-147019. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Clean and Efficient Catalytic Reduction of Perchlorate**

Mahdi M. Abu-Omar,* Lee D. McPherson, Joachin Arias, and Virginie M. Béreau

Even though perchlorate is a strong oxidizing agent thermodynamically [Eq. (1)], its reactions are very slow



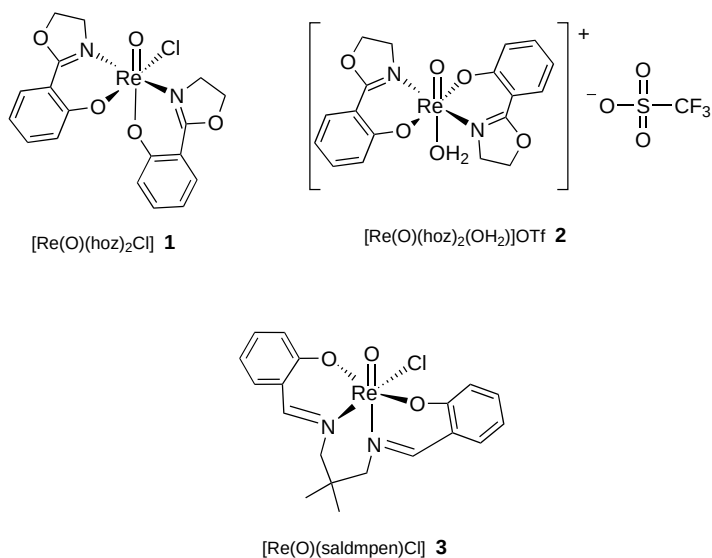
because it is nonlabile.^[1–3] Consequently, perchlorate salts are often used to adjust ionic strength in kinetics and electrochemical investigations. Perchlorate is also a poor complexing (“innocent”) anion.^[4] In 1997, ClO₄[–] was found in ground and surface waters in several U.S. western states at concentrations up to 3700 mg L^{–1}.^[5–7] For example, 30% of the wells sampled

[*] Prof. M. M. Abu-Omar, L. D. McPherson, J. Arias, Dr. V. M. Béreau
Department of Chemistry and Biochemistry
University of California at Los Angeles
405 Hilgard Avenue
Los Angeles, CA 90095–1569 (USA)
Fax: (+1) 310-206-9130
E-mail: mao@chem.ucla.edu

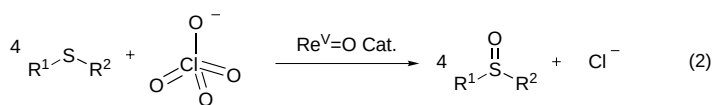
[**] This work was supported by the National Science Foundation (CAREER Award to M.M.A.-O., CHE-9874857), the University of California Toxic Substance Research and Teaching Plan (UCTSR&TP), and the Arnold and Mabel Beckman Foundation (BYI Award to M.M.A.-O.).

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in California were contaminated with perchlorate, and 9% exceeded the state's provisional action level of $18 \mu\text{g L}^{-1}$.^[8] The oxoanion ClO_4^- targets the thyroid gland and muscle tissue.^[9] Most perchlorate in the environment comes from defense and aerospace activities since ammonium perchlorate is an ingredient in rocket fuels.^[6] Perchlorate poses serious challenges for remediation due to the high solubility of its salts (in both water and organic solvents) and its extreme chemical inertness. Therefore, typical water treatment technologies such as ion exchange, carbon adsorption, and air stripping are not effective.^[7] Recent research efforts have focused on bioremediation as a possible treatment for perchlorate-contaminated water.^[10–12] We discovered in our laboratory that the simple oxorhenium(v) coordination complexes **1–3** (hoz = [2-(2'-hydroxyphenyl)-2-oxazoline],



OTf = trifluoromethanesulfonate, saldmpen = [*N,N'*-bis(salicylidene)-1,3-diamino-2,2'-dimethylpropane]) are effective catalysts for the reduction of perchlorate with sulfides under mild conditions [Eq. (2)].



The reduction of perchlorate and other oxoanions by methylrhenium dioxide ($\text{CH}_3\text{ReO}_2 \cdot n\text{H}_2\text{O}$, or MDO) has been reported.^[13, 14] Nevertheless, the need for highly acidic media and the facile polymerization of MDO rendered this system impractical.^[14] Another setback was the fact that the product, methylrhenium trioxide (MTO), required strong reducing agents such as hypophosphorus acid to regenerate MDO.^[14]

The occurrence of the reaction in Equation (2) was established by the following: 1) sulfoxide is the only organic product in the ^1H NMR spectra; 2) the stoichiometry $\text{ClO}_4^-:\text{R}^1\text{SR}^2=1:4$ is based on the yield of sulfoxide as determined by ^1H NMR spectroscopy under conditions in

which ClO_4^- is limiting; 3) the product from perchlorate, Cl^- , is precipitated and isolated as AgCl upon the addition of AgBF_4 at the end of the reaction. The reactivity of the rhenium catalysts **1–3** was probed under steady-state conditions with a variety of sulfides and 0.8% catalyst (Table 1).

Table 1. Catalytic reduction of ClO_4^- with sulfides.^[a]

Entry	Substrate	Catalyst 1 or 2		Catalyst 3	
		<i>t</i> [h]	yield [%] ^[b]	<i>t</i> [h]	yield [%] ^[b]
1	CH_3SCH_3	4	100	48	69
2	$\text{CH}_3\text{CH}_2\text{SCH}_2\text{CH}_3$	4	100	48	62
3	$(\text{C}_6\text{H}_5)\text{SCH}_3$	5	96	48	69
4	$(\text{C}_6\text{H}_5)\text{SCH}_3$ ^[c]	7	83	–	–
5	$(\text{C}_6\text{H}_5)\text{S}(\text{C}_6\text{H}_5)$	7	87	38	57

[a] Conditions: 0.0030 M catalyst, 0.092 M LiClO_4 , 0.50 M sulfide in $\text{CD}_3\text{CN}/\text{D}_2\text{O}$ (v/v 95/5) at 20°C . [b] Yield of sulfoxide. [c] In the presence of 0.10 M LiCl .

Catalysts **1** and **2** gave rise to identical results, indicating that the two precursors yield the same active catalyst. On the other hand, the rhenium–saldmpen compound **3**,^[15] even though reactive, was clearly inferior to **1** and **2**. The catalytic kinetics are independent of the nature of the sulfide; aryl and alkyl sulfides give comparable conversion rates. Thus the rate-determining step (RDS) involves the reaction of perchlorate and oxorhenium(v). Addition of LiCl showed product inhibition (Table 1, entry 4).^[16]

The catalytic reaction proceeds initially through oxidation of the rhenium(v) complex (green) to a cationic dioxorhenium(vii) complex (red),^[17] followed by the rapid reduction of dioxorhenium(vii) back to oxorhenium(v) with organic sulfides. This color change of the rhenium complex is significant because spectrophotometric methods for the detection of perchlorates are nonexistent.^[18]

The reaction of perchlorate with oxorhenium(v) complexes **1** and **2** was studied by stopped-flow spectrophotometry, following the newly formed peak at 500 nm due to the dioxorhenium(vii).^[19] The two rhenium complexes display identical kinetics; compound **1** undergoes chloride dissociation initially to give $[\text{Re}(\text{O})(\text{hoz})_2(\text{OH}_2)]^+$ with Cl^- as the counteranion. The kinetics were first-order in $[\text{Re}]_{\text{T}}$ and saturation in rate was observed with respect to $[\text{ClO}_4^-]$ (Figure 1). The kinetics were conducted in CH_3CN and the rate was independent of the H_2O concentration over a wide range (0.70–5.3 M). At saturation in $[\text{ClO}_4^-]$, k_{max} from the nonlinear least-squares fitting is $0.34 \pm 0.05 \text{ s}^{-1}$. When chlorate (ClO_3^-) was used as the oxidant (in place of perchlorate) the kinetics were first-order in $[\text{Re}]_{\text{T}}$ but zero-order in $[\text{ClO}_3^-]$, even at concentrations as low as 0.0050 M ClO_3^- (Figure 1). It is worth noting that k_{max} at the plateau for both perchlorate and chlorate is the same ($\approx 0.35 \text{ s}^{-1}$), indicating a RDS that is dependent on the catalyst but not the oxoanion [Scheme 1, Eq. (3)].^[20] Indeed, substitution reactions of these oxorhenium(v) complexes are dissociative, and intermediate [I] in Scheme 1 is most likely a five-coordinate complex.

Since the red dioxorhenium(vii) complex persists over a reasonable amount of time (see below), its reaction with sulfides was investigated independently by stopped-flow spectrophotometry. Solutions of dioxorhenium(vii) were pre-

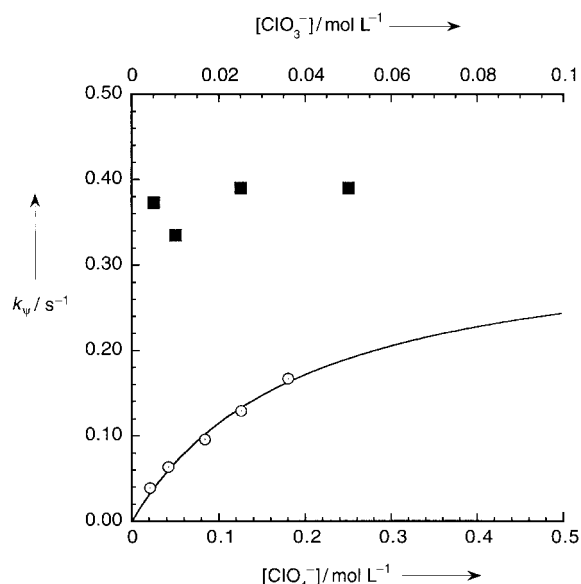
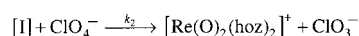
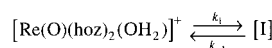


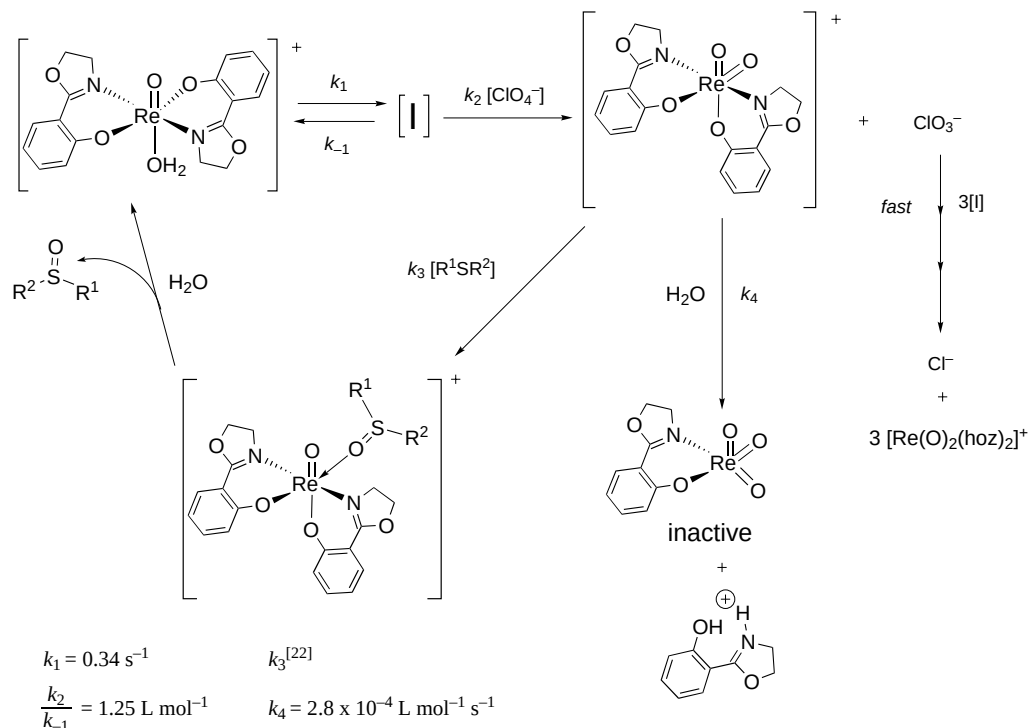
Figure 1. Concentration dependencies for the oxidation of $[\text{Re}(\text{O})(\text{hoz})_2(\text{OH}_2)]^+$ to $[\text{Re}(\text{O})_2(\text{hoz})_2]^+$ by ClO_4^- (○) and ClO_3^- (■) as measured by stopped-flow kinetics.



Scheme 1. Mechanism of ClO_4^- reduction by $[\text{Re}(\text{O})(\text{hoz})_2(\text{OH}_2)]^+$ ($k_1 = 0.34 \pm 0.05 \text{ s}^{-1}$, $k_{-1}/k_2 = 0.80 \pm 0.16 \text{ mol L}^{-1}$). [I] = intermediate.

$$v = \frac{-d[\text{ClO}_4^-]}{dt} = \frac{k_1 k_2 [\text{Re}][\text{ClO}_4^-]}{k_{-1} + k_2 [\text{ClO}_4^-]} \quad (3)$$

pared from the reaction of LiClO_4 or pyridine-*N*-oxide and complex **2** in situ, then mixed with sulfide, and the decay of the peak at 500 nm was monitored.^[21] The second-order rate constants for a few sulfides were determined (Scheme 2).^[22] The trend in the kinetics is in agreement with the cationic dioxorhenium(vii) complex acting as an electrophilic oxygen donor. Sulfoxides are much less reactive than their corresponding sulfides;^[22] this fact is consistent with the observation that under steady-state catalysis sulfoxide is the only product and no sulfone is detected. The second-order rate constants for most sulfides, Ph_2S being the exception, are much larger than k^{app} ($k^{\text{app}} = k_1 k_2 / k_{-1} = 0.43 \text{ L mol}^{-1} \text{ s}^{-1}$, Scheme 1) for the perchlorate reaction with rhenium(v). Thus,



Scheme 2. Mechanism and rate constants for the catalytic reduction of perchlorate.

under catalytic conditions rhenium is dominantly present as oxorhenium(v).

In the absence of a reductant, sulfide, the red dioxorhenium(vii) complex decomposes with first-order dependence on $[\text{Re}]$ and $[\text{H}_2\text{O}]$ ($k_4 = 2.8 \pm 0.2 \times 10^{-4} \text{ L mol}^{-1} \text{ s}^{-1}$; Scheme 2).^[23] The rate of decomposition is independent of $[\text{ClO}_4^-]$. The formed yellow compound $[(\text{hoz})\text{Re}^{\text{VII}}\text{O}_3]$ is inactive; it is reduced by neither sulfides nor phosphanes. This decomposition pathway, however, is insignificant under steady-state conditions since the catalyst is present dominantly ($\geq 99\%$) in the oxorhenium(v) form (see above), which is indefinitely stable, and the reduction of $[\text{Re}^{\text{VII}}(\text{O})_2(\text{hoz})_2]^+$ by most sulfides is faster than decomposition ($k_3 \gg k_4$).

Since aryl sulfides absorb in the UV range much more intensely than sulfoxides, we studied the kinetics of catalytic reduction of perchlorate with thioanisole, PhSMe , at 260 nm. A typical kinetic trace is shown in Figure 2a. The rate is zero-order with respect to $[\text{PhSMe}]$, first-order in $[\text{ClO}_4^-]$, and first-order in catalyst ($[\text{I}]_{\text{T}}$). Thus, the RDS is the reaction of perchlorate with **1**. The rise in absorption following the zero-order consumption of PhSMe is due to the formation of the dioxorhenium(vii) species once PhSMe is depleted. Under these conditions, PhSMe is limiting (1.0–2.0 mM) and ClO_4^- is in excess (0.0050–0.025 M). The program KINSIM^[24] was used to simulate the kinetics under these conditions (Figure 2b). The simulated data clearly show that the major form of the catalyst under steady-state conditions is rhenium(v), and dioxorhenium(vii) only evolves after PhSMe is depleted. From a plot of the initial rate (V_i) versus $[\text{I}]_{\text{T}}[\text{ClO}_4^-]$, an experimental second-order rate constant was computed and found to be $0.33 \pm 0.04 \text{ L mol}^{-1} \text{ s}^{-1}$, which is in excellent agreement with that determined independently by stopped-

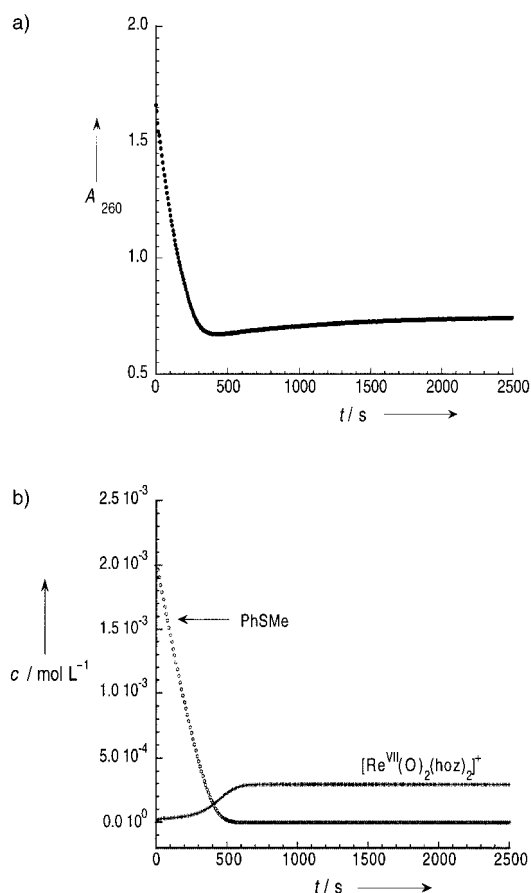


Figure 2. a) Time profile for the oxidation of thioanisole (PhSMc), monitored at 260 nm (pathlength 1.00 mm), with ClO_4^- as catalyzed by complex **1**. Conditions: $[\text{PhSMc}] = 2.0 \text{ mM}$, $[\text{LiClO}_4] = 0.022 \text{ M}$, $[\mathbf{1}] = 3.0 \times 10^{-4} \text{ M}$, $[\text{H}_2\text{O}] = 1.0 \text{ M}$ in CH_3CN at 20.0°C . b) Simulated kinetics using the program KINSIM, showing the evolution of the dioxorhenium(vii) complex after the zero-order consumption of PhSMc.

flow methods for the reaction of **1** with ClO_4^- ($k_1 k_2 / k_{-1}$, Scheme 2).

Complexes **1** and **2** are promising catalysts for the chemical treatment of perchlorate because of the following advantages: 1) They are stable towards air and moisture; 2) they show very little decomposition even after hundreds of turnovers;^[25] 3) they are easily prepared from the readily available compound $[\text{Re}(\text{O})\text{Cl}_3(\text{OPPh}_3)(\text{Me}_2\text{S})]$;^[26] 4) the reduction of perchlorate proceeds cleanly to sulfoxide and chloride;^[27] and 5) the oxazoline ligands employed in these catalysts are easy to make from inexpensive and commercially available starting materials.^[28] Furthermore, the ligands could be easily functionalized by straightforward organic synthesis,^[29] and thus, these catalysts could be tethered to a resin or support, allowing the treatment of perchlorate heterogeneously. In addition to the possible application to remediation, the color change associated with the reaction of **1** with ClO_4^- could be utilized in analysis of perchlorate at submillimolar levels based on the molar absorptivity of the dioxorhenium(vii) product.

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- [20] The oxidation of complex **1** by ClO_4^- is inhibited by other anions such as Cl^- , OTf^- , and PF_6^- . For example, the second-order rate constant $k^{\text{app}} = 0.08 \text{ L mol}^{-1} \text{ s}^{-1}$ in 0.075 M LiOTf and 0.025 M LiClO_4 ($\mu = 0.10$), but $k^{\text{app}} = 0.24 \text{ L mol}^{-1} \text{ s}^{-1}$ with 0.10 M LiClO_4 ($\mu = 0.10$). However, the reaction of **1** with ClO_3^- is unaffected by LiOTf .
- [21] In a typical experiment, compound **2** (1.0 mM) is treated with LiClO_4 or pyridine-*N*-oxide (1.0–5.0 mM), and the resulting red solution is allowed to react on the stopped-flow instrument with the reagent (0.01–0.10 M; for $\text{CH}_3\text{S}(\text{O})\text{CH}_3$ 0.50–2.0 M) at $20.0 \pm 0.2^\circ\text{C}$. An Applied Photophysics SX18.MV stopped-flow spectrophotometer was employed.
- [22] Several values for k_3 [$\text{L mol}^{-1} \text{ s}^{-1}$] (Scheme 2): Me_2S : 7500 ± 300 , Et_2S : 6900 ± 200 , PhSMc: 110 ± 8 , Ph_2S : 1.1 ± 0.1 , $\text{Me}_2\text{S}(\text{O})$: 0.85 ± 0.04 .
- [23] $[\mathbf{1}] = 5.0 \times 10^{-4} \text{ M}$, $[\text{LiClO}_4] = 0.010\text{--}0.025 \text{ M}$, $[\text{H}_2\text{O}] = 1.0\text{--}5.0 \text{ M}$, $\mu = 0.10$, $T = 20.0 \pm 0.2^\circ\text{C}$.
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- [26] See the Supporting Information for detailed synthetic procedures and characterizations of the oxorhenium(v) complexes **1** and **2**.
- [27] Dimethyl sulfoxide (DMSO) is essentially nontoxic, especially at the low concentrations that would be employed in water treatment (LD_{50} orally in rats is greater than 20 g Kg^{-1}), and is used as a topical antiinflammatory agent (*The Merck Index*, 11th ed., Merck & Co., Rahway, NJ, USA, **1989**).
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The First Entry to Complex Bakkanes: A Highly Effective Retroaldol–Aldol-Based Approach to (–)-Bakkenolides III, B, C, and H**

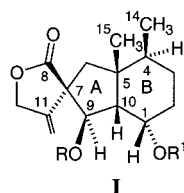
Olivier Hamelin, Yanyun Wang, Jean-Pierre Deprés,*
and Andrew E. Greene

*Dedicated to Prof. Jean Lhomme
on the occasion of his retirement*

Since the first members were isolated and elucidated in the late 1960s, the bakkanes have rapidly increased in number, particularly recently, so as to count now approximately 50.^[1] These hydrindane sesquiterpenes of both terrestrial and marine origin, characterized by their novel spiro γ -butyrolactone function and significant biological effects,^[1m,n,2] have become popular synthetic targets. To date bakkenolide A, homogynolides A and B, palmosalide C, and 9-acetoxylukinanolide have been prepared, most more than once.^[3]

The nonfunctionalized and monofunctionalized bakkenolides, however, are each oxygenated singly and taken together comprise only a relatively minor number of the known bakkanes. Much more representative (ca. 80 %) of this class are the densely functionalized and stereochemically challenging C1,C9-dioxygenated bakkenolides (**1**, **R**, **R**¹ = H, acyl). These compounds, which have significant inhibitory activity toward Hep G2, Hep G2.2,15, and P-388 tumor cell lines,^[1n] as well as PAF, arachidonic acid, and collagen-induced platelet aggregation,^[1m] have been isolated from *Petasites japonicus* Maxim. and *Petasites formosanus* Kitamura (Compositae).^[1b–f, m, n]

Herein we illustrate the first entry to this large group of bakkanes through the preparation of the representative natural bakkenolides III, B, C, and H (**1–4**). We note in



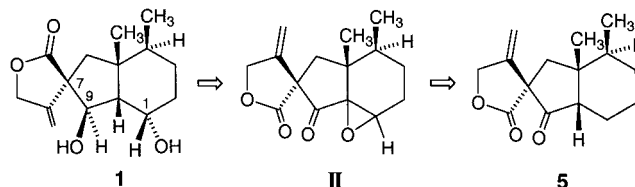
- 1**, **R** = **R**¹ = H Bakkenolide III
2, **R** = H; **R**¹ = COC(CH₃)₂CH(CH₃) Bakkenolide C
3, **R** = COCH₃; **R**¹ = COC(CH₃)₂CH(CH₃) Bakkenolide B
4, **R** = **R**¹ = COCH(CH₃)₂ Bakkenolide H

[*] Prof. Dr. J.-P. Deprés, Dr. O. Hamelin, Dr. Y. Wang, Dr. A. E. Greene
 LEDSS, BP 53X
 Université Joseph Fourier
 38041 Grenoble Cedex (France)
 Fax: (+33)4-76-51-44-94
 E-mail: Jean-Pierre.Depres@ujf-grenoble.fr

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advance that this highly stereocontrolled and efficient approach allows potentially total access to the group.

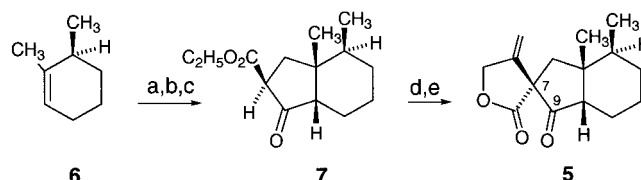
With its six stereocenters in the proper configurations and two hydroxyl groups which could be, in principle, easily differentiated, diol **1** (bakkenolide III) appeared to be the ideal late intermediate for accessing the entire range of known C1,C9-difunctionalized bakkenolides (Scheme 1). It seemed this diol might be secured from epoxy ketone **II** through chemoselective reduction, followed by stereochemical adjustments.



Scheme 1. Retrosynthesis of bakkenolide III (**1**).

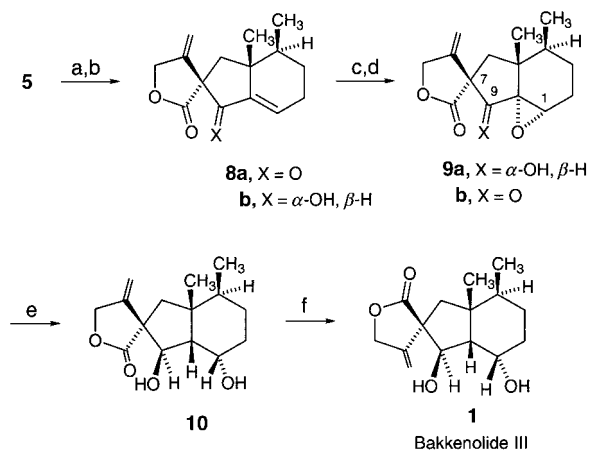
Molecular mechanics calculations^[4] revealed diol **1** to have the lowest global minimum energy of the four C7,C9 diastereomers, thus it could possibly be reached through a retroaldol–aldol equilibration. Epoxy ketone **II**, in turn, was viewed as arising from keto lactone **5**, an intermediate previously prepared in our laboratory from 1,6-dimethylcyclohexene.^[3n, 5]

Keto lactone **5** was synthesized stereoselectively from enantiopure 1,6-dimethylcyclohexene (**6**)^[3d] in five steps and 27 % overall yield^[6] in a manner similar to that used earlier. It was found, however, that ring expansion could be improved by using boron trifluoride as the catalyst and that neutral conditions benefitted the transesterification^[7] (Scheme 2).



Scheme 2. Synthesis of keto lactone **5**. a) Ref. [3d, n]; b) Zn, CH₃CO₂H, 70 °C, 1 h (63 %, 2 steps); c) N₂CHCO₂C₂H₅, BF₃ · O(C₂H₅)₂, (C₂H₅)₂O, 0 °C, 12 h (72 %); d) HC≡CCH₂OH, C₆H₅CH₃, reflux, 24 h (87 %); e) Mn(O₂CCH₃)₃, C₂H₅OH, 20 °C, 2.5 h (68 %).

Of the numerous procedures examined for effecting dehydrogenation of this keto lactone to its $\Delta^{1(10)}$ derivative **8a**, phenylselenenylation/oxidation^[8] was clearly the most efficacious and under carefully optimized conditions the sensitive enone could be isolated in 77 % yield (Scheme 3). Base-catalyzed epoxidations with this derivative invariably resulted in degradation, and so a hydroxyl-directed procedure was next considered. Toward this end, enone **8a** was subjected to a Luche reduction in ethanol,^[9] which led cleanly and highly stereoselectively ($\geq 98:2$) to the retroaldol-prone allylic alcohol **8b**, in which the hydroxyl group had the desired α orientation.^[10] This material was exposed without purification to a [VO(acac)₂]-catalyzed epoxidation with *tert*-butyl hydroperoxide (TBHP) in warm toluene^[11] to provide selectively the required epoxide **9a**, which was isolated in 57 %



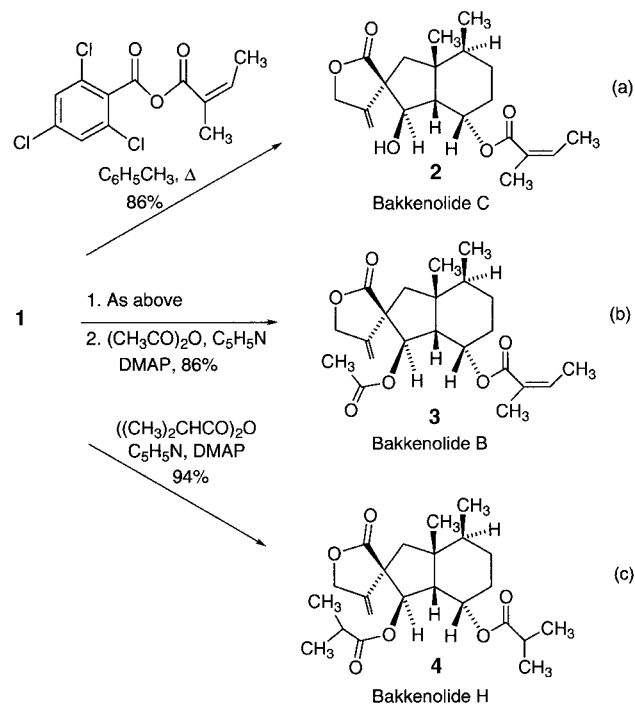
Scheme 3. Synthesis of bakkenolide III (**1**). a) NaHMDS, THF, -65°C , 4 h; $\text{C}_6\text{H}_5\text{SeBr}$, $-65 \rightarrow 0^{\circ}\text{C}$, 1 h; H_2O_2 , $\text{CH}_3\text{CO}_2\text{H}/\text{H}_2\text{O}$, 0°C , 1.5 h (77 %); b) NaBH_4 , CeCl_3 , $\text{C}_2\text{H}_5\text{OH}$, -30°C , 1.5 h; c) $[\text{VO}(\text{acac})_2]$, TBHP, $\text{C}_6\text{H}_5\text{CH}_3$, $50-60^{\circ}\text{C}$, 6 h (57 %, 2 steps); d) $(\text{ClCO})_2$, DMSO, CH_2Cl_2 , -60°C , 15 min; $(\text{C}_2\text{H}_5)_3\text{N}$, $-60 \rightarrow 20^{\circ}\text{C}$, 1 h (83 %); e) SmI_2 , THF/ H_2O , 20°C , 0.5 h (83 %); f) $(\text{C}_4\text{H}_9)_4\text{NF}$, THF, 0°C , 0.5 h (82 %). acac = acetylacetonate; DMSO = dimethyl sulfoxide; HMDS = 1,1,1,3,3,3-hexamethyldisilazide; TBHP = *tert*-butyl hydroperoxide.

yield (2 steps). Swern oxidation then gave efficiently epoxy ketone **9b** and set the stage for reduction to the C1,C9-diol.

Previous experience had shown SmI_2 to be uniquely effective for the reduction of 7-epi-9-oxo-bakkenolide A (**5**).^[3n] It was therefore hoped that this reagent, also known to be able to effect reductive cleavage of α heteroatom substituents in ketone derivatives, used in excess and in a protic medium might produce a chemoselective double reduction of epoxy ketone **9b**.^[12] A clean transformation, in fact, resulted when excess reagent in 1 % aqueous THF was used at room temperature for 0.5 h and delivered only the C1 α ,C9 β -diol derivative **10** in 83 % yield. A single-crystal X-ray analysis corroborated these assignments.^[13]

The success of the entire venture now hinged on the thermodynamics predicted by molecular modeling studies^[4] for the retroaldol–aldol transformation of lactone diol **10**.^[14] To our considerable satisfaction, **10** underwent smooth isomerization, in spite of numerous possible alternative pathways, on exposure to tetrabutylammonium fluoride (TBAF) in THF at 0°C for 0.5 h to provide a single, crystalline lactone diol in high yield. Confirmation that the crucial conversion had in fact occurred as predicted to deliver the key compound, (–)-bakkenolide III (**1**),^[1n] was obtained by direct comparison with the hydrolysis product^[1b,d,f] of (–)-bakkenolide B and through single-crystal X-ray analysis.^[15]

The hydroxyl groups in bakkenolide III are clearly in different steric environments and this was expected to translate into regioselective acylations. Bakkenolide III in the presence of the Yamaguchi mixed anhydride derived from angelic acid and under conditions earlier found to avoid the facile angelic $\rightarrow$ tiglic isomerization^[16] formed (–)-bakkenolide C (**2**)^[1d] in 86 % yield without detectable amounts of C9-acylated material or tiglic ester (Scheme 4a). The bakkenolide C so obtained was identical with a sample secured by partial hydrolysis^[1b,d,f] of natural bakkenolide B.



Scheme 4. Synthesis of a) bakkenolide C (**2**), b) bakkenolide B (**3**), and c) bakkenolide H (**4**). DMAP = 4-dimethylaminopyridine.

In the presence of excess anhydride and 4-dimethylaminopyridine (DMAP), however, C9 acylation could be readily achieved. Thus, (–)-bakkenolide B (**3**)^[1b,d,f,n] could be secured in 86 % yield from (–)-bakkenolide C with excess acetic anhydride in pyridine in the presence of DMAP (Scheme 4b). Likewise, (–)-bakkenolide H (**4**)^[1m,n] which shows significant cytotoxic activity in several test systems, could be prepared in 94 % yield from bakkenolide III by using excess isobutyric anhydride under similar conditions (Scheme 4c).

In summary, enantiopure (–)-bakkenolide III has been efficiently synthesized (>6 % overall yield, 11 steps) through a highly stereocontrolled approach and used as a platform for accessing representative C1,C9-dioxygenated bakkenolides. This first successful entry to these numerous bioactive compounds relies on a novel SmI_2 -promoted chemoselective double reduction of an epoxy ketone to generate the C1 and C9 hydroxyl groups and a thermodynamically governed retroaldol–aldol strategy to install the spiro lactone unit and the C9 hydroxyl group with the proper stereochemistry. Both of these should find additional application.

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- [6] Yields are for purified, chromatographically homogeneous substances. The NMR, IR, and mass spectra are in full accord with the proposed structures and satisfactory combustion analyses and/or high resolution mass spectral data have been obtained for all new compounds. **5**: m.p. 71–71.5 °C (diethyl ether/hexane); [α]_D²⁰ = +426 (*c* = 1.2 in CHCl₃). **7**: [α]_D²⁰ = +62.9 (*c* = 1.8 in CHCl₃). **8a**: m.p. 145–146 °C (ethyl acetate/hexane); [α]_D²⁰ = –1.1 (*c* = 2.5 in CHCl₃). **8b**: m.p. 145–146 °C (diethyl ether/hexane); [α]_D²⁰ = +50.1 (*c* = 1.6 in CHCl₃). **9a**: [α]_D²⁰ = +32.0 (*c* = 4.1 in CHCl₃). **9b**: m.p. 166.5–167 °C (chloroform/hexane); [α]_D²⁰ = –91.2 (*c* = 1.3 in CHCl₃). **10**: m.p. 131–131.5 °C (dichloromethane/hexane); [α]_D²⁰ = +100.0 (*c* = 1.4 in CHCl₃). **1**: m.p. 177–178 °C (dichloromethane/hexane); [α]_D²⁰ = –71.7 (*c* = 1.0 in CHCl₃), [α]_D²² = –79.7 (*c* = 1.0 in CH₃OH). **2**: m.p. 165–165.5 °C (dichloromethane); [α]_D²⁰ = –76.2 (*c* = 0.8 in CHCl₃), [α]_D²² = –156.1 (*c* = 0.7 in CH₃OH). **3**: m.p. 101.5–102 °C; [α]_D²² = –118.1 (*c* = 0.8 in CHCl₃), [α]_D²² = –149.3 (*c* = 1.1 in CH₃OH). **4**: m.p. 130–130.5 °C (diethyl ether/hexane); [α]_D²⁰ = –105.6 (*c* = 1.0 in CHCl₃), [α]_D²² = –124.5 (*c* = 1.1 in CH₃OH).
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- [12] For a review, see G. A. Molander, *Org. React.* **1994**, *46*, 211–367. This type of double reduction with SmI₂ is apparently unprecedented; however, the less chemoselective Li-NH₃-NH₄Cl system has been used for this purpose. See: A. A. Devreese, M. Demuyne, P. J. De Clercq, M. Vandewalle, *Tetrahedron* **1983**, *39*, 3039–3048; C. S. Swindell, S. J. deSolms, *Tetrahedron Lett.* **1984**, *25*, 3801–3804. The *cis* ring fusion was anticipated based on precedent^[3b,i] the C9 β isomer was expected to result since SmI₂ is known to deliver in general the more stable isomer ($\Delta E_{9\beta-9\alpha}$ = –2.0 kcal mol⁻¹[4]). The configuration at C9 in **10**, however, is irrelevant in that it too is subjected to equilibration in the retroaldol–aldol reaction.
- [13] Crystal data for ($\pm$)-**10** (from parallel studies):^[17] C₁₅H₂₂O₄, tetragonal, *I*₄/a, *a* = 20.220(7), *c* = 13.427(7) Å, *V* = 5490(3) Å³, *Z* = 16, ρ_{calcd} = 1.289 Mg m⁻³, *F*(000) = 2304, θ = 2.0–30°, 5466 measured reflections, 5274 (*R*(int) = 0.017) independent reflections, *R*(*F*) (*F* > 2 σ (*F*)) = 0.0487, *wR* (all data) = 0.0512, GOF = 1.965.
- [14] See: J. D. White, N. S. Cutshall, T.-S. Kim, H. Shin, *J. Am. Chem. Soc.* **1995**, *117*, 9780–9781 and ref. [3n].
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A Tandem Sulfur Transfer/Reduction/Michael Addition Mediated by Benzyltriethylammonium Tetrathiomolybdate**

Kandikere Ramaiah Prabhu, Pennadam S. Sivanand, and Srinivasan Chandrasekaran*

Disulfides and sulfur containing organic compounds are important functional groups widely present in nature and have commercial significance.^[1] Therefore, the synthesis of disulfides, sulfides, and ω -thio ketones is not only attractive but also finds numerous applications.^[2] Studies directed

[*] Prof. Dr. S. Chandrasekaran, Dr. K. R. Prabhu, P. S. Sivanand
Department of Organic Chemistry
Indian Institute of Science
Bangalore 560 012 (India)
Fax: (+91) 80-360-0529
E-mail: scn@orgchem.iisc.ernet.in

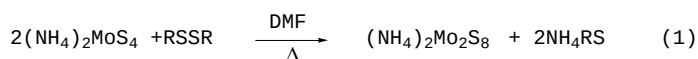
[**] This work was supported by the Department of Science and Technology, New Delhi.

towards the cleavage of disulfide bonds in proteins are an important determinant in understanding the structural activity and structural domains of proteins, this helps in the design and development of new therapeutic agents and the conversion of large proteins into smaller fragments that are more amenable for sequencing.^[3] The sulfur–sulfur bond in organic disulfides may be cleaved by nucleophilic, electrophilic, and radical processes. Several transition metal complexes have also been shown to cleave sulfur–sulfur bonds in organic disulfides.^[4]

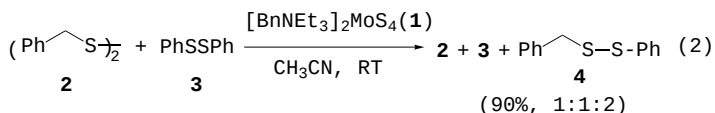
We have reported the usefulness of benzyltriethylammonium tetrathiomolybdate (**1**) as an efficient sulfur transfer reagent in organic synthesis.^[5] Further work revealed that the tetrathiomolybdate **1** mediates reductive dimerization of organic thiocyanates and selenocyanates to the corresponding disulfides and diselenides.^[6]

In continuing our quest to exploit the redox chemistry associated with Mo–S systems we find that **1** can mediate not only the formation of the disulfide bond but can also cleave the disulfide bond under appropriate reaction conditions. Herein, we present our findings that **1** can mediate disulfide interchange reactions and that the intermediate generated in situ in the cleavage of the disulfide bond undergoes a facile conjugate addition to suitable Michael acceptors.

In work on induced redox reactions of ammonium tetrathiomolybdate Stiefel and co-workers^[7] demonstrated that under forcing conditions (DMF, 90 °C) alkyl and aryl disulfides undergo cleavage to produce thiolates and Mo₂S₈²⁻ [Eq. (1)]. Since we have reported that **1** effects sulfur transfer to alkyl halides to form disulfide bonds very efficiently, and in light of Stiefel's observations, it was of interest to find out whether **1** would effect an exchange reaction of a mixture of disulfides.

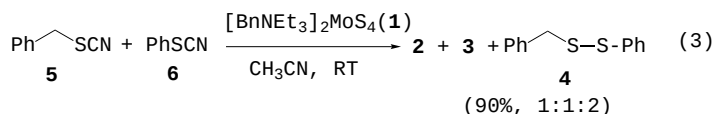


A 1:1 mixture of dibenzyl disulfide (**2**) and diphenyl disulfide (**3**) in CH₃CN was reacted with **1** (2 equiv) at room temperature for 15 h. Interestingly, the unsymmetrical disulfide, benzylphenyl disulfide (**4**) was obtained as the major product along with the symmetrical disulfides **2** and **3** in the ratio of 2:1:1 (90%, [Eq (2)]). This reaction clearly indicates that **1** is mediating an exchange reaction of disulfides which must involve cleavage of the disulfide bond.



As tetrathiomolybdate **1** converts alkyl halides and organic thiocyanates to disulfides,^[5, 6a] it was decided to find out whether two disulfide precursors (like two alkyl halides or thiocyanates) in the presence of excess of **1** would also lead to unsymmetrical disulfides in an exchange reaction. Thus, a mixture of benzyl thiocyanate (**5**; 1 equiv) and phenyl

thiocyanate (**6**; 1 equiv) was treated with **1** (2 equiv) [Eq (3)]. As expected, the unsymmetrical disulfide **4** was isolated as the major product (90%) along with **2** and **3** in the ratio 2:1:1.



These results clearly suggest that disulfides undergo cleavage of the disulfide bond with an excess of **1** and undergo exchange reactions to produce unsymmetrical disulfides. Encouraged by these findings, we were interested to find out whether alkyl and aryl disulfides in the presence of **1** would undergo 1,4-addition to suitable Michael acceptors. If this were successful, it would avoid the use of free thiols in the Michael reactions.

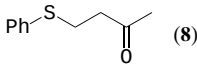
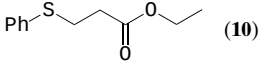
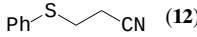
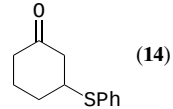
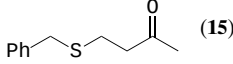
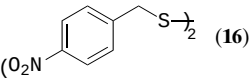
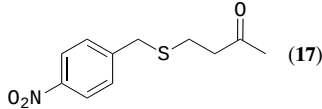
In the initial experiments diphenyl disulfide (**3**) was treated with **1** in the presence of a number of Michael acceptors and as anticipated the corresponding Michael adducts were formed in very good yields (Table 1). Thus, in the reaction of **3** (0.5 equiv) and methyl vinyl ketone (**7**) (1 equiv) with **1** (1.1 equiv), the β -ketosulfide **8**^[8] was isolated in 85% yield. Similarly, the reaction of **1** with **3** in the presence of either ethyl acrylate (**9**) or acrylonitrile (**11**) or 2-cyclohexenone (**13**) resulted in the formation of the corresponding Michael adducts **10**,^[8] **12**,^[9] **14**^[10] respectively in very good yields (Table 1). In the reaction of **2** and methyl vinyl ketone **7** in the presence of **1**, the ketosulfide **15** was obtained as the exclusive product.^[11] When *p*-nitrobenzyl disulfide **16** was treated with **7** in the presence of **1** the corresponding product **17** was isolated in good yield (Table 1). This appears to be a general reaction with wide applications.

Having successfully executed the one-pot reduction of disulfide followed by Michael reaction the stage was set for effecting a three-step tandem reaction starting from an alkyl halide, wherein the first step is the sulfur transfer reaction mediated by **1**. Thus, when benzyl bromide (**18**) was allowed to react with **1** (2 equiv) and **7**, the corresponding ketosulfide **15** was isolated in 86% yield (Table 2). Clearly in one pot we have been able to effect sulfur transfer, reduction of disulfide, and conjugate addition of thiolate to **7**. A similar reaction of *p*-nitrobenzyl bromide **19** with two equivalents of **1** and an equivalent of **7** led to the formation of the corresponding ketosulfide **17** in 91% yield (Table 2).

Having found that the tandem reactions work well, it was then decided to apply this one pot methodology to intramolecular reactions (Table 2). When the chloroenone **20** (1 equiv) was treated with **1** (2 equiv) the corresponding spiro compound, **21**^[12] was isolated in 80% yield, while the bromoenone **22** under similar conditions afforded the corresponding β -ketosulfide **23**.

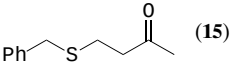
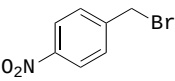
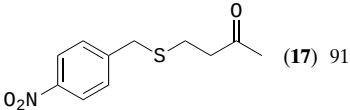
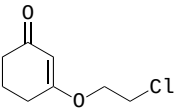
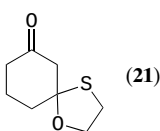
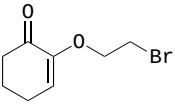
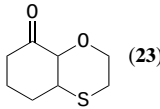
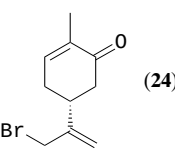
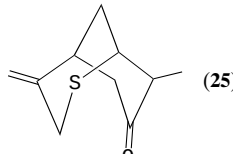
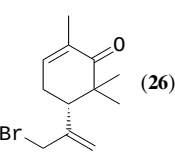
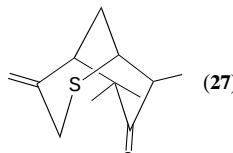
The same methodology was then extended to the synthesis of bicyclo[3.3.1]nonane skeletons. In the reaction of bromoenones^[13] **24** and **26** with **1** (2.2 equiv), the corresponding bicyclic adducts **25** and **27** were obtained in near quantitative yields.

Table 1. Cleavage of disulfides and conjugate addition to a Michael acceptor in the presence of **1**.

Substrate/s	Time [h]	Product	Yield [%] ^[a]
PhSSPh (3)	5	 (8)	85
3	8	 (10)	81
3	5	 (12)	86
3	5	 (14)	80
(PhCH ₂ S) ₂ (2)	6	 (15)	88
 (16)	4	 (17)	82

[a] Yield refers to pure, isolated products.

Table 2. Tandem sulfur transfer/reduction/Michael addition in one pot.

Substrate/s	Time [h]	Product	Yield [%] ^[a]
PhCH ₂ Br (18) + 7	10	 (15)	86
 (19) + 7	8	 (17)	91
 (20)	10	 (21)	80
 (22)	8	 (23)	75
 (24)	8	 (25)	94
 (26)	6	 (27)	98

[a] Yield refers to pure, isolated products.

The chemistry described herein demonstrates that the tandem sulfur transfer/reduction/Michael addition strategy mediated by tetrathiomolybdate **1** is very efficient in the

synthesis of variety of useful synthetic intermediates. The highlights of this methodology are the one-pot reactions, and that there is no free thiol involved in the reaction.

Experimental Section

Typical experimental procedure for the tandem sulfur transfer reduction Michael addition: Benzyl triethylammonium tetrathiomolybdate **1** (1.135 g, 1.86 mmol) was added to a solution of bromoenone **26** (0.200 g, 0.85 mmol) in acetonitrile (3 mL) and stirred at room temperature (25 °C) for 6 h. The solvent was evaporated under reduced pressure, the black residue was extracted with dichloromethane:diethyl ether (1:5, 25 mL × 5), and filtered through a Celite pad. The filtrate was concentrated and purification on a silica gel column eluting with ethyl acetate:hexane (1:10) furnished the pure compound **27** as a white solid (0.175 g, 98%); m.p. 84–86 °C; IR (nujol): $\bar{\nu}$ = 2980, 2900, 1680, 1630, 1440, 1400, 1380, 1360, 1320, 1280 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, 25 °C, TMS): δ = 4.75 (s, 1 H), 4.71 (s, 1 H), 3.14 (d, J = 14 Hz, 1 H), 3.05–2.99 (m, 2 H), 2.8 (dt, J = 13.6, 3.4 Hz, 1 H), 2.65 (d, J = 14.4 Hz, 1 H), 2.50 (s, 1 H), 2.34 (d, J = 13.6 Hz, 1 H), 1.25 (s, 6 H), 1.19 (d, J = 6.6 Hz, 3 H); ¹³C NMR (125 MHz, CDCl₃, 25 °C): δ = 216.3, 145.5, 111.0, 53.10, 44.83, 44.6, 35.7, 28.0, 27.9, 23.21, 13.3; low resolution MS m/z : 210 [M^+], 195, 182, 177, 163, 149, 140, 124, 111, 93; high resolution MS: calcd for C₁₂H₁₈OS. 210.1078; found 210.1080; [α]_D²⁰ = 211° (c = 0.010 g in CH₂Cl₂).

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Transition Metal Complexes as Photosensitizers for Near-Infrared Lanthanide Luminescence**

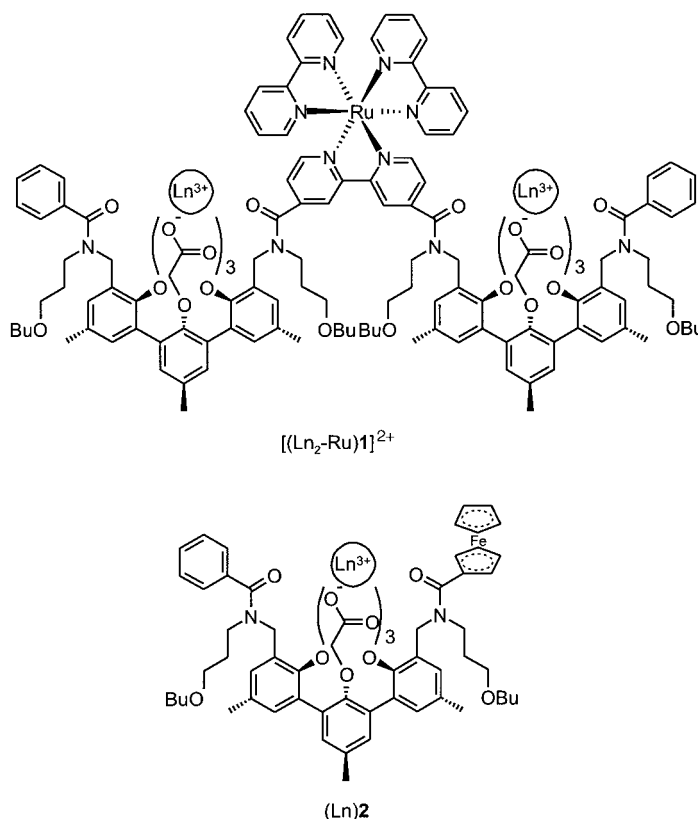
Stephen I. Klink, Henk Keizer, and Frank C. J. M. van Veggel*

The design of sensitizer-functionalized lanthanide complexes that exhibit efficient luminescence in the near-infrared (NIR) region is currently of interest because of potential applications in fluoroimmuno-assays,^[1] in laser systems,^[2] and in polymer-based optical signal amplifiers.^[3,4] NIR luminescence of complexes of the lanthanide ions erbium(III), neodymium(III), and ytterbium(III) is readily observed in solution at room temperature, only when the ions are excited *indirectly* by energy transfer from the triplet state^[5] of an incorporated organic chromophore (the antenna or sensitizer). Direct excitation is more demanding because of the forbidden

optical transitions within the 4f subshells of lanthanide ions. As a result, the absorption coefficients are very low (typically 1–10 M⁻¹ cm⁻¹) and the lifetimes of the excited states are relatively long (microseconds to milliseconds).

Herein, we report the first example of Nd^{III} and Yb^{III} luminescence sensitized by the transition metal complexes ruthenium(II) tris(bipyridine) ([Ru(bpy)₃]²⁺) and ferrocene. Investigations of sensitizers for NIR lanthanide luminescence have focused on conjugated organic molecules, nevertheless, there are only a few examples of sensitizers that enable visible light excitation instead of near-UV excitation.^[1,4,6,7] The [Ru(bpy)₃]²⁺ complex not only has an intersystem-crossing quantum yield near unity, but it should enable excitation of Nd^{III} and Yb^{III} with visible light of up to 500 nm. Compared to [Ru(bpy)₃]²⁺, ferrocene has weaker absorption bands in the visible region,^[8] but its low-lying triplet state ($E_T = 13\,300\text{ cm}^{-1}$)^[9] closely matches the receiving luminescent states of Nd^{III} (11 300 cm⁻¹) and Yb^{III} (10 250 cm⁻¹).

Our systems [(Ln₂-Ru)**1**]²⁺ and (Ln)**2** are based on *m*-terphenyl-based lanthanide (Ln) complexes^[10–12] that are covalently linked to the transition metal complexes. Experimental and physical data can be found in the Supporting Information.



Excitation of the [(Nd₂-Ru)**1**]²⁺ and [(Yb₂-Ru)**1**]²⁺ complexes in deoxygenated [D₆]DMSO (10⁻⁵ M) with visible light resulted in Ln^{III}-centered NIR luminescence. Figure 1 shows the emission bands of [(Nd₂-Ru)**1**]²⁺ at 1060 and 1330 nm corresponding to the ⁴F_{3/2} → ⁴I_{11/2} and ⁴I_{13/2} transitions of Nd^{III}, respectively, and the emission band of [(Yb₂-Ru)**1**]²⁺ at

[*] Dr. ir. F. C. J. M. van Veggel, Dr. S. I. Klink, ir. H. Keizer
Laboratory of Supramolecular Chemistry & Technology
and MESA⁺ Research Institute
University of Twente
P.O. Box 217, 7500 AE Enschede (The Netherlands)
Fax: (+31) 53-489-4645
E-mail: F.C.J.M.vanVeggel@ct.utwente.nl

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

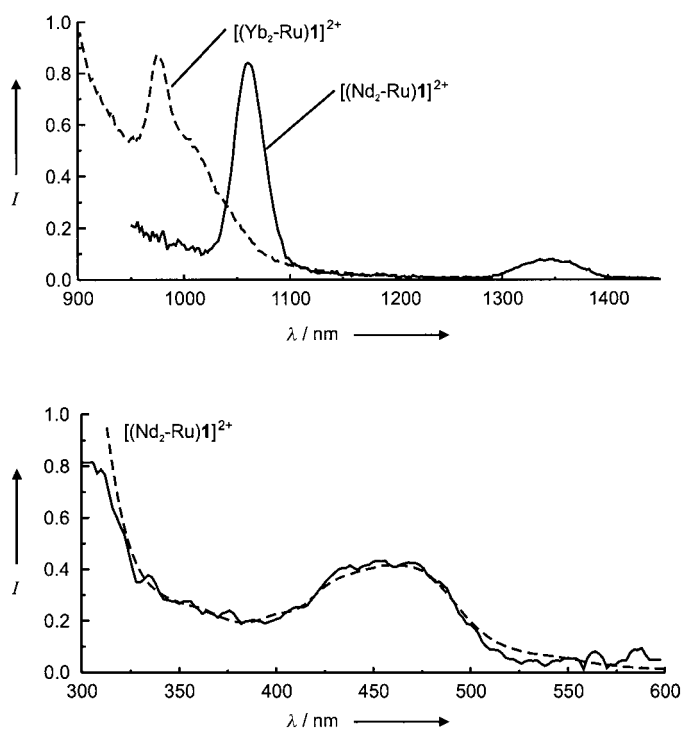


Figure 1. Top: the NIR luminescence spectrum of $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$ and $[(\text{Yb}_2\text{-Ru})\mathbf{1}]^{2+}$ in $[\text{D}_6]\text{DMSO}$ (10^{-5} M) upon excitation at 450 nm. The spectra have been corrected for the absorbance of the samples. Bottom: the excitation spectrum of the Nd^{3+} luminescence of $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$ while monitoring the 1060 nm emission band (solid line). The dashed line is the absorption spectrum of $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$.

975 nm corresponding to the $^4\text{F}_{5/2} \rightarrow ^4\text{F}_{7/2}$ transition of Yb^{III} . The 1060 nm band of $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$ and the 975 nm band of $[(\text{Yb}_2\text{-Ru})\mathbf{1}]^{2+}$ are superimposed on the tail of the weak Ru(bpy)-antenna luminescence. The sensitized Yb^{III} luminescence of $[(\text{Yb}_2\text{-Ru})\mathbf{1}]^{2+}$ is less than the sensitized Nd^{III} luminescence of $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$. The excitation spectra closely resemble the absorption spectrum of the Ru(bpy) antenna, this observation clearly shows that the Ln^{III} ions are excited by the $^1\text{MLCT}$ (metal-to-ligand charge transfer) state of the Ru(bpy) antenna. The excitation and absorption spectra of $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$ are shown in Figure 1. The $[(\text{Yb}_2\text{-Ru})\mathbf{1}]^{2+}$ complex has identical excitation and absorption spectra.

Time-resolved luminescence measurements of the antenna and Nd^{III} demonstrated that the pathway of the sensitized emission indeed involves the $^3\text{MLCT}$ state of the antenna. During the time that the energy transfer from the antenna triplet state to the lanthanide ion is in progress, the lanthanide luminescence intensity is expected to rise with a rate equal to the decay rate of antenna triplet state. Upon excitation of $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$, antenna luminescence was observed at 660 nm originating from the $^3\text{MLCT}$ state and decayed with a time-constant of 420 ± 10 ns. At the same time, the Nd^{III} emission at 1060 nm rose with the same time-constant of 420 ± 20 ns, after which it decayed with a time-constant of 2.1 ± 0.2 s, which is the lifetime of the Nd^{III} luminescence.^[11]

As a reference compound, the $[(\text{Gd}_2\text{-Ru})\mathbf{1}]^{2+}$ complex allows the study of the antenna luminescence in the absence of energy transfer, because Gd^{III} has no energy levels below 32000 cm^{-1} , and therefore cannot accept any energy from the

antenna triplet state. As expected, the antenna luminescence intensity of $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$ is less than the antenna luminescence intensity of $[(\text{Gd}_2\text{-Ru})\mathbf{1}]^{2+}$ (Figure 2). Also the antenna luminescence lifetime of $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$ is shorter than the

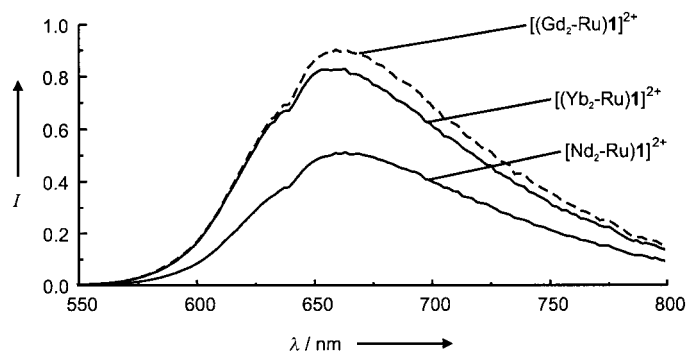


Figure 2. Emission spectra of the Ru(bpy)-centered luminescence of $[(\text{Gd}_2\text{-Ru})\mathbf{1}]^{2+}$, $[(\text{Yb}_2\text{-Ru})\mathbf{1}]^{2+}$, and $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$ in $[\text{D}_6]\text{DMSO}$ (10^{-5} M) upon excitation at 450 nm. The spectra have been corrected for the absorbance of the samples.

770 ± 20 ns of $[(\text{Gd}_2\text{-Ru})\mathbf{1}]^{2+}$. Since the antenna luminescence lifetime of $[(\text{Gd}_2\text{-Ru})\mathbf{1}]^{2+}$ represents the $^3\text{MLCT}$ -state lifetime in the absence of energy transfer, the energy transfer rate (k_{ET}) in the $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$ complex can thus be calculated from $k_{\text{ET}} = 1/\tau_{\text{RuNd}} - 1/\tau_{\text{RuGd}} = 1.1 \times 10^6\text{ s}^{-1}$, where τ = luminescence lifetime.

Closer examination of the antenna luminescence properties of $[(\text{Yb}_2\text{-Ru})\mathbf{1}]^{2+}$ revealed that the antenna luminescence intensity (see Figure 2) and lifetime of $[(\text{Yb}_2\text{-Ru})\mathbf{1}]^{2+}$ are equal, within the experimental error, to the antenna luminescence intensity and lifetime of $[(\text{Gd}_2\text{-Ru})\mathbf{1}]^{2+}$. This indicates that the rate of the energy transfer process in $[(\text{Yb}_2\text{-Ru})\mathbf{1}]^{2+}$ is at least an order of magnitude lower than 10^6 s^{-1} , the decay rate of the $^3\text{MLCT}$ level of the antenna. The rise-time of the Yb^{III} luminescence at 980 nm could not be measured because the first microseconds (after the laser pulse) of the time-resolved emission spectrum are dominated by the decay of the Ru(bpy)-antenna luminescence. This is not surprising since the emission spectrum shows that there is an appreciable amount of antenna luminescence at 980 nm (see Figure 1). The luminescence decay of $[(\text{Yb}_2\text{-Ru})\mathbf{1}]^{2+}$ at 980 nm could be fitted with a bi-exponential curve with lifetimes of 780 ± 20 ns (corresponding to the antenna luminescence) and $18.2 \pm 0.8\text{ }\mu\text{s}$ (corresponding to the Yb^{III} luminescence).^[11]

In light of the competing antenna processes the energy transfer rates in $[(\text{Nd}_2\text{-Ru})\mathbf{1}]^{2+}$ ($\sim 10^6\text{ s}^{-1}$) and $[(\text{Yb}_2\text{-Ru})\mathbf{1}]^{2+}$ ($\leq 10^5\text{ s}^{-1}$) are too low for a complete energy transfer. The energy transfer process is most likely to involve an electron-exchange mechanism^[13] that requires a physical overlap between the 4f orbitals of the lanthanide ion and the ground state HOMO and LUMO of the antenna. Although the antenna ground state LUMO is probably localized on the amide-functionalized bpy moiety,^[14] which is closest to the lanthanide ions (separation $\sim 5\text{ }\text{\AA}$), the antenna ground state HOMO is localized on the Ru^{II} center,^[15] which is further away from the lanthanide ions ($> 5\text{ }\text{\AA}$). This results in a weaker electron-exchange interaction and thus a slow energy-

transfer rate. The energy-transfer rate is further determined by the so-called spectral overlap of the antenna and the lanthanide ion, that is, how well the donating and receiving energy levels match. The energy-transfer rate in $[(Yb_2-Ru)1]^{2+}$ will be even slower than in $[(Nd_2-Ru)1]^{2+}$, because the antenna triplet state ($E_T = 17400\text{ cm}^{-1}$)^[16] matches the $Nd^{III} {}^4G_{5/2}$ state at 17100 cm^{-1} , whereas the overlap with the only Yb^{III} energy level, the ${}^2F_{5/2}$ state which is at 10240 cm^{-1} , is much smaller.

Deoxygenated $[D_6]DMSO$ solutions of the ferrocene-functionalized **(Nd)2** and **(Yb)2** complexes ($10^{-4}M$) exhibit the typical linelike lanthanide emission upon excitation of the ferrocene antenna at 320 nm .^[17] At room temperature, sensitized emission at 1060 and 1330 nm (${}^4F_{3/2} \rightarrow {}^4I_{11/2}$, and ${}^4I_{13/2}$ transitions, respectively) is observed for **(Nd)2**, and at 980 nm (${}^2F_{5/2} \rightarrow {}^2F_{7/2}$ transition) for **(Yb)2** (see Figure 3). The

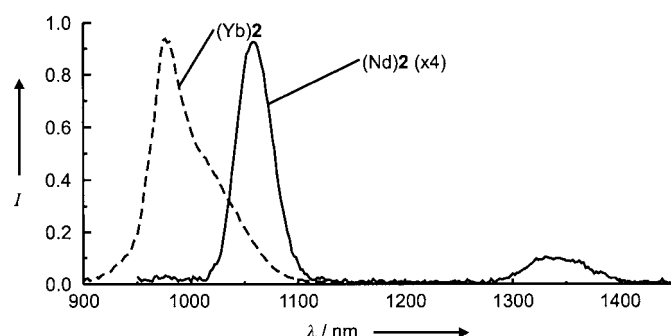


Figure 3. Emission spectra of **(Yb)2** and **(Nd)2** in $[D_6]DMSO$ ($10^{-4}M$) upon excitation at 320 nm . The spectra have been corrected for the absorbance of the samples.

excitation spectra of **(Nd)2** and **(Yb)2** (not shown), closely follow the absorption spectrum of the ferrocene antenna and confirm unambiguously that excitation takes place through the antenna. Upon excitation at 337 nm the lanthanide luminescence decay curves could be fitted mono-exponentially with lifetimes of $2.0 \pm 0.2\text{ }\mu\text{s}$ for **(Nd)2** and $18.8 \pm 0.8\text{ }\mu\text{s}$ for **(Yb)2**.

In conclusion, the transition metal complexes $[Ru(bpy)_3]^{2+}$ and ferrocene are able to sensitize both Nd^{III} and Yb^{III} . Moreover, these sensitizers enable excitation with visible light. The luminescent nature of the $[Ru(bpy)_3]^{2+} {}^3MLCT$ state has allowed a direct study of the sensitization process. It has been established for the $[(Nd_2-Ru)1]^{2+}$ complex that the energy transfer takes place in a “classical way” through the antenna triplet state with a rate of $1.1 \times 10^6\text{ s}^{-1}$. The sensitization process of $[(Yb_2-Ru)1]^{2+}$ follows the same pathway, although the energy transfer rate is lower ($\leq 10^5\text{ s}^{-1}$).

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A Novel Iron-Based Catalyst for the Biphasic Oxidation of Benzene to Phenol with Hydrogen Peroxide

Daniele Bianchi,* Rossella Bortolo, Roberto Tassinari, Marco Ricci, and Rodolfo Vignola*

Direct oxidation of benzene to phenol is an important reaction in organic chemistry. Often, however, its selectivity is rather poor since phenol is more reactive toward oxidation than benzene itself, and substantial formation of over-oxygenated by-products (catechol, hydroquinone, benzoquinones, and tars) also occurs.^[1]

Similar over-oxidation problems are efficiently solved in biological systems by segregating catalysts and products into

- [*] Dr. D. Bianchi, Dr. R. Bortolo, R. Tassinari, Dr. M. Ricci
EniChem S.p.A.-Centro Ricerche Novara “Istituto G. Donegani”
Via Fauser 4, 28100 Novara (Italy)
Fax: (+390)321-447425
E-mail: daniele.bianchi@enichem.it
- Dr. R. Vignola
EniTecnologie
Via Ramarini 32, 00016 Monterotondo (Italy)
Fax: (+390)6-90673243
E-mail: rvignola@enitecnologie.eni.it

different environments. The active sites of several oxygenases are deeply buried in hydrophobic pockets^[2] where lipophilic substrates are readily oxidized, while the more hydrophilic reaction products are promptly released into the surrounding aqueous environment. Likewise, the cellular membranes have important functions as selective barriers around the cell that allow the import of components and the export of products.^[3]

In the course of industrial research aimed at developing a new process for the one-step production of phenol, we wondered whether a biphasic system could mimic these important features of biological systems. Here we report on a new method for the selective biphasic oxidation of benzene to phenol with hydrogen peroxide as oxidant, an iron complex as catalyst, and trifluoroacetic acid as cocatalyst.

The first aspect that we investigated was the solvent system. The importance of this component is well illustrated by the history of hydrocarbon oxidation with hydrogen peroxide. For example, water is the solvent in the Fenton system,^[4] while pyridine is characteristic of the Gif chemistry widely studied by Barton et al. in the last ten years.^[5] We found that the use of an aqueous/organic reaction medium dramatically affects the selectivity of the reaction. Screening was carried out with FeSO₄ (a conventional Fenton catalyst) and a series of organic solvent/water mixtures as reaction media.

As shown in Figure 1, a remarkable enhancement of selectivity was obtained with the biphasic system water/acetonitrile (1/1) in the presence of benzene. In this medium the concentration of benzene in the aqueous phase increased from 0.18 % (solubility of benzene in water)^[6] to 0.76 %, and the phenol was largely extracted (85 %) into the organic phase. Thus, the biphasic process minimizes over-oxidation by reducing the contact between phenol and the catalyst, which is soluble in the aqueous phase. This hypothesis was confirmed on comparing the benzene/phenol molar ratios in the aqueous phase of water/benzene and water/acetonitrile/benzene reaction mixtures, which were 0.25 and 3.7, respectively. Such enhancement is not possible in the case of solvents that are too hydrophobic, such as *n*-octane, which quantitatively extract benzene from the aqueous phase, or with those that

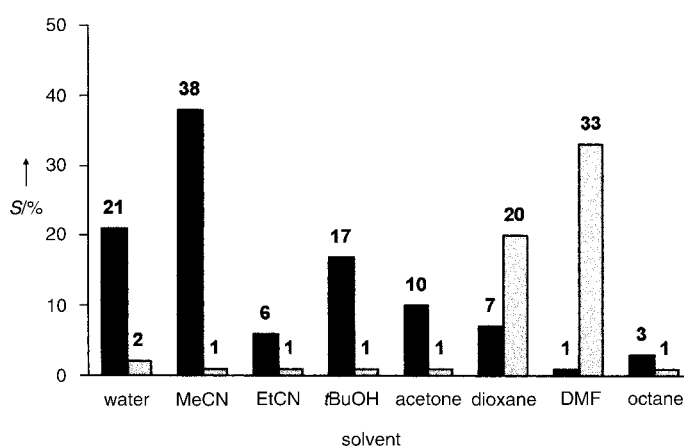


Figure 1. Effect of the reaction medium on the oxidation of benzene. The reaction was carried out under the conditions described in the Experimental Section with FeSO₄ as catalyst in absence of ligands. The composition of the reaction medium was water/solvent/benzene 45/45/10 (v/v/v). Black bar: Fraction of H₂O₂ oxidizing benzene to phenol; gray bar: fraction of H₂O₂ oxidizing benzene to dioxxygenated products (hydroquinone, catechol, and 1,4-benzoquinone). The concentration of over-oxidation products (tars) was not determined. S = selectivity based on H₂O₂.

are too hydrophilic, such as dimethylformamide (DMF), which do not form a two-phase system.

The identification of a more efficient and selective catalyst, which is an essential requirement for industrial application, was the second key aspect of the study. After testing a series of bidentate N,N, N,O and O,O ligands for iron (Table 1), 5-carboxy-2-methylpyrazine-*N*-oxide (**1**; entry 15) was selected as the most efficient catalyst precursor. The nature of the ligand plays a crucial role in determining both the catalyst reactivity and selectivity. In general, complexes with N,N ligands (entries 2–5) were almost inactive, those with O,O ligands (entries 6–8) displayed good activity but poor selectivity, and those with N,O ligands,

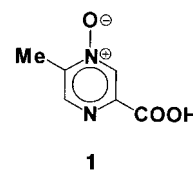


Table 1. Oxidation of benzene: selection of the ligand for iron.^[a]

Entry	Ligand	H ₂ O ₂ conversion [%]	Selectivity on H ₂ O ₂ ^[b] [%]	Selectivity on C ₆ H ₆ ^[c] [%]
1	none	77	38	33
2	2,2'-bipyridine	6	< 1	n.d.
3	1,10-phenanthroline	6	< 1	n.d.
4	2,6-di(2-pyridyl)pyridine	0	0	–
5	2,3-di(2-pyridyl)pyrazine	4	< 1	n.d.
6	1,2-dihydroxybenzene	100	44	60
7	1,2-dihydroxynaphthalene	100	25	n.d.
8	1,2-dihydroxynaphthalene-4-sulfonic acid	100	3	n.d.
9	8-hydroxyquinoline	0	0	–
10	pyridine-2-carboxylic acid	13	15	n.d.
11	pyridine-2,6-dicarboxylic acid	2	< 1	–
12	quinoline-2-carboxylic acid	90	33	n.d.
13	pyrazinecarboxylic acid	78	70	81
14	pyrazine-2,3-dicarboxylic acid	90	52	78
15	5-carboxy-2-methylpyrazine- <i>N</i> -oxide (1)	94	78	85

[a] Screening was carried out under the reaction conditions described in the Experimental Section with a molar ratio Fe/ligand/CF₃COOH of 1/4/1. The reaction medium was water/acetonitrile/benzene (5/5/1). [b] Selectivity based on H₂O₂: (moles of phenol produced) × 100/(moles of converted hydrogen peroxide). [c] Selectivity based on benzene: (moles of phenol produced) × 100/(moles of converted benzene). n.d.: not determined.

especially the pyrazinecarboxylic acid derivatives (entries 13–15), gave the best results.

The cocatalyst was selected from a series of acids, including acetic, phosphoric, sulfuric, *p*-toluenesulfonic, and trifluoroacetic acids. The latter gave the best results and increased the phenol selectivity (based on H₂O₂) from 68 (in the absence of any acid cocatalyst) to 78%. Conversely, complete inhibition of the catalytic activity occurred with phosphoric acid.

With FeSO₄, ligand **1**, and trifluoroacetic acid, in the selected water/acetonitrile/benzene biphasic system, under optimized reaction conditions (a further increase in selectivity was obtained by lowering the temperature from 50 to 35 °C), we achieved a benzene conversion of 8.6% with selectivities of 97% (based on benzene) and 88% (based on H₂O₂). These values are higher than those reported for other iron-based catalysts such as the Fenton^[7] and Gif systems.^[8] In the former case, high selectivities based on H₂O₂ (up to 83%) were only obtained at low benzene conversion (<1%). In the latter, a series of iron complexes with pyridine-2-carboxylic acid derivatives were reported to be completely ineffective in the oxidation of benzene under Gif reaction conditions.

It is also noteworthy that in the biphasic system, only a negligible amount (<1%) of biphenyl was detected among the secondary products, whereas in the classical Fenton oxidation, this compound is formed by radical dimerization of hydroxycyclohexadienyl radicals in a yield of 8–39%.^[9]

This different reactivity and selectivity was confirmed in the oxidation of toluene. In the biphasic system, this reaction afforded a mixture of hydroxylation products (*o*- and *p*-cresol) and benzylic oxidation products (benzylic alcohol, benzaldehyde, benzoic acid) in 76 and 23% overall yield, respectively (see Experimental Section). Conversely, under Fenton conditions, the reported product distribution was: hydroxylation 4%, benzylic oxidation 56%, and radical dimerization to bibenzyl 30%.^[9]

The catalyst system is also effective in the oxidation of alkanes, including a substrate as poorly reactive as methane, which is mainly converted (70 °C, 5 MPa) to formic acid (46% based on hydrogen peroxide); in this case, a biphasic system is not expected to form.

In conclusion, a new iron-based homogeneous catalyst for the oxidation of benzene to phenol with 97% selectivity was discovered. An attractive feature of the process is the operation in a biphasic system, with the quantitative distribution of the catalyst in the aqueous phase, that can be separated and reused in several consecutive cycles as a “working solution” without any appreciable loss of activity. In the light of these promising results, the development of a continuous process, more suitable for industrial application, is presently under investigation.

Experimental Section

1 was synthesised according to a literature procedure.^[10] All other chemicals (Aldrich) were used as purchased.

Solvent screening: FeSO₄·7H₂O (0.06 mmol) and CF₃COOH (0.06 mmol) were dissolved in water (8 mL), and the solution added to a mixture of an organic solvent (acetonitrile, propionitrile, *tert*-butyl alcohol, acetone, dioxane, dimethylformamide, or *n*-octane; 8 mL) and benzene (1.6 mL, 18 mmol). The resulting biphasic system was stirred at 50 °C, and an

aqueous solution of hydrogen peroxide (30% w/w, 1.8 mmol) was added stepwise over 1 h. At the end of the reaction, the hydrogen peroxide concentration in the aqueous phase was determined by titration with potassium permanganate, and the concentration of oxidation products in the aqueous and organic phases was determined by HPLC analysis.

Catalyst screening: FeSO₄·7H₂O (0.06 mmol), CF₃COOH (0.06 mmol), and one of the ligands listed in Table 1 (0.24 mmol) were dissolved in water (8 mL), and the reaction mixture was stirred for 1 h at 25 °C. The resulting solution was added to a mixture of acetonitrile (8 mL) and benzene (1.6 mL, 18 mmol). The reactions were carried out as described for solvent screening.

Optimized conditions for benzene oxidation: FeSO₄·7H₂O (0.4 mmol), CF₃COOH (0.4 mmol), and **1** (0.4 mmol) were dissolved in water (109 mL), and the reaction mixture was stirred for 1 h at 25 °C. The resulting solution was added to a mixture of acetonitrile (109 mL) and benzene (22 mL, 248 mmol). The resulting biphasic system was stirred at 800 rpm at 35 °C, and an aqueous solution of hydrogen peroxide (30% w/w, 24 mmol) was progressively added with a peristaltic pump over 4 h. At the end of the reaction, the concentrations of hydrogen peroxide and of the oxidation product were measured as described above. Hydrogen peroxide and benzene conversions were 95 and 8.4%, respectively, with selectivities to phenol of 88 and 97% based on hydrogen peroxide and benzene, respectively. The molar product distribution was: phenol 97.1, hydroquinone 0.5, catechol 0.6, 1,4-benzoquinone 0.1, biphenyl 0.1, and tars 1.5%.

Toluene oxidation: The reaction was carried out as described above for benzene, with water (109 mL), acetonitrile (109 mL), FeSO₄·7H₂O (0.4 mmol), CF₃COOH (0.4 mmol), **1** (0.4 mmol), toluene (240 mmol), and hydrogen peroxide (30% w/w; 24 mmol). After 4 h at 50 °C the hydrogen peroxide and toluene conversions were 60 and 3.1%, respectively. The molar product distribution was: *o*-cresol 44, *p*-cresol 32, 2-methyl-1,4-benzoquinone 1, benzyl alcohol 5, benzaldehyde 15, and benzoic acid 3%.

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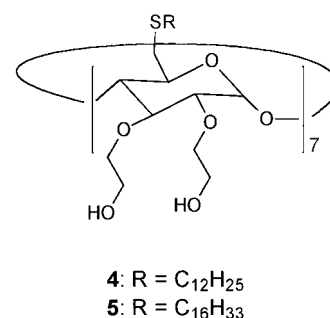
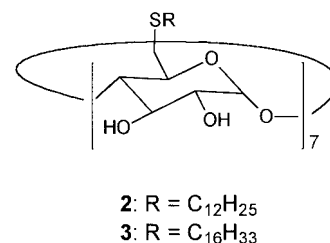
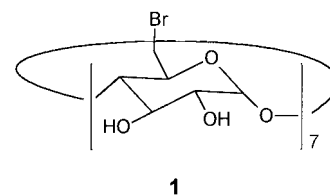
Cyclodextrin Bilayer Vesicles**

Bart Jan Ravoo and Raphael Darcy*

Over the past decade, several groups have reported amphiphilic cyclodextrin derivatives that display a range of lyotropic and thermotropic mesophases. Amphiphilic cyclodextrins have been shown to form monolayers at the air–water interface^[1] and micelles in water.^[2] Thermotropic liquid crystals of cyclodextrins were also described.^[3] Amphiphilic cyclodextrins can be admixed in limited percentages to phospholipid monolayers^[4] as well as liposomes,^[5] and they can be dispersed as “nanoparticles” of pharmaceutical interest.^[6] However, with few exceptions, these materials have poor water solubility. We have now prepared the first examples of bilayer vesicles composed entirely of amphiphilic cyclodextrins. These vesicles combine the properties of liposomes and macrocyclic host molecules, and create new possibilities for the development of advanced host–guest carrier and delivery systems. Some examples of vesicles composed of amphiphilic macrocycles such as calixarenes^[7] and cryptands^[8] were recently described.

Heptakis(6-alkylthio)- β -cyclodextrins^[3] display thermotropic mesophases and form monolayers at the air–water interface,^[1,3,9] but are practically insoluble in water. The poor water solubility of these cyclodextrins most likely results from intramolecular hydrogen bonding of the secondary hydroxyl groups, as is the case for unmodified β -cyclodextrin. Attempts to “break” this hydrogen-bond network by per-substitution of the secondary side by methyl and acetyl groups only slightly improved the water solubility.^[9] However, we demonstrate herein that substitution of the secondary side with hydrophilic hydroxyethyl groups dramatically improves the water solubility (as observed previously for random and low-degree substitution of β -cyclodextrin^[10]), and provides cyclodextrins with pronounced amphiphilic character.

Cyclodextrins **4** and **5** were obtained in a three-step synthesis from β -cyclodextrin. β -Cyclodextrin was per-brominated at the C₆ positions,^[11] and the resulting heptakis(6-bromo-6-deoxy)- β -cyclodextrin (**1**) yielded the 6-alkylthioethers **2** and **3** by reaction with potassium *n*-dodecylthiolate and *n*-hexadecylthiolate, respectively.^[3] Subsequently, we developed an efficient modification of the hydroxyethylation process for β -cyclodextrin,^[10] which uses ethylene carbonate as the alkylating agent. We found that **2** and **3** can be efficiently hydroxyethylated in tetra-*N*-methylurea (TMU) at 150 °C using an excess of ethylene carbonate and adding 10% of K₂CO₃ as base.^[12] The elevated temperature is essential for rapid and extensive substitution. Cyclodextrin **4** was purified



by size-exclusion chromatography and cyclodextrin **5** was purified by crystallization from methanol. The structures of **4** and **5** were confirmed using ¹H NMR and ¹³C NMR spectroscopy, microanalysis, and electrospray-MS.^[12] The most intense peaks in the electrospray mass spectra correspond to 13-fold hydroxyethylation of **4** and 10-fold hydroxyethylation of **5**, with peaks for products with higher and lower degrees of substitution spaced 44 mass units apart, as expected for (CH₂CH₂O)_{*n*}. Line broadening of critical signals such as the anomeric proton in the ¹H NMR spectra and both C₁ and C₄ in the ¹³C NMR spectra indicate that the cyclodextrin molecules lose their symmetry upon hydroxyethylation. Further details of the synthesis and characterization of these and similar cyclodextrins will be published in the near future.

Cyclodextrins **4** and **5** were dispersed in water by sonication of a thin film (cast by slow rotary evaporation of a solution of the cyclodextrins in chloroform) by using a sonication bath at 50 °C for 1 h.^[13] The solutions obtained in this way are rather turbid, which indicates the presence of cyclodextrin aggregates larger than 200 nm. At high concentrations (5–10 mg mL^{−1}), the cyclodextrins partly flocculate upon standing for 3–5 h at room temperature, but the precipitate is readily dispersed upon slight heating and agitation. No flocculation occurs if the cyclodextrin concentration is below 1 mg mL^{−1}.

Dynamic light scattering of solutions of **4** and **5** in water indicated the presence of cyclodextrin aggregates with an average diameter of 170 nm.^[14] These aggregates were also examined by transmission electron microscopy.^[14] Spherical vesicles with diameters of 50–300 nm were observed using

[*] Dr. R. Darcy, Dr. B. J. Ravoo
Department of Chemistry
University College Dublin
Belfield, Dublin 4 (Ireland)
Fax: (+353) 1-7062127
E-mail: raphael.darcy@ucd.ie

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uranyl acetate as a negative staining agent (Figure 1A). A monodisperse solution of spherical vesicles with an average diameter of 60 nm was obtained upon prolonged sonication (9 h) of a dispersion of **4** in water (Figure 1B). The structure of the vesicles is reminiscent of that of conventional liposomes, which can be prepared in a similar manner, and which show a similar correlation of particle size and sonication time.^[13]

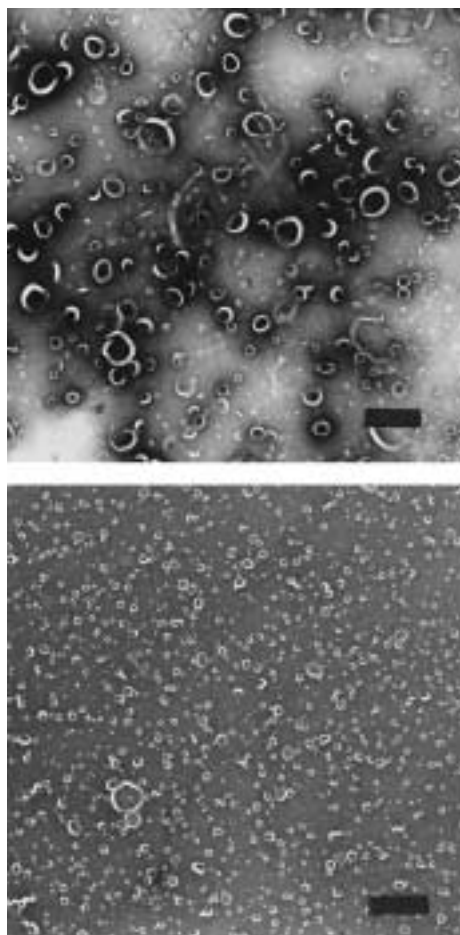


Figure 1. Electron micrographs of vesicles of **4** and **5**. Top: Vesicles of cyclodextrin **5** obtained after 2 h sonication at 50 °C. Bottom: Vesicles of cyclodextrin **4** obtained after 9 h sonication at room temperature. The scale bars represent 500 nm.

Further evidence for the formation of vesicles of **4** and **5** was obtained by encapsulation of the fluorescent dye carboxyfluorescein (CF) in the aqueous interior of the vesicles.^[13] Vesicles of **4** and **5** were prepared in a buffered solution of CF at a self-quenching concentration. The CF entrapped in the vesicles of **4** and **5** was separated from free CF by gel filtration on Sephadex G25. The elution profiles are shown in Figure 2A. The results are presented as the ratio of fluorescence intensity after (F_{TX}) and before (F_{init}) the addition of 0.1 % of Triton TX-100 (which solubilizes the cyclodextrin vesicles and causes release of their contents), which is a direct measure of the presence of entrapped CF. Independent absorbance measurements indicated that “empty” vesicles of **4** and **5** elute at 5–8 mL, whereas free CF elutes beyond 16 mL. The

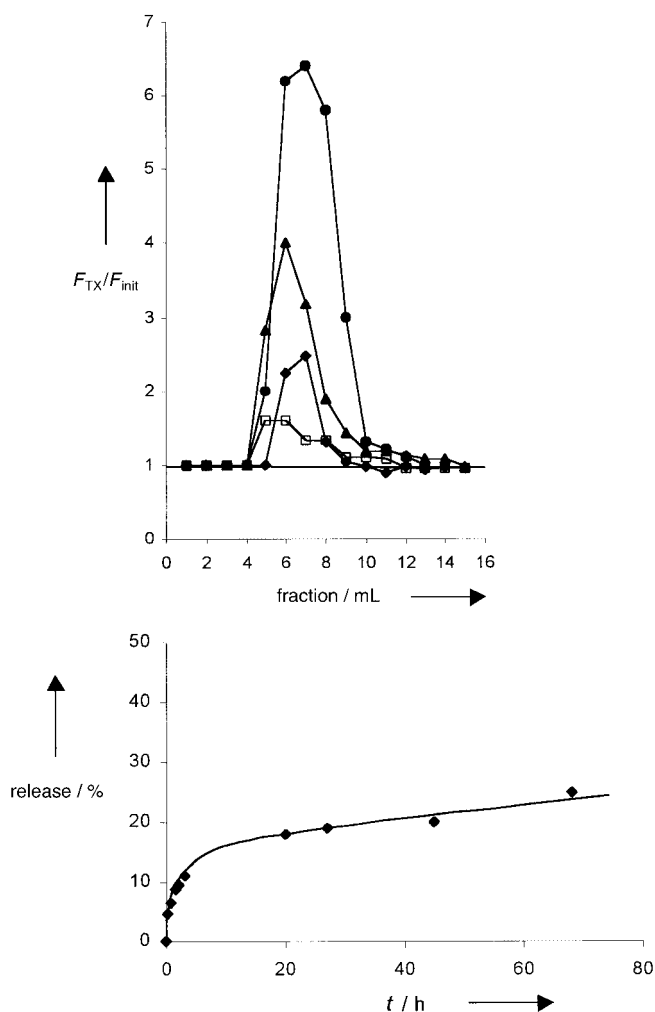


Figure 2. Encapsulation and release of carboxyfluorescein (CF) from vesicles of cyclodextrins **4** and **5**. Top: Elution profiles of vesicles of cyclodextrins **4** and **5** loaded with CF on a 18 × 1 cm Sephadex G25 column (void volume 4 mL). The relative fluorescence intensity scale F_{TX}/F_{init} indicates encapsulated CF only, as explained in the text. ●: **5** at 20 mg mL⁻¹; ▲: **5** at 8 mg mL⁻¹; ◆: **5** at 5 mg mL⁻¹; □: **4** at 5 mg mL⁻¹. Bottom: Leakage of CF from vesicles of cyclodextrin **5**.

coincidence of the peak of entrapped CF with the elution of vesicles of **4** and **5** confirms the existence of an aqueous inner compartment within the vesicles. As anticipated, the amount of entrapped CF in vesicles of **5** correlates with the concentration of **5**. The encapsulation of CF in vesicles of **4** was much less efficient, which may be a consequence of the shorter alkyl chains in **4** compared to in **5**. The spontaneous release of CF from vesicles of **5** was measured over time (Figure 2B): CF leakage is limited, and the vesicles still retain more than 75 % of CF after three days. Finally, vesicles of **5** were prepared in a solution of fluorescein-labeled dextran with an average molecular weight of 4000 daltons. Encapsulated (3–5 %) and free dextran were separated by gel filtration on Sephadex G50. We contend that the encapsulation procedure can be optimized and extended to larger and more significant solutes, such as proteins.

Vesicles of **5** were further characterized by a study of the temperature dependence of the fluorescence polarization of diphenylhexatriene (DPH) absorbed in vesicles of **5**.^[13]

Diphenylhexatriene has been extensively used to probe the main thermotropic phase transition of lipid bilayers.^[13] This L_{β} - L_{α} transition is typical of lamellar lyotropic phases, and its occurrence provides strong evidence for the formation of bilayers. The fluorescence polarization of DPH in vesicles of cyclodextrin **5** shows a sharp decrease from 0.38 to 0.27 around 50 °C. This decrease is most likely the result of an L_{β} - L_{α} transition of the bilayer of **5**. Lipids of similar hydrophobic chain length display this transition at comparable temperatures.^[15] The DPH fluorescence remains rather high above 50 °C, possibly because of partial inclusion of DPH in the hydrophobic cavity of **5** which restricts the DPH mobility.

Finally, a sample of aqueous cyclodextrin **5** was analyzed by differential scanning calorimetry (DSC).^[16] This technique enables the quantification of heat effects that accompany the L_{β} - L_{α} transition. Indeed, the heating scan in the DSC study displays a reproducible endothermic phase transition of a 10% (w/w) dispersion of **5** in water. The transition occurs around 48–49 °C and the enthalpy of transition amounts to 59 kJ mol⁻¹ for cyclodextrin. The transition temperature coincides with the decrease in the DPH fluorescence polarization. The enthalpy of transition is high compared to the typical transition enthalpy for phospholipid bilayers (30–40 kJ mol⁻¹).^[15] However, it is much lower when calculated per mole of hydrocarbon chain: 8.5 kJ mol⁻¹ for cyclodextrin **5** (with seven hexadecyl chains) compared with 15–20 kJ mol⁻¹ for a phospholipid (with two hexadecyl chains). The lower transition enthalpy may indicate the rigidity of the macrocyclic amphiphile relative to a phospholipid in an L_{α} bilayer.

In summary, we have developed novel non-ionic amphiphilic hydroxyethylated cyclodextrins that form bilayer vesicles in water. The encapsulation of solutes in such vesicles creates exciting new possibilities for the use of cyclodextrins as carrier materials.

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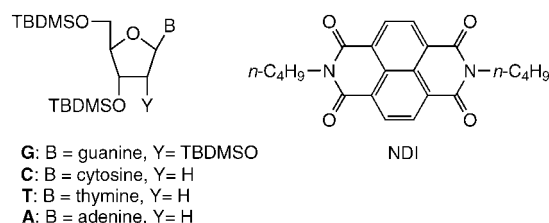
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Selective Enhancement of the One-Electron Oxidation of Guanine by Base Pairing with Cytosine**

Kiyohiko Kawai,* Yasuharu Wata, Nobuyuki Ichinose, and Tetsuro Majima*

The one-electron oxidation of nucleobases has been extensively studied since it leads to strand scission and oxidative lesions.^[1] Guanine, with the lowest oxidation potential,^[2] is the base subjected to most oxidation and which leads to the formation of mutagenic 8-oxoguanine and imidazolone.^[1a] Various studies suggest there is a lower oxidation potential for guanine in duplex DNA.^[3] The π -stacking interactions, especially by the adjacent 5' guanine residue, have been demonstrated to decrease the ionization potential of guanine.^[4] A computational experiment has suggested that the ionization potential of the hydrogen-bonded guanine–cytosine pair is lower than that of guanine alone.^[5] Modulation of electron transfer by hydrogen-bonding interactions has been suggested to play an important role in electron-transfer systems such as photosystem II in green plants.^[6] However, there have been few experimental studies specifically addressing the effects of hydrogen bonding on the rate of electron transfer in nucleic acids. Since solvation also affects the redox properties of nucleobases,^[7] it is difficult to evaluate the effect of hydrogen bonding by comparing the oxidation properties between single-stranded DNA and duplex DNA where the solvation and base stacking are significantly different. Therefore, in order to separate hydrogen bonding from stacking effects we have designed an experiment in dichloromethane which mimics the hydrophobic environment of the base moiety in duplex DNA. We report here the first experimental results of the effect of hydrogen bonding on the one-electron oxidation of nucleobases.

To facilitate the solubility of the nucleosides in dichloromethane substituted nucleoside derivatives with a *tert*-butyldimethylsilyl (TBDMS) group on the ribose unit, that is, tri-*tert*-butyldimethylsilylguanine (**G**), di-*tert*-butyldimethylsilylcytosine (**C**), di-*tert*-butyldimethylsilyladenine (**A**), and di-*tert*-butyldimethylsilylthymine (**T**), were prepared. *N,N'*-di-butynaphthalldiimide (NDI) in the triplet excited state (³NDI*) was selected as an oxidant for the selective and guanine-specific single electron oxidation that proceeds slower than the diffusion-controlled rate in dichloromethane (the diffusion-controlled rate constant $k_{\text{diff}} = 1.6 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$) under the present experimental conditions.^[8] The third-harmonic oscillation (355 nm, 4 ns, 15 mJ) from a Q-switched



Nd:YAG laser was used for laser flash excitation of NDI, and the rate constants of the electron transfer from nucleosides to ³NDI* were measured.

A transient absorption spectrum with two bands with maxima at 455 and 485 nm was observed 20 ns after excitation of a solution of NDI in dichloromethane with a laser flash pulse with a wavelength of 355 nm. A depletion of the two absorption bands at 360 and 380 nm corresponding to NDI in the ground state were also evident in the spectrum. The spectrum formed is characteristic of the T_1 – T_n absorption of *N,N'*-alkylatednaphthalldiimide derivatives in organic solvents and was assigned to ³NDI* (Figure 1 a).^[8] Concomitant with the decay of the transient absorption peaks of ³NDI* at 455 and 485 nm (Figure 1 b), the formation of a transient absorption in the range of 470–600 nm was observed at 200 ns. The transient absorption spectrum was identical to that observed during the quenching of ³NDI* by the well-known electron

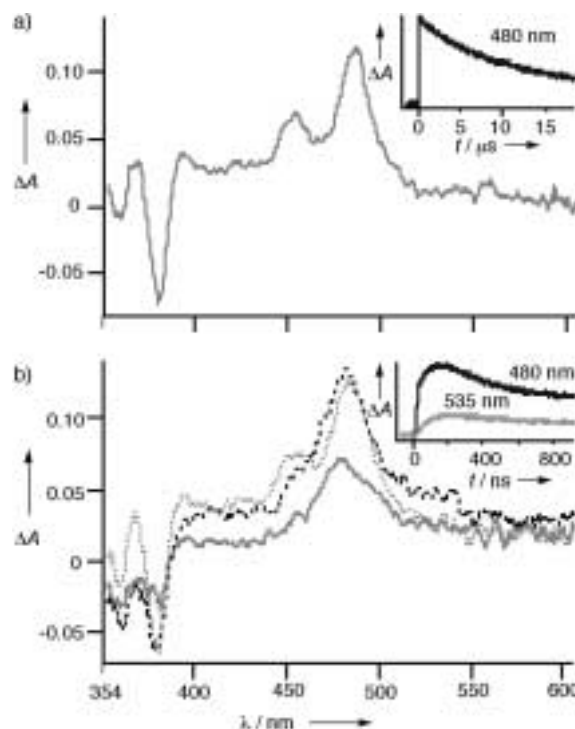


Figure 1. a) Transient absorption spectrum observed 20 ns after the pulsed excitation of 20 μM NDI in argon-saturated dichloromethane. The inset shows the triplet decay observed at 480 nm. b) Time-dependent transient absorption spectra recorded for 20 μM NDI in argon-saturated dichloromethane containing 9 mM **G** and **C** at 20 ns (••••), 200 ns (---), and 1 μs (—) after the pulsed excitation ($\lambda_{\text{exc}} = 355 \text{ nm}$). The inset shows time profiles of the transient absorption peaks at 480 and 535 nm which correspond to ³NDI* and NDI^{•–}, respectively.

[*] Dr. K. Kawai, Prof. T. Majima, Y. Wata, N. Ichinose
 The Institute of Scientific and Industrial Research (SANKEN)
 Osaka University
 Mihogaoka 8-1, Ibaraki, Osaka 567-0047 (Japan)
 Fax: (+81) 6-6879-8499
 E-mail: kiyohiko@sanken.osaka-u.ac.jp,
 majima@sanken.osaka-u.ac.jp

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donor 1,4-diazobicyclo[2,2,2]octane (DABCO). Thus the product from the quenching by the nucleosides is identified as the one-electron reduced forms of NDI ($\text{NDI}^{\bullet-}$).^[9]

The bimolecular quenching plots for the electron-transfer reaction of $^3\text{NDI}^*$ with electron donors (D) were obtained from the rate constants for the decay (k_{obs}) at various concentrations of donors such as the nucleosides **G**, **C**, **A**, **T**, DABCO, 1,2,4-trimethoxybenzene (1,2,4-TMB), and 1,4-dimethoxybenzene (1,4-DMB; Figure 2a). The rate constants (k_q) for the electron transfer in the forward reaction (from D to $^3\text{NDI}^*$) were calculated from the slope of the linear plots (Table 1).

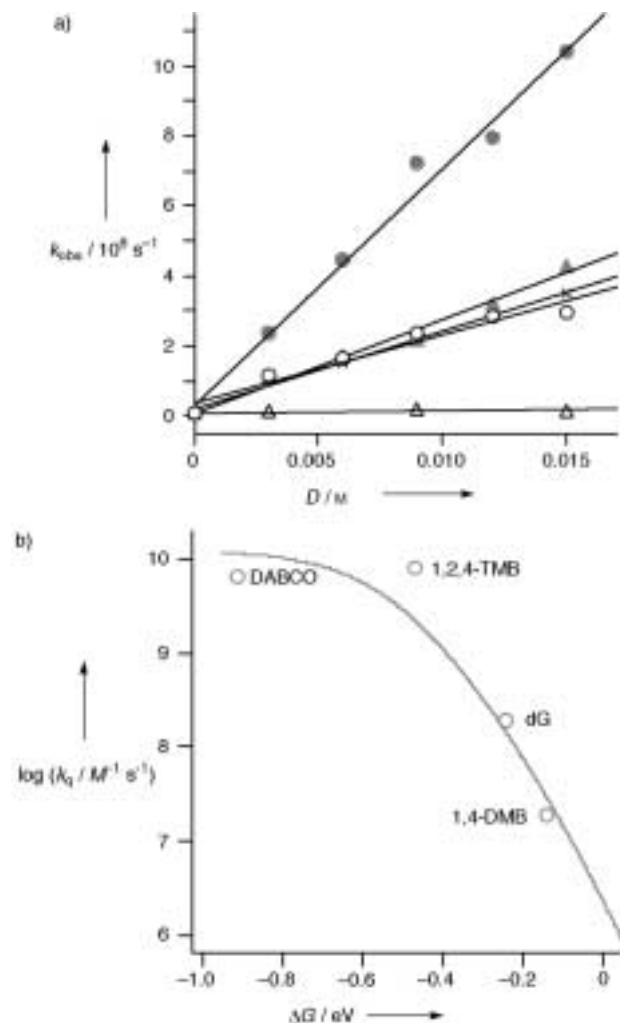


Figure 2. a) Bimolecular quenching plots for the electron-transfer reaction between $^3\text{NDI}^*$ and electron donors (D): **G** (○), **C** (△), **G** + **T** (▲), **G** + **A** (×), and **G** + **C** (●). b) Correlation between the intermolecular quenching rate constants (k_q) and free-energy change of the electron transfer ($-\Delta G$). The solid line was calculated according to the Marcus Equation assuming $\lambda = 1.0$ eV and $\bar{\nu}K_D = 5.0 \times 10^{10} \text{ cm}^{-1}$.^[15]

Quenching of $^3\text{NDI}^*$ by **G** was performed in the presence of **C**, **T**, and **A** to verify the effect of hydrogen bonding between **G** and other nucleosides on the rate constant. Of special interest was that an acceleration of the electron transfer was observed selectively for the **G**:**C** pair, whereas addition of **T** and **A** gave only negligible changes. The association constant

Table 1. Bimolecular rate constant (k_q) for the electron-transfer reaction between $^3\text{NDI}^*$ and nucleoside analogues together with the electron donors (D) in dichloromethane, oxidation potential ($E_{1/2}$), and free-energy change for the electron transfer (ΔG).

D	k_q [$10^8 \text{ M}^{-1} \text{ s}^{-1}$]	$E_{1/2}$ [V] ^[a]	ΔG [eV]
DABCO	65	0.81 ^[12]	−0.92
1,2,4-TMB	80	1.26 ^[13]	−0.47
G + C	6.8	1.37 ^[b]	−0.36
G + T	2.7	1.44 ^[b]	−0.29
G + A	2.2	1.46 ^[b]	−0.27
G	1.9	1.47 ^[7]	−0.26
1,4-DMB	0.19	1.59 ^[13]	−0.14
A	< 0.05	1.94 ^[7]	0.22
C	< 0.05	2.1 ^[7]	0.38
T	< 0.05	2.1 ^[7]	0.38

[a] Potential versus NHE in CH_3CN . [b] Estimated from k_q according to the relation between k_q and $-\Delta G$ (Figure 2b).

of **G** with **C** was measured to be $6.1 \times 10^3 \text{ M}^{-1}$ in CDCl_3 by the NMR titration method.^[10] Therefore, 80 to 95% of **G** is estimated to form the selective hydrogen bond with **C** under the present experimental conditions. Changing the concentration of **C** from 6 to 15 mM while the concentration of **G** was kept constant at 6 mM had only a negligible effect on k_q .^[11] This observation corresponds to a 1:1 complexation between **G** and **C**. Thus, the observed acceleration of the one-electron oxidation of **G** in the presence of **C** is clearly attributed to the presence of the hydrogen bond between **C** and **G**.

Thermodynamic driving forces of the electron transfer (ΔG) were estimated from the Rehm–Weller Equation [Eq. (1)] by using the reported reduction potential (1.81 V versus the normal hydrogen electrode (NHE))^[8] of $^3\text{NDI}^*$, and oxidation potentials of solutions of the donors D in acetonitrile (Table 1).^[7, 12, 13] The electrostatic interaction term was estimated to be 0.085 eV.^[14]

$$\Delta G = [E(\text{D}^+/\text{D}) - E(^3\text{NDI}^*/\text{NDI}^{\bullet-})] + 0.085 \quad (1)$$

The k_q values increased as the value of $-\Delta G$ for the electron transfer increased, which is consistent with the theoretical line obtained from the Marcus equation when it is assumed that $\lambda = 1.0$ eV and $\bar{\nu}K_D = 5.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$.^[15] From this relationship between k_q and $-\Delta G$ for the donors studied here, $E_{1/2}$ values were estimated for **G** in the presence of **C**, **A**, and **T**. Thus, **G**:**C** base pairing is found to lower the oxidation potential of **G** by 100 mV. The effects of hydrogen-bond interactions between the electron donor, a bacteriochlorophyll dimer, and histidine residues in the bacterial reaction center has been investigated by site-directed mutational experiments.^[16] It has been demonstrated that the midpoint potential was increased by 60–125 mV for the addition of each hydrogen bond to histidine. In this case histidine serves as a hydrogen-bond donor which leads to the destabilization of the oxidized state of the electron donor. Since cytosine possess a donor-acceptor-acceptor hydrogen-bonding pattern, it behaves overall as a hydrogen-bond acceptor and results in a decrease in the oxidation potential of guanine upon hydrogen bonding.

In the present study, we have clarified that the oxidation potential of guanine is lowered by selective hydrogen bonding

with cytosine. Our results suggest that the rate and direction of electron transfer could be regulated by hydrogen-bonding recognition. Hence, hydrogen bonding may play an important role in biological systems involving electron-transfer processes.

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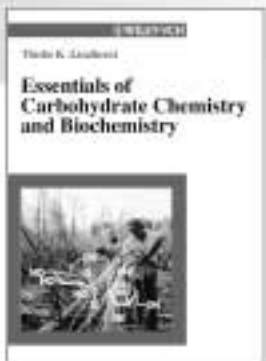
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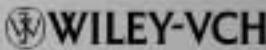
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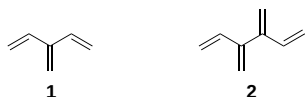
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First Synthesis of the Dendralene Family of Fundamental Hydrocarbons**

Simon Fielder, Daryl D. Rowan, and Michael S. Sherburn*

Of all the possible structural classes of conjugated polyene hydrocarbons, the dendralenes have received the least attention.^[1] These compounds are typified by an acyclic polyolefinic structure in which both the degree of unsaturation and level of cross conjugation are of the highest order. The simplest member of the group, [3]dendralene (3-methylene-1,4-pentadiene **1**) is a volatile substance which dimerizes

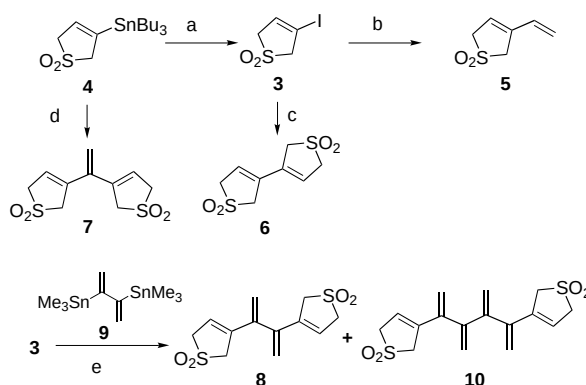


readily.^[2] [3]Dendralene has been prepared through either low-yielding, classical flow-tube thermolyses of ester derivatives,^[3a–d] by Hofmann elimination,^[3e] by thermal isomerization of 1,2-hexadien-5-yne followed by partial hydrogenation^[3f] and by flash vacuum pyrolysis of 3-vinyl-3-sulfolene.^[3g] Polymerization-prone [4]dendralene (3,4-bismethylene-1,5-hexadiene, **2**) has been prepared in low yields by thermolytic isomerization and elimination processes^[1] and, more recently, through high-temperature fragmentation of a sulfolene derivative.^[4] Surprisingly, none of the higher members of this fundamental class of compounds have been reported, and spectroscopic data for the parent triene **1** and tetraene **2** are still not complete.^[5] Herein we report the synthesis and spectroscopic properties of the [*n*]dendralene family (*n* = 3–6) using a unified strategy. We also demonstrate the applicability of this approach for the synthesis of a higher member of the group.

In light of the documented instability of [3]- and [4]dendralene (**1** and **2**, respectively), we formulated a synthetic approach involving the masking of each terminal butadiene residue of the dendralene target as its 3-sulfolene^[6] derivative. Assuming that thermolytic cracking of SO₂ from the masked derivatives could be achieved, the problem of [*n*]dendralene

synthesis was thus reduced to the construction and union of suitably functionalized ethylene and butadiene building blocks. We targeted Stille cross-coupling^[7] for the union of these C₂ and C₄ units, due to the anticipated ease of preparation of the requisite vinylstannane/vinyl iodide precursors and the ease with which the functionalities of the two reacting partners can be interchanged. Moreover, the mildness of the Stille reaction conditions was not expected to cause premature cheletropic elimination of SO₂ from the masked dendralene products and thereby lead to product decomposition.

Synthetic approaches to masked [3]-, [4]-, [5]-, and [6]dendralenes are shown in Scheme 1. The key building block, 3-iodo-3-sulfolene (**3**)^[8] was readily prepared by iodolysis of



Scheme 1. The preparation of masked dendralenes. a) I₂, CH₂Cl₂, Ar, 1 h, 80 %; b) Bu₃Sn–CH=CH₂ (1.2 equiv), [PdCl₂(CH₃CN)₂] (0.05 equiv), DMF, RT, Ar, 2 h, 92 %; c) **4** (1.0 equiv), [PdCl₂(CH₃CN)₂] (0.05 equiv), DMF, RT, Ar, 18 h, 95 %; d) CH₂=CBr₂ (0.5 equiv), [PdCl₂(CH₃CN)₂] (0.05 equiv), DMF, 40 °C, Ar, 36 h, 11 %; e) **9** (1.0 equiv) [PdCl₂(CH₃CN)₂] (0.10 equiv), DMF, 60 °C, Ar, 72 h, 43 % of **8** and 30 % of **10**.

3-tributylstannyl-3-sulfolene (**4**).^[9] The [3]dendralene precursor, 3-vinyl-3-sulfolene (**5**)^[3g] was prepared in high yield by Stille coupling of **3** with vinyltributylstannane. The [4]dendralene precursor **6**^[8] was prepared in high yield by coupling equimolar mixtures of **3** and **4**. [5]Dendralene precursor **7**^[8] was prepared by twofold Stille coupling of stannane **4** with 1,1-dibromoethene.^[10, 11] The masked [6]dendralene **8**^[8] was prepared by twofold cross-coupling of **3** with 2,3-bis(trimethylstannyl)-1,3-butadiene (**9**)^[12], a reaction which also furnished [8]dendralene precursor **10**^[8] as a by-product.

Masked [*n*]dendralenes **5–8** and **10** are white crystalline solids that are stable for extended periods of time (> 1 month) under nitrogen at ambient temperature. Whilst their insolubility precludes solution-phase thermolysis, each of the compounds **5–8** undergoes smooth cheletropic elimination of SO₂ upon heating to 450 °C at atmospheric pressure under argon to afford a spectroscopically homogeneous sample of the dendralene (Scheme 2). [8]Dendralene was prepared from **10** by thermolysis in vacuo. In each case the hydrocarbon was collected in a trap cooled to liquid N₂ temperature and, after warming to room temperature, the colorless oil was quickly rinsed into an NMR tube with CDCl₃. The dendralenes polymerize only slowly in dilute solution in CDCl₃ and

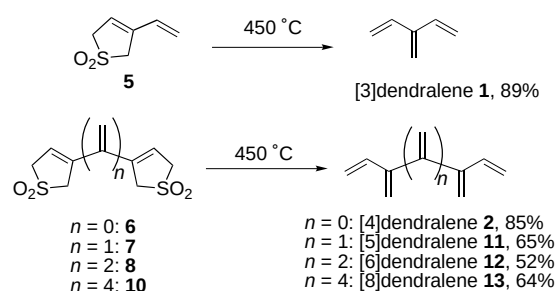
[*] Dr. M. S. Sherburn
School of Chemistry
University of Sydney
Sydney, NSW 2006 (Australia)
Fax: (+61) 2-9351-6650
E-mail: M.Sherburn@chem.usyd.edu.au

Dr. S. Fielder,^[+] Dr. D. D. Rowan
Horticulture and Food Research Institute of New Zealand Ltd
Private Bag 11030, Palmerston North (New Zealand)

[+] Further address:
Institute of Fundamental Sciences
Massey University
Private Bag 11222, Palmerston North (New Zealand)

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Scheme 2. Unmasking the dendralenes.

full spectral data for each compound was readily obtained (Table 1).

All members of the family exhibited excellent thermal stability: traces from GC/EI-MS analyses consisted of a single peak, from which a molecular ion formulating for the expected species was obtained. Dendralene fragmentation under EI conditions is characterized by a successive loss of methylene groups.

Table 1. Selected spectroscopic data for the dendralenes.

[3]dendralene 1 : $R_f = 0.60$ (SiO ₂ , hexane); IR (CDCl ₃ solution): $\tilde{\nu}_{\text{max}} = 3092, 3009, 2978, 1601, 1585, 990\text{ cm}^{-1}$; ¹ H NMR (270 MHz, CDCl ₃): $\delta = 6.47$ (dd, $^3J = 17.6, 11.2\text{ Hz}$, 2H), 5.42 (dd, $^3J = 17.6, ^2J = 1.5\text{ Hz}$, 2H), 5.17 (br. d, $^3J = 11.2\text{ Hz}$, 2H), 5.17 (br. s, 2H); ¹³ C NMR (68 MHz, CDCl ₃) $\delta = 144.5$ (quaternary C), 135.7 (CH), 115.6 (CH ₂), 115.4 (CH ₂); UV/Vis (0.4% CDCl ₃ in EtOH): $\lambda_{\text{max}} (\epsilon) = 232.2\text{ nm}$ (8200); GCMS (EI, 70 eV): m/z (%): 80 (71) [M^+], 79 (100) [$M^+ - H$]; HR-MS (EI, 70 eV): calcd for C ₆ H ₈ : 80.0626; found: 80.0630.	
[4]dendralene 2 : $R_f = 0.60$ (SiO ₂ , hexane); IR (CDCl ₃ solution): $\tilde{\nu}_{\text{max}} = 3092, 3009, 2974, 1583, 990\text{ cm}^{-1}$; ¹ H NMR (270 MHz, CDCl ₃): $\delta = 6.44$ (dd, $^3J = 17.4, 10.6\text{ Hz}$, 2H), 5.25 (m, 2H), 5.19 (br. d, $^3J = 17.4\text{ Hz}$, 2H), 5.11 (br. d, $^3J = 10.6\text{ Hz}$, 2H), 5.07 (m, 2H); ¹³ C NMR (68 MHz, CDCl ₃) $\delta = 146.4$ (quaternary C), 137.4 (CH), 117.7 (CH ₂), 116.5 (CH ₂); UV/Vis (0.4% CDCl ₃ in EtOH): $\lambda_{\text{max}} (\epsilon) = 222.8\text{ nm}$ (13500); GC-MS (EI, 70 eV): m/z (%): 106 (35) [M^+], 105 (45) [$M^+ - H$], 91 (100) [$M^+ - CH_3$]; HR-MS (EI, 70 eV): calcd for C ₈ H ₁₀ : 106.0782; found: 106.0779.	
[5]dendralene 11 : $R_f = 0.48$ (SiO ₂ , hexane); IR (CDCl ₃ solution): $\tilde{\nu}_{\text{max}} = 3092, 1580, 1131, 991\text{ cm}^{-1}$; ¹ H NMR (270 MHz, CDCl ₃): $\delta = 6.44$ (dd, $^3J = 17.4, 10.6\text{ Hz}$, 2H), 5.34 (dd, $^3J = 17.4; ^2J = 1.5\text{ Hz}$, 2H), 5.26 (s, 2H), 5.23 (m, 2H), 5.14 (br. d, $^3J = 10.6\text{ Hz}$, 2H), 5.12 (m, 2H); ¹³ C NMR (68 MHz, CDCl ₃) $\delta = 146.9$ (quaternary C), 146.5 (quaternary C), 136.9 (CH), 116.8 (CH ₂), 116.4 (CH ₂), 116.2 (CH ₂); UV/Vis (0.4% CDCl ₃ in EtOH): $\lambda_{\text{max}} (\epsilon) = 219.8\text{ nm}$ (22000); GC-MS (EI, 70 eV): m/z (%): 132 (13) [M^+], 131 (48) [$M^+ - H$], 117 (100) [$M^+ - CH_3$]; HR-MS (EI, 70 eV): calcd for C ₁₀ H ₁₂ : 132.0939; found: 132.0914.	
[6]dendralene 12 : $R_f = 0.49$ (SiO ₂ , hexane); IR (CDCl ₃ solution): $\tilde{\nu}_{\text{max}} = 3092, 2978, 2875, 1581, 1113, 989\text{ cm}^{-1}$; ¹ H NMR (270 MHz, CDCl ₃): $\delta = 6.46$ (ddd, $^3J = 17.4, 10.5; ^4J = 0.9\text{ Hz}$, 2H), 5.29 (br. d, $^3J = 17.4\text{ Hz}$, 2H), 5.28 (m, 2H), 5.26 (m, 2H), 5.13 (dm, $^3J = 10.5\text{ Hz}$, 2H), 5.09 (m, 4H); ¹³ C NMR (68 MHz, CDCl ₃) $\delta = 147.3$ (quaternary C), 146.4 (quaternary C), 137.8 (CH), 117.7 (CH ₂), 117.4 (CH ₂), 116.2 (CH ₂); UV/Vis (0.4% CDCl ₃ in EtOH): $\lambda_{\text{max}} (\epsilon) = 220.0\text{ nm}$ (25000); GC-MS (EI, 70 eV): m/z (%): 158 (16) [M^+], 157 (53) [$M^+ - H$], 143 (56) [$M^+ - CH_3$], 129 (100) [$M^+ - C_2H_5$]; HR-MS (EI, 70 eV): calcd for C ₁₂ H ₁₄ : 158.1095; found: 158.1090.	
[8]dendralene 13 : $R_f = 0.40$ (SiO ₂ , hexane); IR (CDCl ₃ solution): $\tilde{\nu}_{\text{max}} = 3093, 3008, 2930, 1577, 1319, 1131, 990\text{ cm}^{-1}$; ¹ H NMR (270 MHz, CDCl ₃): $\delta = 6.46$ (dd, $^3J = 17.4, 10.5\text{ Hz}$, 2H), 5.36 – 5.25 (m, 8H), 5.12 (m, 8H); ¹³ C NMR (68 MHz, CDCl ₃) $\delta = 147.5$ (quaternary C), 147.3 (quaternary C), 146.9 (quaternary C), 137.7 (CH), 117.8 (CH ₂), 117.5 (CH ₂), 117.2 (CH ₂), 116.2 (CH ₂); UV/Vis (0.4% CDCl ₃ in EtOH): $\lambda_{\text{max}} (\epsilon) = 220.6\text{ nm}$ (25000); GCMS (EI, 70 eV): m/z (%): 210 (10) [M^+], 209 (31) [$M^+ - H$], 195 (100) [$M^+ - CH_3$]; HR-MS (EI, 70 eV): calcd for C ₁₆ H ₁₈ : 210.1409; found: 210.1405.	

The ¹H NMR spectra^[13] contain the expected ABX system of a monosubstituted alkene, with the C2 methine proton appearing furthest downfield ($\delta = 6.44$ – 6.47) as an isolated doublet of doublets ($^3J = 17.4$ – $17.6, 10.5$ – 11.2 Hz). The remaining signals ($\delta = 5.09$ – 5.42) are well resolved and first-order at 270 MHz for [3]dendralene but increase in complexity with higher members of the family. The ¹³C NMR spectra of the dendralenes^[13] are particularly informative, with signals appearing in three distinct regions: $\delta = 115.4$ – 117.7 (methylene C atoms), 135.7 – 137.8 (methine C atoms), and 144.5 – 147.3 (quaternary C atoms).

The dendralenes display a single absorption maximum in the UV/Vis region^[13] at $\lambda_{\text{max}} = 219.8$ – 232.2 nm (0.4% CDCl₃ in ethanol), confirming the nonplanar, nonconjugated arrangement of *s-trans*-1,3-butadiene and ethylene units in these molecules.^[14] Indeed, there is an approximate correlation between the extinction coefficient of each dendralene and the number of 1,3-butadiene moieties that makes up its structure.

In summary, the first four members of the dendralene family have been prepared by a modular strategy in which the more exposed terminal 1,3-butadiene residues are masked as 3-sulfolene derivatives. The applicability of this approach for the synthesis of higher family members has been demonstrated by the synthesis of [8]dendralene. Access to this fundamental (yet neglected^[1]) class of cross-conjugated hydrocarbons sets the scene for studies into their structure, reactivity, and properties.^[15, 16]

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The Hexaazidogermanate(IV) Ion: Syntheses, Structures, and Reactions**

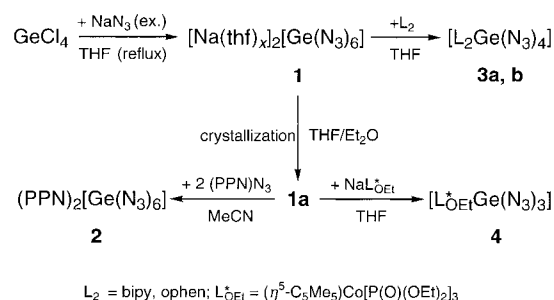
Alexander C. Filippou,* Peter Portius,
Dirk U. Neumann und Klaus-Dieter Wehrstedt

*Dedicated to Professor Herbert Schumann
on the occasion of his 65th birthday*

The synthesis, isolation, and structural characterization of high-energy compounds is an experimental challenge.^[1] Binary azides of the main Group IV elements belong to this class of compounds, however, very little is known about their structure and reactivity.^[2] To date only the ions $[\text{C}(\text{N}_3)_3]^{+2d}$ and $[\text{E}(\text{N}_3)_6]^{2-}$ (E = Sn, Pb),^[2e,h] as well as $\alpha\text{-Pb}(\text{N}_3)_2$ ^[2f] have been structurally characterized; in comparison there is no structural information for the analogous silicium and germa-

nium compounds. We recently reported the syntheses, structures, and oxidation reactions of Ge^{II} azides with HN_3 ,^[3] hypercoordinate triazidogermane compounds of the type $[\text{L}_3\text{Ge}(\text{N}_3)_3]$ ($\text{L}_3 = [\text{CpCo}(\text{P}(\text{O})(\text{OEt})_2)_3]$, $\text{HB}(\text{pz}^*)_3$; $\text{Cp} = \text{C}_5\text{H}_5$; $\text{pz}^* = 3,5\text{-dimethylpyrazol-1-yl}$) could be prepared and structurally characterized.^[4] As an extension to this work on germanium compounds with a high nitrogen content^[5] we report herein the formation, structure, and first reactions of the hexaazidogermanate(IV) ion to give derivatives of $\text{Ge}(\text{N}_3)_4$.

The reaction of GeCl_4 with excess NaN_3 in boiling THF leads selectively to disodium hexaazidogermanate(IV) **1**, which, after workup and crystallization, was isolated as colorless, hydrolysis-sensitive, single crystals of composition $[\text{Na}_2(\text{thf})_3(\text{Et}_2\text{O})][\text{Ge}(\text{N}_3)_6]$ (**1a**; Scheme 1).^[6] The crystal



Scheme 1. Synthesis of $\text{Ge}(\text{N}_3)_4$ derivatives starting from GeCl_4 .

lattice of **1a** is composed of distorted octahedral $[\text{Ge}(\text{N}_3)_6]^{2-}$ ions of C_1 symmetry and two symmetry independent sodium ions, of which one is solvated by two THF molecules (Na1-O1 2.327(4), Na1-O2 2.287(5) Å) and the other by one THF and one Et_2O molecule (Na2-O3 2.265(5), Na2-O4 2.304(11) Å; Figure 1).^[7] The Na–O bond lengths are in the range reported for other Na–THF/ Et_2O complexes.^[8] Short Na–N contacts exist between the Na^+ ions and the N_α and N_γ atoms of the azide groups of the $[\text{Ge}(\text{N}_3)_6]^{2-}$ ions. These contacts link each $[\text{Ge}(\text{N}_3)_6]^{2-}$ ion with two Na1 and Na2 cations and each sodium cation with two $[\text{Ge}(\text{N}_3)_6]$ anions to give a layer structure. This arrangement results in a strongly distorted octahedral coordination geometry of the Na1 cations and a coordination polyhedron for the pentacoordinate Na2 cations that can be rationalized using the Kepert model for chelate complexes.^[9] The Na–N distances (2.410(5)–2.636(6) Å) are in the range found for Na–N coordinate bonds^[10] and increase as the coordination number of the Na^+ ions and of the azide nitrogen atoms increases. The short interionic Na–N contacts in **1a** have only a slight influence on the average bonding parameters of the $[\text{Ge}(\text{N}_3)_6]^{2-}$ ion. This point is shown by a comparison with the crystal structure of the salt $(\text{PPN})_2[\text{Ge}(\text{N}_3)_6]$ **2** ($(\text{PPN})^+ = [\text{N}(\text{PPh}_3)_2]^+$), which contains less-polarizing cations (Table 1). Compound **2** is formed in the reaction of **1a** with $(\text{PPN})\text{N}_3$ in MeCN (Scheme 1) and was fully characterized. It was isolated in 55 % yield as a colorless microcrystalline powder that is very soluble in CH_2Cl_2 but insoluble in THF. In the solid state **2** is air stable for a short time, whereas in solution it is rapidly hydrolyzed releasing HN_3 and $(\text{PPN})\text{N}_3$. IR spectroscopy of **2** in MeCN at

[*] Prof. Dr. A. C. Filippou, Dipl.-Chem. P. Portius,
Dipl.-Chem. D. U. Neumann
Institut für Chemie der Humboldt-Universität zu Berlin
Hessische Strasse 1–2, 10115 Berlin (Germany)
Fax: (+49) 30-2093-6939
E-mail: filippou@chemie.hu-berlin.de

Dr. K.-D. Wehrstedt
Bundesanstalt für Materialforschung und -prüfung (BAM)
Unter den Eichen 87, 12205 Berlin (Germany)

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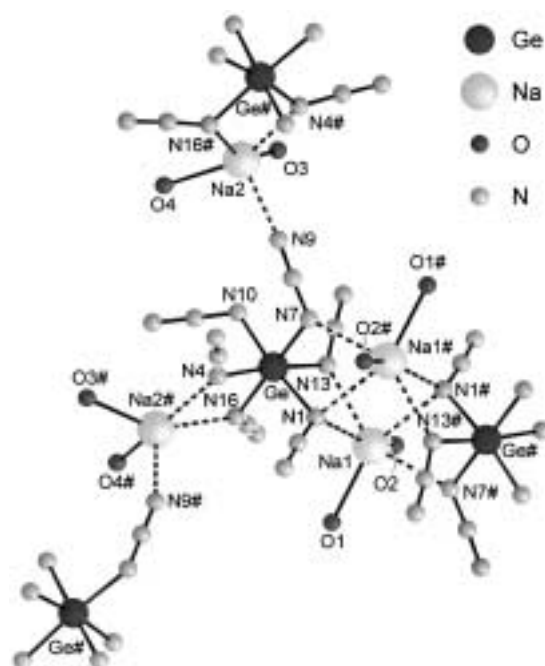


Figure 1. View of the crystal structure of **1a** that shows the interionic interactions between the $[\text{Ge}(\text{N}_3)_6]^{2-}$ ion and the solvated cations Na1 and Na2. For clarity, only the O atoms of the solvent molecules are shown. Selected distances [Å] and angles [°]: Ge–N1 1.984(5), Ge–N4 1.983(4), Ge–N7 1.990(6), Ge–N10 1.985(5), Ge–N13 1.959(4), Ge–N16 1.985(6), Na1–N1 2.552(5), Na1–N1# 2.636(6), Na1–N7# 2.487(6), Na1–N13 2.523(4), Na2–N4# 2.473(7), Na2–N9 2.410(5), Na2–N16# 2.450(5); N1–Ge–N10 173.3(2), N4–Ge–N13 173.7(2), N7–Ge–N16 176.5(2).

Table 1. Experimentally determined average bond lengths [Å] and angles [°] of the azide groups in compounds **1a–4** (standard deviations in parentheses) and calculated values for the S_6 -symmetric minimum structure of $[\text{Ge}(\text{N}_3)_6]^{2-}$ with various DFT methods/basis sets.

Compound	Ge– N_α	N_α – N_β	N_β – N_γ	Ge– N_α – N_β	N_α – N_β – N_γ
1a	1.981(5)	1.204(7)	1.132(8)	119.3(4)	176.9(6)
2	1.974(2)	1.212(3)	1.147(3)	119.8(2)	175.9(2)
3a	1.955(2)	1.216(2)	1.140(3)	117.7(1)	176.8(2)
3b ^[a]	1.949(2)	1.221(2)	1.136(3)	117.1(1)	176.1(2)
4 ^[a]	1.92(1)	1.20(2)	1.17(2)	117(1)	174(1)
$[\text{Ge}(\text{N}_3)_6]^{2-}$					
BP86/dzp	2.022	1.213	1.168	123.7	174.5
BP86/svp	2.016	1.211	1.168	124.9	174.8
BLYP/dzp	2.044	1.218	1.172	123.4	174.5
BLYP/svp	2.036	1.216	1.171	124.6	174.8

[a] A. C. Filippou, P. Portius, unpublished results.

different concentrations gave no evidence of a dissociation of **2** into $\text{Ge}(\text{N}_3)_4$ and $(\text{PPN})\text{N}_3$. Compound **2** is not impact sensitive, as demonstrated in experiments with the drop hammer of the Bundesanstalt für Materialforschung und -prüfung (BAM Fallhammer),^[11] and is thermally astoundingly stable. Thus it melts in vacuum, without decomposition, at 211 °C. In addition, a thermal analysis of **2** by differential scanning calorimetry (DSC) showed that the melting of **2** (extrapolated onset temperature $T_e = 194$ °C, peak temperature $T_p = 213$ °C, $\Delta H_f = +46 \text{ J g}^{-1}$) follows a strongly exothermic decomposition, which takes place in two steps ($T_e = 256$ °C, $\Delta H = -503 \text{ J g}^{-1}$; $T_e = 312$ °C, $\Delta H = -344 \text{ J g}^{-1}$). The crystal structure of **2**^[7] reveals the presence of separated

$[\text{Ge}(\text{N}_3)_6]^{2-}$ ions of S_2 symmetry (roughly S_6 symmetry; Figure 2) enclosed by the voluminous $(\text{PPN})^+$ ions.^[12] This encapsulation makes the $[\text{Ge}(\text{N}_3)_6]^{2-}$ ions “phlegmatic” (inert) and offers an explanation for the thermal and mechanical

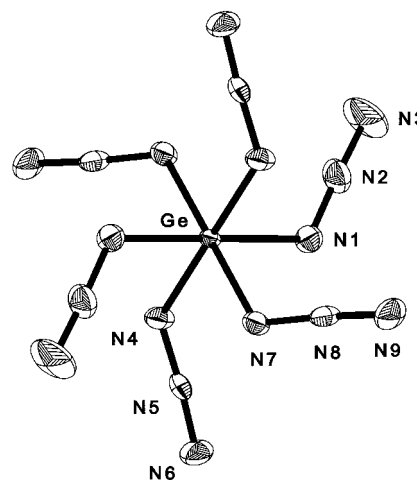


Figure 2. ZORTEP drawing of the molecular structure of the $[\text{Ge}(\text{N}_3)_6]^{2-}$ ion in the crystal lattice of **2**. The thermal ellipsoids are set at the 50% probability level. Selected bond lengths [Å] and angles [°]: Ge–N1 1.980(2), Ge–N4 1.969(2), Ge–N7 1.974(2), N1–N2 1.213(3), N2–N3 1.152(4), N4–N5 1.214(3), N5–N6 1.143(3), N7–N8 1.210(3), N8–N9 1.146(3); N1–Ge–N4 89.2(1), N1–Ge–N7 90.4(1), N4–Ge–N7 90.9(1), Ge–N1–N2 118.4(2), Ge–N4–N5 122.7(2), Ge–N7–N8 118.4(2), N1–N2–N3 176.6(3), N4–N5–N6 175.0(2), N7–N8–N9 176.1(2).

stability of **2**. Density functional theory (DFT) calculations using various methods predict a S_6 -symmetric minimum structure for the $[\text{Ge}(\text{N}_3)_6]^{2-}$ ion and show a very good agreement between the calculated and experimentally determined bond lengths and angles (Table 1).^[13] These results suggest a reduction of symmetry of the anion in the crystal through packing effects. The solution IR and Raman spectra (including the polarization measurements) of **2** are also consistent with S_6 -symmetric anion, and allow in combination with the calculated IR and Raman spectra an assignment of the individual fundamental vibrations of the $[\text{Ge}(\text{N}_3)_6]^{2-}$ ion (see Experimental Section). In addition, the ^{73}Ge NMR spectrum of **2** displays a sharp signal at $\delta = -290.6$ ($\Delta\nu_{1/2} = 15 \text{ Hz}$), which suggests a highly symmetrical environment for the germanium atom.^[14] The bond lengths and angles of the $[\text{Ge}(\text{N}_3)_6]^{2-}$ ion in **2** are in good agreement with those of other hexa-coordinate Ge^{IV} azide compounds (Table 1) and indicate the presence of polar, covalent Ge–N bonds.^[4] Correspondingly two well-resolved singlets at $\delta = -288.9$ and -208.0 are found in the ^{14}N NMR spectrum of **2** in MeCN, which, by comparison with the data of other covalent azide compounds,^[15] can be assigned to the N_α and N_γ atoms of the azide groups (the N_β signal of **2** is masked by the solvent signal at $\delta = -136.3$).

Whereas **2** has proved so far to be relatively unreactive, **1** and **1a** react readily with nucleophiles. Thus the reactions of **1** with 2,2'-bipyridine (bipy) and 1,10-phenanthroline (ophen) leads to the Lewis-base adducts of $\text{Ge}(\text{N}_3)_4$, **3a** and **3b**, respectively, and the reaction of **1a** with $\text{NaL}^*_{\text{OEt}}$ gives the hexacoordinate triazidogermane **4** (Scheme 1). The com-

pounds were isolated in good yields as surprisingly thermally stable colorless crystals (**3a**, **3b**·0.5 MeCN) or as a yellow powder (**4**); these compounds were fully characterized and their molecular structures determined by single-crystal X-ray diffraction (Table 1, Figure 3).^[7]

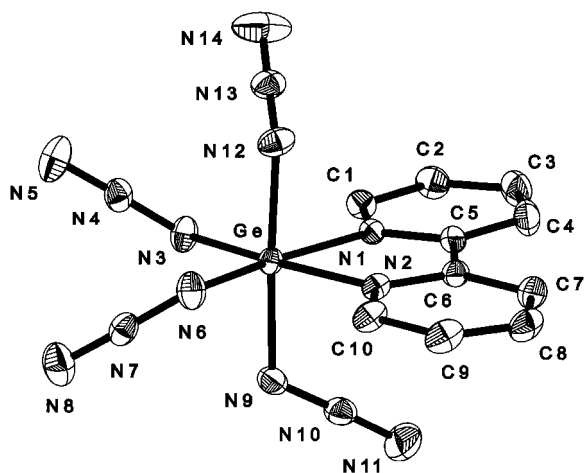


Figure 3. ZORTEP drawing of the molecular structure of **3a**. The thermal ellipsoids are set at the 50% probability level. The hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Ge-N1 2.057(2), Ge-N2 2.046(2), Ge-N3 1.958(2), Ge-N6 1.925(2), Ge-N9 1.972(2), Ge-N12 1.966(2), N3-N4 1.218(2), N4-N5 1.139(3), N9-N10 1.208(2), N10-N11 1.142(3); N1-Ge-N6 170.02(6), N2-Ge-N3 170.22(6), N9-Ge-N12 176.55(6), Ge-N3-N4 116.9(1), Ge-N9-N10 119.2(1), N3-N4-N5 176.7(2), N9-N10-N11 177.6(2).

The complexes **1a–3b** are not just examples of a very rare class of hypercoordinate compounds containing a GeN_6 framework,^[4] but are also the first structurally characterized derivatives of the long sought after species $\text{Ge}(\text{N}_3)_4$ and are thus promising starting materials for the preparation of new binary germanium nitride compounds.^[16]

Experimental Section

The experiments were performed using appropriate safety procedures (conditions see ref [4]) because of the risk of explosion in handling azides (armored glass, face shield, metal gauntlets, protective clothing). GeCl_4 ^[17] and $(\text{PPN})\text{N}_3$ ^[18] were prepared by literature methods. The solution IR spectra were recorded in a NaCl cell under rigorous exclusion of moisture. (Bruker-IFS 55 Spectrometer). Raman spectra: Bruker-RFS-100 FT Raman Spectrometer, Nd:YAG Laser, power 500 mW. The NMR spectra were recorded at room temperature; ^1H , $^{13}\text{C}\{^1\text{H}\}$, and $^{31}\text{P}\{^1\text{H}\}$ NMR: Bruker-AM-300 Spectrometer; ^{14}N and ^{73}Ge NMR: Bruker-DRX-500 Spectrometer; ^{31}P , ^{14}N and ^{73}Ge NMR signals relative to $\delta(\text{H}_3\text{PO}_4)_{\text{ext}}$, $\delta(\text{CH}_3\text{NO}_2)_{\text{ext}}$, and $\delta(\text{GeMe}_4)_{\text{ext}}=0$, respectively; coupling constants [Hz] of the ring protons of **3a**: $^3J(\text{H}_3, \text{H}_4)=8.0$, $^4J(\text{H}_3, \text{H}_5)=1.1$, $^5J(\text{H}_3, \text{H}_6)=0.9$, $^3J(\text{H}_4, \text{H}_5)=7.7$, $^4J(\text{H}_4, \text{H}_6)=1.5$, $^3J(\text{H}_5, \text{H}_6)=5.6$; **3b**: $^3J(\text{H}_2, \text{H}_3)=5.1$, $^4J(\text{H}_2, \text{H}_4)=1.3$, $^3J(\text{H}_3, \text{H}_4)=8.2$. Mass spectrometry: Hewlett-Packard 5995A Spectrometer, m/z values relative to the ^{74}Ge isotope. The DSC measurement was carried out in a high-pressure stainless-steel vessel with a gold coated copper sealing disk (7 mg weight of sample, heating rate 5 K min^{-1}) using a Perkin-Elmer differential scanning calorimeter "Pyris 1"; T_{e} = extrapolated onset temperature, T_{p} = peak temperature.

1a: Into a reflux apparatus, which had been thoroughly baked-out, was placed NaN_3 (5.30 g, 81.5 mmol) suspended in THF (41 mL). The mixture was treated with GeCl_4 (1.16 mL, 2.14 g, 9.98 mmol). The suspension was stirred vigorously and heated under reflux for 140 h. After cooling an IR

spectrum of a diluted sample of the supernatant solution was recorded and showed the selective formation of **1** [$\bar{\nu}=2123$ (vw), 2089 cm^{-1} (vs), $(\nu_{\text{as}}(\text{N}_3))$].^[6] The colorless solution was separated from the white precipitate by filtration; the precipitate weighed 4.47 g after drying. The filtrate was used directly in the synthesis of **3a, b** or worked up as follows for the isolation of **1a**: the volume of the solution was reduced under vacuum, until a clear oil remained from which, after the addition of Et_2O (10 mL) a solid (**1a**) started to slowly crystallize radially. The supernatant solution was decanted, the residue was dissolved in THF (10 mL) and separated from the white insoluble material by filtration. The filtrate was crystallized again as above. The resulting crystal mass of **1a** was washed with a little Et_2O and dried for a few minutes under fine vacuum. The resulting powder did not contain chlorine;^[6] yield 2.93 g (approximately 44% from GeCl_4). Single crystals of **1a** were obtained from this powder by crystallization from THF/ Et_2O .

2: A solution of $(\text{PPN})\text{N}_3$ (1.01 g, 1.74 mmol) in acetonitrile (11 mL) was added dropwise to a stirred solution of **1a** (661 mg, 1.00 mmol) in acetonitrile (20 mL) at room temperature. A suspension was formed immediately and became more cloudy during the course of the reaction. After 0.5 h the IR spectrum of the reaction solution showed that the $\nu_{\text{as}}(\text{N}_3)$ absorption band of $(\text{PPN})\text{N}_3$ at 2005 cm^{-1} had vanished and only the $\nu_{\text{as}}(\text{N}_3)$ absorption band of **2** could be identified. The white suspension was filtered, the clear, colorless filtrate collected and the solvent removed by vacuum. The resulting crude product (1.06 g) was dissolved in acetonitrile (20 mL), the cloudy solution was filtered to remove insoluble material, and the filtrate concentrated to a few mL and left to stand for 2 d at -30°C . The colorless crystals thus obtained were washed with pentane (10 mL) and dried for 0.5 h under high vacuum; yield 771 mg (55% from **1a**); elemental analysis calcd (%) for $\text{C}_{72}\text{H}_{60}\text{GeN}_{20}\text{P}_4$ (1401.91): C 61.69, H 4.31, N 19.98, P 8.84; found: C 61.84, H 4.60, N 20.04, P 8.39; the substance did not contain chlorine; IR (MeCN): $\bar{\nu}([\text{Ge}(\text{N}_3)_6]^{2-})=2083$ (vs) ($\nu_{\text{as}}(\text{N}_3)$, E_u and A_u), 1297 cm^{-1} (w) ($\nu_s(\text{N}_3)$, E_u and A_u); Raman (MeCN, RT; p = polarized, dp = depolarized): $\bar{\nu}([\text{Ge}(\text{N}_3)_6]^{2-})=2113$ (m, p) ($\nu_{\text{as}}(\text{N}_3)$, A_g), 2082 (w, dp) ($\nu_{\text{as}}(\text{N}_3)$, E_g), 1307 (m, p) ($\nu_s(\text{N}_3)$, A_g), 1085 (m, dp), 414 cm^{-1} (vs, p) ($\nu(\text{Ge-N})$, A_g); ^1H NMR (CD_3CN): $\delta=7.44\text{--}7.50$ (m, 24H, *m*-H, Ph), $7.54\text{--}7.68$ (m, 36H, *o*- and *p*-H, Ph); $^{13}\text{C}\{^1\text{H}\}$ NMR (CD_3CN): $\delta=128.2$, 130.4 , 133.3 , 134.6 (each m, Ph); $^{31}\text{P}\{^1\text{H}\}$ NMR (CD_3CN): $\delta=22.3$ (s); ^{73}Ge NMR (CH_3CN): $\delta=-290.6$ (s, $\Delta\nu_{1/2}=15\text{ Hz}$); ^{14}N NMR (CH_3CN): $\delta=-208.0$ (s, N_γ), -288.9 (s, N_α).

3a: Yield 50% (from GeCl_4), colorless crystals, m.p. 197°C ; 273°C (detonation); elemental analysis calcd (%) for $\text{C}_{10}\text{H}_8\text{GeN}_{14}$ (396.88): C 30.26, H 2.03, N 49.41; found: C 30.22, H 2.61, N 49.14; the substance did not contain chlorine; selected spectroscopic data: IR (MeCN): $\bar{\nu}=2120$ (s), 2097 (s, sh), 2091 (vs) ($\nu_{\text{as}}(\text{N}_3)$), 1607 cm^{-1} (w) ($\nu_{\text{ring}}(\text{bipy})$); ^1H NMR ($[\text{D}_8]\text{THF}$): $\delta=8.06$ (ddd, 2H, H5), 8.52 (ddd, 2H, H4), 8.81 (ddd, 2H, H3), 9.35 (ddd, 2H, H6); $^{13}\text{C}\{^1\text{H}\}$ NMR ($[\text{D}_8]\text{THF}$): $\delta=124.2$, 128.9 , 142.7 , 144.4 , 146.0 (each s, bipy); EI-MS (70 eV): m/z (%): 242 (1) [$M^+ - \text{bipy}$], 200 (22) [$M^+ - \text{bipy} - \text{N}_3$], 156 (100) [bipy^+], 116 (20) [$M^+ - \text{bipy} - 3\text{N}_3$], 88 (8) [$M^+ - \text{bipy} - 3\text{N}_3 - \text{N}_2$], 74 (11) [Ge^+].

3b·0.5 MeCN: Yield 45% (from GeCl_4), colorless needles, $125\text{--}130^\circ\text{C}$ (crepitation), m.p. 205°C (decomp); elemental analysis calcd (%) for $\text{C}_{13}\text{H}_{9.5}\text{GeN}_{14.5}$ (441.41): C 35.37, H 2.17, N 46.01; found: C 35.55, H 2.41, N 45.67; the substance did not contain chlorine; selected spectroscopic data: IR (MeCN): $\bar{\nu}=2120$ (vs), 2093 (vs, br) ($\nu_{\text{as}}(\text{N}_3)$), 1523 cm^{-1} (w) ($\nu_{\text{ring}}(\text{ophen})$); ^1H NMR ($[\text{D}_8]\text{THF}$): $\delta=8.34$ (dd, 2H, H3/H8), 8.41 (s, 2H, H5/H6), 9.08 (dd, 2H, H4/H7), 9.59 (dd, 2H, H2/H9); $^{13}\text{C}\{^1\text{H}\}$ NMR ($[\text{D}_8]\text{THF}$): $\delta=126.9$, 128.5 , 130.7 , 134.3 , 142.9 , 146.6 (each s, ophen); EI-MS (70 eV): m/z (%): 242 (1) [$M^+ - \text{ophen}$], 200 (25) [$M^+ - \text{ophen} - \text{N}_3$], 180 (100) [ophen^+], 116 (21) [$M^+ - \text{ophen} - 3\text{N}_3$], 88 (9) [$M^+ - \text{ophen} - 3\text{N}_3 - \text{N}_2$], 74 (16) [Ge^+].

4: Yield 66% (from **1a**), light yellow powder, m.p. 229°C (decomp); elemental analysis calcd (%) for $\text{C}_{22}\text{H}_{45}\text{CoGeN}_5\text{O}_5\text{P}_3$ (804.12): C 32.86, H 5.64, N 15.68; found: C 32.83, H 5.50, N 15.14; IR (CH_2Cl_2): $\bar{\nu}=2112$ (m), 2093 cm^{-1} (vs) ($\nu_{\text{as}}(\text{N}_3)$); ^1H NMR (CD_2Cl_2): $\delta=1.31$ (t, $^3J(\text{H,H})=7.1\text{ Hz}$, 18 H , $6 \times \text{CH}_3$), 1.71 (q, $^4J(\text{P,H})=2.4\text{ Hz}$, 15 H , C_5Me_3), 4.22 (m, 12 H , $6 \times \text{CH}_2$); $^{31}\text{P}\{^1\text{H}\}$ NMR (CD_2Cl_2): $\delta=116.7$ (s); $^{13}\text{C}\{^1\text{H}\}$ NMR (CD_2Cl_2): $\delta=10.1$ (s, C_5Me_3), 16.2 (pseudo-q, $^3J(\text{P,C})=6.5\text{ Hz}$, CH_2CH_3), 63.3 (pseudo-q, $^2J(\text{P,C})=10.6\text{ Hz}$, CH_2CH_3), 102.5 (q, $^2J(\text{P,C})=1.5\text{ Hz}$, C_5Me_3).

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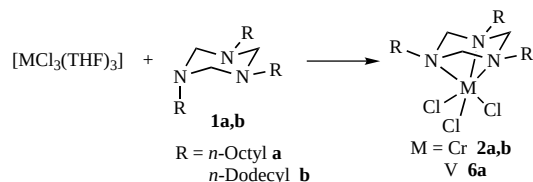
Selective Trimerization of α -Olefins with Triazacyclohexane Complexes of Chromium as Catalysts**

Randolf D. Köhn,* Matthias Haufe, Gabriele Kociok-Köhn, Siegfried Grimm, Peter Wasserscheid, and Wilhelm Keim

Dedicated to Professor Herbert Schumann on the occasion of his 65th birthday

$\text{CrO}_3/\text{SiO}_2$ -based heterogeneous Phillips catalysts^[1] for the polymerization of ethene produce a large fraction of the world production of high-density polyethylene (HDPE).^[2] Under certain conditions the $\text{CrO}_3/\text{SiO}_2$ -based catalysts show an unusual selectivity for the trimerization of ethene to 1-hexene and homogeneous systems based on a mixture of chromium complexes, pyrrols, and alkylaluminum compounds have been optimized to produce the trimer selectively.^[3, 4] Contrary to the Ziegler–Natta systems, very little is known about the mechanism and nature of the active species in these chromium systems although some model systems suggest a route via metallacycles.^[5, 6] Another homogeneous system $[\text{Cp}^*\text{TiMe}_3]/\text{B}(\text{C}_6\text{F}_5)_3$ ($\text{Cp}^* = \text{C}_5\text{Me}_5$) has recently been found that can also trimerize ethene and “cotrimerize” styrene with ethene.^[7] So far, none of these systems has been able to selectively trimerize α -olefins.

For several years we have been investigating the coordination chemistry of 1,3,5-triazacyclohexanes **1**. We were able to isolate CrCl_3 and alkylchromium(III) complexes as well as cationic alkylzinc complexes^[8a–e] with ligands having very small steric demand due to the small N-metal-N angle. The toluene-soluble *n*-octyl- and *n*-dodecyl-substituted complexes **2a, b** can be prepared according to Scheme 1.^[8e]



Scheme 1. Syntheses of the complexes **2** and **6**.

[*] Dr. R. D. Köhn
Department of Chemistry, University of Bath
Bath BA2 7AY (UK)
Fax: (+44) 1225-826231
E-mail: r.d.kohn@bath.ac.uk

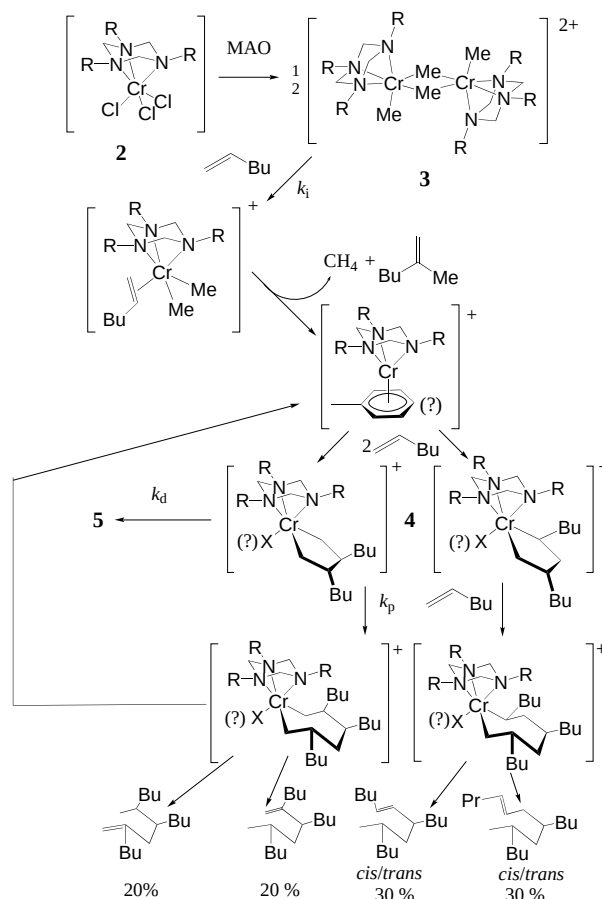
Dr. M. Haufe, Dr. G. Kociok-Köhn
Institut für Anorganische und Analytische Chemie
Technische Universität Berlin (Germany)
S. Grimm, Dr. P. Wasserscheid, Prof. Dr. W. Keim
Institut für Technische und Makromolekulare Chemie
RWTH Aachen (Germany)

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

Activation of **2** with methylaluminoxane (MAO) gives a yellow-green solution that can polymerize ethene and also shows some trimerization activity.^[8e] Now, we have found that α -olefins (propene, 1-hexene, and styrene) can be transformed in a new catalytic reaction selectively to a mixture of isomers of the trimers.^[9] At room temperature, the catalytic trimerization ceases after a few hours at up to 80% conversion (at maximal chromium concentration of approximately 10 mM) or about 1000 turnovers (in neat hexene). At 0 °C, this catalyst decomposition can be avoided and trimerization to >90% conversion is possible after several days in the refrigerator. The system is highly selective for α -olefins. Internal olefins (*cis* or *trans*) and conjugated dienes are not trimerized. Donor-functionalized olefins as well as donor solvents deactivate the system.

We have investigated the reaction with 1-hexene in more detail and our observations are in agreement with the mechanism shown in Scheme 2 which is similar to that



Scheme 2. Proposed mechanism for the trimerization and distribution of the major isomers.

proposed and supported by Briggs and Jolly.^[3, 5] Compound **2** is alkylated by MAO and a methyl/chloride anion is abstracted to give a cationic complex **3**. After insertion of the first olefin, β -H and reductive alkane elimination gives a Cr^{I} complex. Oxidative addition of two olefins gives the metallacyclic steady-state complex **4**. The rate-determining insertion of a third olefin molecule into one of the $\text{Cr}-\text{C}$ bonds of **4**

gives a metallacycloheptane which is unstable towards β -H and reductive alkane elimination to the Cr^{I} complex and finally **4** after oxidative addition of two more olefin molecules to complete the cycle. Compound **4** slowly decomposes to give an inactive complex **5**.

Besides unreacted 1-hexene the trimer can be isolated in high selectivity. The formation of the major isomers can be explained according to Scheme 2. Dimers and tetramers are found in less than 1%. Small amounts of methane and 2-methyl-1-hexene can be identified by NMR spectroscopy as the products of the initiation.

The homogeneous reaction can be monitored by UV/Vis and NMR spectroscopy. The UV/Vis spectrum of **2** (720 and 510 nm) changes to a new set of absorptions for **3** typical for Cr^{III} (at 600 and 430 nm) after addition of 100 equivalents of MAO. Compound **3** reacts within several minutes with added 1-hexene to give a new complex **4** (620 and 430 nm) which decomposes over several hours to give a complex **5** with absorptions at 550 and <400 nm.

The paramagnetism of the chromium complexes does not allow a useful observation of the complexes by ^1H or ^{13}C NMR spectroscopy. However, the ring-deuterated complex $[\text{D}_6]\text{-2a}$ is observable by ^2H NMR spectroscopy. η^3 -coordinated $[\text{D}_6]\text{-1a}$ shows a set of two signals of equal intensity for the two different deuterium atoms in the triazacyclohexane ring. The paramagnetically shifted and broadened signal pair at $\delta = 28$ and 5 for $[\text{D}_6]\text{-2a}$ moves to $\delta = 65$ and 5 for **3** after addition of 100 equivalents of MAO. Addition of 1-hexene gives a new signal pair at $\delta = 50$ and 5 for **4**. Then, a slow decomposition can be observed with the appearance of equal amounts of **5** with a signal pair at $\delta = 140$ and 5 and a diamagnetic triazacyclohexane **1** with a sharp signal at $\delta = 3.6$ indicating decomplexation.

The kinetics of the 1-hexene consumption can be determined by ^1H NMR spectroscopy and can be described by the rate law given in Equation (1) (based on Scheme 2), with the

$$\ln\{[\text{hexene}]/[\text{hexene}]_0\} = -3k_p[\text{Cr}]/k_d(1 - \exp(-k_d t)) \quad (1)$$

rate constant for the olefin insertion into **4** of $k_p = 1 \times 10^{-2} \text{ s}^{-1} \text{ M}^{-1}$ and the decomposition rate constant of $k_d = 2 \times 10^{-4} \text{ s}^{-1}$ at room temperature. Matching kinetics observed by the UV/Vis and NMR investigations of the complexes show that a triazacyclohexane complex **4** carries the trimerization and that the ligand is partially lost only upon decomposition.

During the NMR studies, the average magnetic moment can be determined by the Evans method.^[10] By using the concentrations of the individual chromium complexes from the kinetic investigations, the magnetic moments can be determined: $3.9 \mu_B$ for **2**, $3.1 \mu_B$ for **3**, $4.1 \mu_B$ for **4**, and $3.3 \mu_B$ for **5**. The magnetic moments for **2** and **4** are typical for mononuclear Cr^{III} complexes, while the reduced magnetic moments for **3** and **5** are in the range of bridged, dinuclear Cr^{III} complexes.

So far, there is only spectroscopic evidence for Cr^{III} complexes, and the Cr^{I} complex proposed in Scheme 2 is mostly based on the observation of the products of reductive elimination. The analogous V^{III} system **6a**/MAO also polymerizes ethene but does not react with 1-hexene under the

same conditions. Usually analogous V^{III} complexes show similar reactions when no redox reaction is involved. Additionally, the isomer distribution of the trimers depends on the substitution patterns of the arene solvent used and may indicate a direct involvement of the solvent.

X-ray crystallography on crystals of **2a** at 110 K allowed the structure determination without disorder in the octyl substituents (Figure 1).^[11]

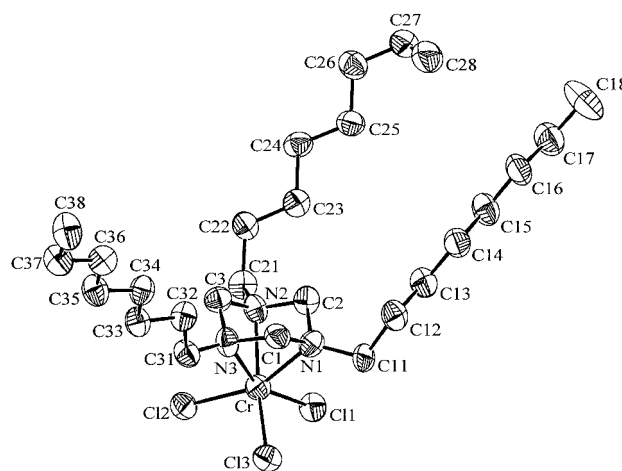


Figure 1. ZORTEP plot of the molecular structure of **2a** (thermal ellipsoids for 50% probability). Selected bond lengths [Å] and angles [°]: Cr–N1 2.095(7), Cr–Cl1 2.288(3), N1–Cr–N2 65.9(3), Cl1–Cr–Cl2 99.74(10), Cr–N1–C11 128.0(5), C1–N1–C11 114.6(7), C1–N1–C2 110.6(7).

The Cr–N bond length is at the lower limit of the range observed in other Cr^{III} –triazacyclohexane complexes (2.10–2.30 Å).^[8b,c, 12] This Cr–N bond is also shorter than that in an analogous tri-*n*-butyl-substituted 1,4,7-triazacyclononane complex (2.15 Å).^[13] This comparison shows that the much lower steric demand of triazacyclohexanes can overcome the electronic weakening of the bond caused by the nearly 30° mis-directed nitrogen lone pairs. Analogous methyl-substituted triazacyclononane or -dodecane complexes are not able to trimerize 1-hexene under the same conditions. This shows the importance of the low steric demand of the triazacyclohexane complexes for the reactivity towards α -olefins.

This unprecedented selective trimerization of α -olefins by the system **2**/MAO should allow access to new products from simple α -olefins. We are currently investigating the influence of the N substituents and the solvent on the isomer ratios.

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Identification of a New Sex Pheromone from the Silk Dragline of the Tropical Wandering Spider *Cupiennius salei***

Mirjam Papke, Stefan Schulz,* Harald Tichy, Ewald Gingl, and Rudolf Ehn

Dedicated to Professor Wittko Francke on the occasion of his 60th birthday

The chemistry of pheromones of arachnids and particularly spiders has not received the same attention as that of the pheromones of insects,^[1] despite the widespread distribution

of spiders, their importance in many ecosystems, and their species-rich taxon. The silk is known to play a role as substrate for pheromones in various spiders,^[2] but so far only one pheromone has been identified.^[3] The large tropical wandering spider *Cupiennius salei* (Ctenidae) uses its silk dragline as a chemical signpost, which it leaves behind in the environment. A single thread of a female initiates courtship behavior in conspecific males. The males recognize the inherent chemical signal by examining the silk thread with their pedipalps and send vibratory signals through the substrate which are returned by the female.^[4] In the current study we report on the identification and synthesis of the female courtship-inducing pheromone.

Silk can be obtained from the spiders by using an electrically driven reeling machine which draws the silk thread out of the silk glands of an immobilized, but awake spider. About 5–10 mg silk can be obtained in a week from a single individual. A bioassay was developed to guide in the identification of the active principle. In short, the spiders wandered along a glass duct covered with filter paper and encountered natural or chemically modified silk threads on their way. The characteristic vibratory courtship behavior could be elicited by silk samples from adult females.^[5] In contrast, silk from males or juvenile females was not active. Active silk was extracted with different solvents and the extracts were then applied on inactive silk from male spiders to test their activity. Methanol extracts showed good responses and were used for further analyses.

By use of GC-MS methods usually employed in pheromone research only methyl pyroglutamate could be identified, but both enantiomers did not evoke any response in the bioassay. We then analyzed extracts by NMR spectroscopy. Surprisingly, active extracts prepared with deuterated methanol showed the presence of only one prominent compound, which was absent in inactive silk samples (Figure 1). The spectrum

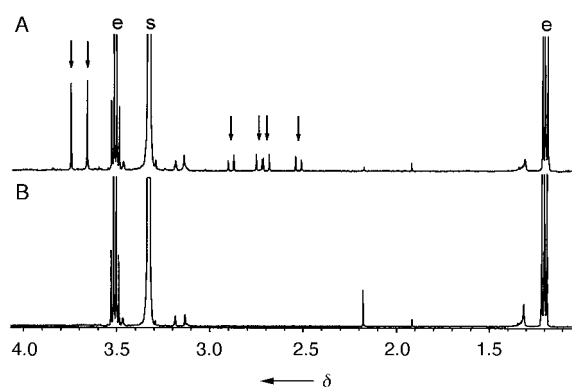


Figure 1. ¹H NMR spectrum of a silk extract (CD₃OD) from adult female (A) and male (B) *Cupiennius salei* spiders. Arrows indicate characteristic signals; e: ethanol (contaminant), s: solvent.

showed two different CH₃ groups and two different CH₂ groups, each of them with diastereotopic H atoms. The spectrum is consistent with the structure of the asymmetric dimethyl citrate **1**. This ester, to the best of our knowledge not known from nature before, was then synthesized as racemate to confirm the assignment.^[6]

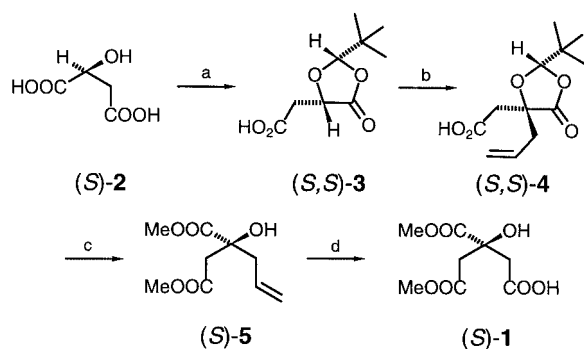
[*] Prof. Dr. S. Schulz, Dr. M. Papke
Institut für Organische Chemie
Technische Universität Braunschweig
Hagenring 30, 38106 Braunschweig (Germany)
Fax: (+49) 531-391-5272
E-mail: stefan.schulz@tu-bs.de
Prof. Dr. H. Tichy, Mag. E. Gingl, Dr. R. Ehn
Institut für Zoologie
Universität Wien
Althanstrasse 14, 1090 Vienna (Austria)

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Comparison of the spectra of the synthetic asymmetric dimethyl citrate and the natural compound revealed some differences in chemical shift values of the CH₂ groups. Because these values depend on the pH value and owing to the presence of ions in the solution, mixing experiments were performed. A natural sample was blended with the same amount of synthetic material, using benzene as internal standard. The NMR spectrum showed only one set of signals, thus confirming that the natural compound is indeed the asymmetric dimethyl citrate **1**.

Both enantiomers were then synthesized for assignment of the absolute configuration of the natural compound (Scheme 1). Both (*R*)- and (*S*)-malic acids **2** were converted with pivalaldehyde into the respective dioxolanones **3**, which



Scheme 1. Synthesis of (*S*)-cupilure (*S*)-**1**. a) H⁺, pivalaldehyde; b) LiHMDS, then CH₂=CH-CH₂Br; c) 5 equiv BF₃·Et₂O, MeOH; d) RuCl₃, NaIO₄, CCl₄, MeCN, H₂O. HMDS = hexamethyldisilazane.

were subsequently alkylated with allyl bromide, according to the method developed by Seebach et al.^[7] The resulting allyl dioxolanones **4** exhibited a high enantiomeric ratio in excess of 96:4. Treatment with a large excess of BF₃·OEt₂ in methanol cleaved the acetal and esterified the acid groups in one step. Final oxidative cleavage of the double bond in **5** with RuO₄^[8] yielded the desired enantiomers (*R*)- and (*S*)-**1** in a high enantiomeric ratio (96:4).

Comparison of the synthetic samples with the natural compound by gas chromatography on a chiral phase proved the predominant *S* configuration (e.r. = 95:5) of the natural ester (Figure 2). Separation was possible only after transformation of **1** into its isopropyl ester **6** with isopropyl alcohol/acetyl chloride. The conditions of the derivatization did not alter the enantiomeric ratio of the compounds. Only the cyclodextrin phase heptakis(6-*O*-TBDMS-2,3-di-*O*-acetyl)-β-cyclodextrin (in 50% OV1701)^[9] was able to separate the racemate (TBDMS = *tert*-butyldimethylsilyl), while several other phases tested failed.

Behavioral experiments with the synthetic material applied to inactive silk samples proved that (*S*)-**1** stimulates male courtship behavior, while the respective *R* enantiomer is inactive.^[5] The synthetic (*S*)-**1** enabled for the first time the electrophysiological identification of a pheromone sensilla in a spider.^[5] These sensilla are contact chemoreceptors located on the pedipalps. Single-cell recordings were made by inserting an electrolytically sharpened tungsten wire into the shaft of the sensillum; the second electrode was introduced into the tip of the pedipalpus. The area of a single sensillum

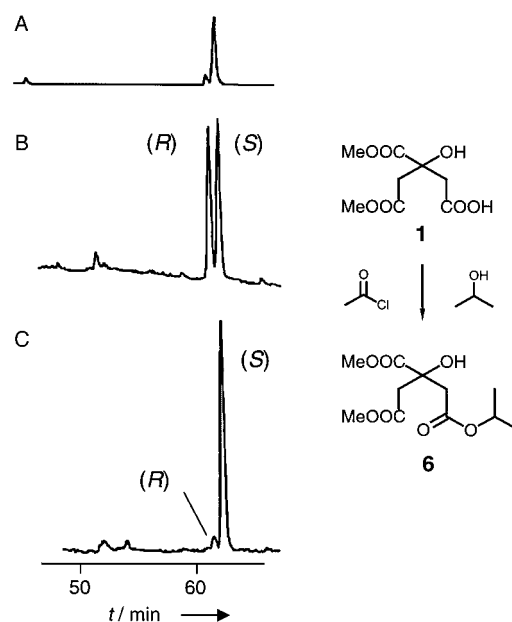


Figure 2. Gas chromatographic separation of dimethyl citrate **1** on a chiral phase (25 m 50% heptakis(6-*O*-TBDMS-2,3-di-*O*-acetyl)-β-cyclodextrin and 50% OV1701,^[10] 110 °C isothermal) after transformation into the respective isopropyl esters **6**. Synthetic (*S*)-**1** (A), *rac*-**1** (B), and natural silk extract containing **1** (C). The stereochemical designators refer to the parent compound **1**.

that contacts the silk dragline is 0.03 μm². This area contains at most 170 000 pheromone molecules, as we extracted a maximum of 1 μg pheromone from 20 m dragline silk.

Our results from chemical analysis, from electrophysiology of pheromone-receptive sensilla, and from the behavioral assay clearly demonstrate that **1**, for which we propose the name cupilure, is a contact sex pheromone bound to the silk of female *Cupiennius salei* spiders. Cupilure is a unique compound, structurally not related to known pheromones. The only other contact spider pheromone identified so far is (*R,R*)-3-(3-hydroxybutyryloxy)butyric acid, which induces web reduction by the males of *Linyphia triangularis*.^[3] Both compounds are biosynthetically very close to the primary metabolism: cupilure can be easily formed by double methylation of ubiquitous citric acid, while the *Linyphia* compound is a simple condensation product of (*R*)-3-hydroxybutyric acid, an important intermediate of the fatty acid biosynthesis. Such close links to primary metabolites are not known from insect pheromones. Whether this is a general feature of spider pheromones, which reflects their carnivorous life style, will require further studies. Recently we have identified a volatile spider pheromone, 8-methyl-2-nonanone, which resembles known insect pheromones.^[10]

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Analysis of the Silkworm Moth Pheromone Binding Protein–Pheromone Complex by Electrospray-Ionization Mass Spectrometry

Neil J. Oldham,* Jürgen Krieger, Heinz Breer, Annette Fishedick, Michal Hoskovec, and Aleš Svatoš

In the four decades since Butenandt et al. identified bombykol ((10*E*,12*Z*)-hexadeca-10,12-dien-1-ol) as the female sex attractant of the silkworm moth, *Bombyx mori*,^[1] considerable progress has been made in our understanding of pheromone chemistry and biology. Throughout this time, the *B. mori* system has continued to serve as a useful model for unraveling the intricacies of chemical communication. Indeed, a complete picture is slowly emerging, from bombykol biosynthesis and regulation in females to olfactory detection and catabolism in male antennae.^[2] The study of mechanisms involved in pheromone detection is particularly active, and has produced a detailed model of events at the molecular

level. Lipophilic pheromone molecules enter the antennal sensilla (sensory hairs) through cuticular pores, where they come into contact with the aqueous lymph medium that surrounds the receptor (nerve) cells. Here they are solubilized by a pheromone binding protein (PBP; a small (<20 kDa), water-soluble lipid binding protein),^[3] and transported through the lymph to the nerve cell membrane. The pheromone receptor itself resides in this membrane, and there is strong evidence that it is G-protein-coupled.^[4] Activation of the receptor, therefore, stimulates a secondary messenger cascade and ion-channel opening, thereby generating a receptor potential.

Progress towards total structural characterization of the *B. mori* PBP (BmPBP) has been rapid, from production of cDNA clones and deduction of the amino acid sequence,^[5] to determination of the disulfide bridging pattern,^[6] culminating in the elucidation of the X-ray crystal structure of BmPBP complexed with bombykol.^[7] In an attempt to probe the interaction between pheromone and binding protein further, we examined the system by electrospray-ionization mass spectrometry (ESI-MS) under nondenaturing conditions. By careful instrumental optimization, use of near-neutral spraying buffer, and low desolvation gas temperature (30 °C), it is possible to observe a variety of noncovalently bound protein–ligand complexes in the gas phase.^[8] Examples of bound ligands seen using this method include enzyme cofactors,^[9] substrates,^[10] and inhibitors.^[11]

We postulated that it should be possible to extend this approach to the BmPBP–bombykol system, and thereby observe the noncovalent complex in the gas phase. Figure 1 shows the (transformed) native ESI mass spectrum of recombinant BmPBP alone, and the spectrum after exposure of BmPBP to bombykol (20 molar equivalents, overnight).^[12] The largest peak in the upper trace (Figure 1a) displayed a mass of 15878 Da, a value identical to the theoretical mass deduced from the amino acid sequence of BmPBP, and to the measured mass using standard (denaturing) ESI-MS conditions (spraying solvent: water/acetonitrile (80/20) with 0.1 % formic acid; data not shown). A second significant peak, at 16160 Da, only appeared under nondenaturing conditions and, therefore, was attributed to a noncovalent adduct of the BmPBP with an unknown contaminant of mass 282 Da. The impurity was probably introduced during expression, or purification, of the recombinant protein. It is interesting to note that oleic acid has a mass of 282 Da, and treatment of the PBP with an excess of this common lipid did lead to a significant increase in the 16160 Da peak. To date, however, we have not been able to confirm oleic acid as the contaminant.

When BmPBP was incubated with bombykol, a third ion was observed in the ESI mass spectrum (Figure 1b). In addition to the masses at 15878 (BmPBP) and 16160 Da (unknown BmPBP adduct), a large peak at 16116 Da was visible. This species was reproducibly 238 Da (=the molecular mass of bombykol) larger than the mass of BmPBP and was identified as the noncovalent BmPBP–bombykol complex [BmPBP+bombykol]. Lower molar excesses of bombykol produced a similar result (minimum excess measured was 5-fold).

[*] Dr. N. J. Oldham

Max-Planck-Institut für Chemische Ökologie
Carl-Zeiss-Promenade 10, 07745 Jena (Germany)
Fax: (+49) 3641-643-665
E-mail: oldham@ice.mpg.de

Dr. J. Krieger, Prof. Dr. H. Breer, A. Fishedick
Institut für Physiologie
Universität Stuttgart-Hohenheim
Garbenstrasse 30, 70593 Stuttgart (Germany)

Dr. M. Hoskovec, Dr. A. Svatoš
Ústav organické chemie a biochemie
Akademie věd České Republiky
Flemingovo nám. 2, Prag 6-Dejvice, 16610 (Czech Republic)

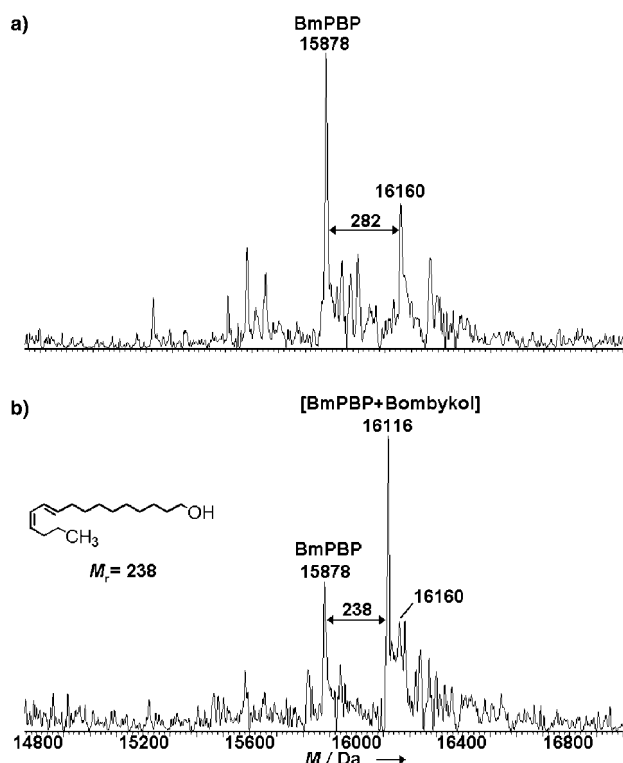


Figure 1. Transformed ESI mass spectrum of: a) BmPBP alone and b) BmPBP after exposure to bombykol. Data clearly show the presence of a [BmPBP+bombykol] complex at 16116 Da.^[12]

Figure 2 shows the result of varying the electrospray cone voltage V , the drying gas temperature T , and the pH value of the spraying buffer on the ratio of [BmPBP+bombykol] complex to free BmPBP. The electrospray sample cone voltage, which directly controls the acceleration of ions through the atmospheric pressure source into the high vacuum of the mass spectrometer, was found to be a critical parameter for observation of the noncovalent complex. Due to the effect of atmospheric-pressure collision processes, very little [BmPBP+bombykol] complex was observed at a cone voltage above 30 V. The temperature of the drying gas (nitrogen), used to evaporate the electrospray solvent droplets, also had an effect on the stability of the complex. Temperatures above 50 °C resulted in a rapid drop in the ratio of bound to free BmPBP, presumably caused by thermal denaturation of the protein. The spraying-buffer pH value exhibited a distinct optimum between pH 6.5 and 7.0, and the dramatic decrease in intact [BmPBP+bombykol] complex below pH 6.0 is consistent with the observed loss of the binding protein's rigid tertiary structure at low pH values.^[13]

One potentially very useful application of this work is in studying the specificity of pheromone (or general odorant) binding proteins. Figure 3 shows that BmPBP does bind ligands that are structurally related to bombykol, such as (11*E*)- and (11*Z*)-hexadec-11-en-1-ol, but no evidence of a complex with the structurally unrelated alcohol 3-phenylpropan-1-ol was seen.^[14] In addition, not only was it possible to distinguish between the ligand binding efficiencies of the monoene alcohols and bombykol, but even the geometric isomers of 11-hexadecenol gave significantly different

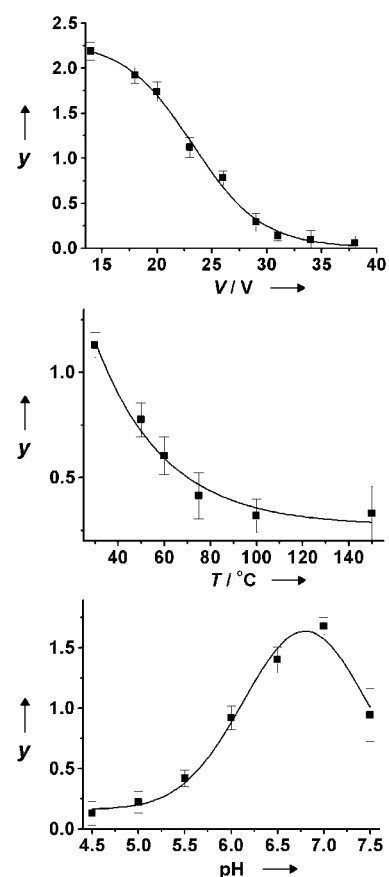


Figure 2. Effect of electrospray source parameters (cone voltage V , drying gas temperature T , and the pH value of the spraying buffer) on the stability of the [BmPBP+bombykol] complex (y-axis shows ratio of [BmPBP+bombykol] complex to free BmPBP).^[12] Each data point is the mean value from five repeat injections (standard errors are indicated by the bars).

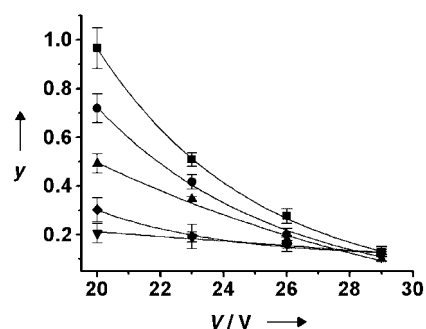


Figure 3. Comparison of BmPBP binding efficiencies with a range of ligands: ■ = bombykol, ● = (11*E*)-hexadec-11-en-1-ol, ▲ = (11*Z*)-hexadec-11-en-1-ol, ◆ = control, ▼ = 3-phenylpropan-1-ol.^[14] The y-axis shows the ratio of [BmPBP+ligand] complex to free BmPBP, V = cone voltage. Each data point is the mean value from 6–8 repeat injections (standard errors are indicated by the bars).

[BmPBP+ligand]:BmPBP ratios. Figure 3 illustrates that the 11*E* monoene appeared to bind better to the BmPBP than the 11*Z* form. Intriguingly, X-ray crystal structure data show that the C11–C12 bond of bound bombykol adopts a *transoid* conformation.^[7]

The nondenaturing ESI-MS technique is a potentially powerful tool for assessment of the specificity of interactions between odorant molecules and olfactory binding proteins.

Plans exist to extend this methodology to develop a competitive binding assay.

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Chemo-, Regio-, and Stereoselective Cyclization of 1,3-Bis(trimethylsilyloxy)-1,3-butadienes with Functionalized Epoxides**

Peter Langer* and Tobias Eckardt

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- [12] Mass spectra were recorded on a Micromass Quattro II mass spectrometer (Micromass, Manchester, UK) fitted with a Z-Spray electrospray source. NH_4OAc (2.5 mM, pH 7.0 unless otherwise stated) was used as a spraying buffer at a flow rate of 5 $\mu\text{L min}^{-1}$. The BmPBP solution (5 mg mL^{-1} , 2 μL , in 2.5 mM NH_4OAc , pH 7.0) was introduced into the flow using an injection valve. The cone voltage was 20 V and the drying gas temperature was 30 °C, unless otherwise stated. Recombinant BmPBP was produced and purified as previously described, except the hydroxylapatite column step was replaced by dialysis against 10 mM tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8.0) with a Slide-A-lyzer cassette (Pierce, Rockford, IL, USA).^[5, 13] BmPBP was concentrated and transferred into NH_4OAc buffer (see above) with a Vivaspin concentrator (molecular weight cut-off 5000 Da; Vivascience, Lincoln, UK). Data was analyzed with MassLynx 3.1 (Micromass) software which had MaxEnt (maximum entropy based) software embedded.
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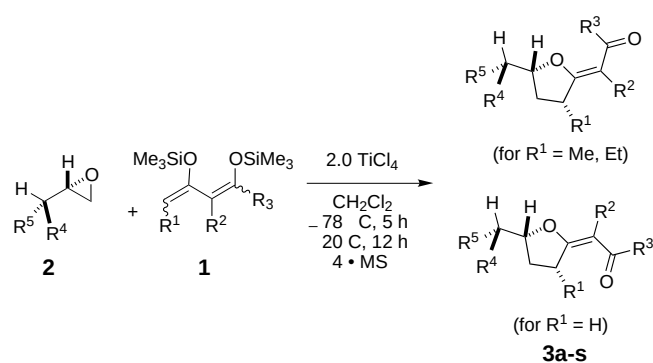
Domino reactions are of interest in organic chemistry since they enable the rapid assembly of complex products in a one-pot process.^[1] Despite the simplicity of the idea, only few reactions of 1,3-dianions and 1,3-dianion equivalents with 1,2-dielectrophiles have been reported so far.^[2] Several drawbacks hinder these reactions: on the one hand, dianions are highly reactive compounds that can react both as a nucleophile and a base; on the other hand, 1,2-dielectrophiles often represent rather labile compounds, which can undergo a series of side reactions (formation of open-chain 2:1 products, single-electron transfer (SET) reactions, elimination, polymerization, decomposition, fragmentation). In the course of work on the development of domino reactions of dianions and dianion equivalents,^[3] we recently developed the first cyclization reaction of dilithiated 1,3-dicarbonyl compounds with oxalic acid dielectrophiles.^[4] These reactions provide an efficient, regio- and stereoselective route to the pharmacologically important class of γ -alkylidenebutenolides.

Herein, we report, to our knowledge, the first Lewis acid mediated cyclizations of 1,3-bis(trimethylsilyloxy)-1,3-butadienes, electroneutral equivalents of 1,3-dicarbonyl dianions,^[5] with epoxides. These reactions allow, for the first time, a highly efficient and chemoselective synthesis of 2-alkylidenetetrahydrofurans with a great variety of substitution patterns and functional groups.^[6, 7] The cyclizations not only proceed with very good chemo-, but also with very good regio- and stereoselectivities. The products are useful precursors for the synthesis of pharmacologically relevant tetrahydrofuran derivatives and natural products.^[8] The preparative usefulness of the new cyclization reaction was demonstrated by the synthesis of methyl nonactate, a known precursor to the natural product nonactin.

Our first attempts to induce a cyclization reaction of propenoxide **2a** with 1,3-bis(trimethylsilyloxy)-1,3-butadiene **1a**, which was prepared in two steps from ethyl acetoacetate,^[5c] were unsuccessful (Scheme 1, Table 1). The use of $\text{BF}_3 \cdot \text{OEt}_2$ as the Lewis acid resulted in formation of a complex reaction mixture. Only starting materials were isolated when trimethylsilyl trifluoromethanesulfonate ($\text{Me}_3\text{-SiOTf}$) was employed. Equally disappointing results were obtained when the reaction was carried out at 20 °C in the presence of ZnCl_2 . A complex mixture was obtained when the reaction was carried out at 0 → 20 °C using TiCl_4 as the Lewis acid (Table 1, entry 4).

[*] Dr. P. Langer, T. Eckardt
Institut für Organische Chemie
Georg-August-Universität Göttingen
Tammannstrasse 2, 37077 Göttingen (Germany)
Fax: (+49) 551-399475
E-mail: planger@uni-goettingen.de

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Scheme 1. Cyclization of epoxides **2** with 1,3-bis(trimethylsilyloxy)-1,3-dienes **1**.

Table 1. Optimization of the reaction of epoxide **2a** with diene **1a**.

Entry	Lewis acid (equiv)	2a [equiv]	<i>t</i> [h] ^[a]	Yield [%] ^[b]
1	BF ₃ ·Et ₂ O (2.0)	1	5 + 12	0
2	Me ₃ SiOTf (2.0)	1	5 + 12	0
3	ZnCl ₂ (2.0)	1	0 + 12	0
4	TiCl ₄ (2.0)	1	0 + 12 ^[c]	0
5	TiCl ₄ (2.0)	1	5 + 0	12
6	TiCl ₄ (2.0)	1	5 + 5	57
7	TiCl ₄ (2.0)	1	5 + 12	70
8	TiCl ₄ (2.0)	1.5	5 + 12	62
9	TiCl ₄ (1.0)	1.0	5 + 12	24

[a] Reaction time at $-78 \rightarrow 20^\circ\text{C}$ + reaction time at 20°C . [b] Yield of isolated product. [c] The reaction was started at 0°C .

The reaction of diene **1a** with epoxide **2a** at $-78 \rightarrow 20^\circ\text{C}$ (5 h) gave 2-alkylidenetetrahydrofuran **3a** in low yield (Table 1, entry 5). After much experimentation (Table 1), optimal yields (up to 70%) were obtained when the reaction was stirred at $-78 \rightarrow 20^\circ\text{C}$ for 5 h and subsequently at 20°C for 12 h and when 1.0 equivalent of the epoxide and 2.0 equivalents of TiCl₄ were used (Table 1, entry 8). The reaction proceeded by attack of the terminal carbon atom of the diene on the sterically less hindered carbon atom of the epoxide and subsequent regioselective cyclization at the oxygen atom (vide infra). Interestingly, excellent selectivity was observed in favor of the product containing an *E*-configured exocyclic double bond.

The preparative scope of the new cyclization reaction was investigated by systematically varying the substituents of the 1,3-bis(trimethylsilyloxy)-1,3-diene and the epoxide (Scheme 1, Table 2). Reaction of diene **1a** with 1,2-butenoxide (**2b**) and 1-benzyloxy-2,3-propenoxide (**2c**) afforded the *E*-configured 2-alkylidenetetrahydrofurans **3b** and **3c**, respectively, in good yields and with very good stereoselectivities. Reaction of **1a** with 1-chloro- and 1-bromo-2,3-propenoxide (**2d** and **2e**) afforded the *E*-configured 2-alkylidenetetrahydrofurans **3d** and **3e**, respectively, in good yields and with very good chemo-, regio-, and *E*-selectivities. Reaction of diene **1a** with *threo*-3-bromo-1,2-butenoxide **2f** afforded the diastereomerically pure 2-alkylidenetetrahydrofuran **3f** in good yield and with very good *E*-selectivity. No epimerization could be detected in the side chain attached to carbon C5. Reaction of diene **1a** with ethyl 3,4-epoxy-3-butenate (**2g**) and ethyl 4,5-epoxy-4-pentenoate (**2h**) af-

Table 2. Synthesis of 2-alkylidenetetrahydrofurans **3a–s**.

1	2	3	R ¹	R ²	R ³	R ⁴	R ⁵	Yield [%] ^[a,b]	<i>E</i> : <i>Z</i>
a	a	a	H	H	OEt	H	H	70	> 98:2
a	b	b	H	H	OEt	Me	H	62	> 98:2
a	c	c	H	H	OEt	OBn	H	58	> 98:2
a	d	d	H	H	OEt	Cl	H	52	> 98:2
a	e	e	H	H	OEt	Br	H	48	> 98:2
a	f	f	H	H	OEt	Br	Me	41	> 98:2
a	g	g	H	H	OEt	CO ₂ Et	H	50	> 98:2
a	h	h	H	H	OEt	CH ₂ CO ₂ Et	H	51	> 98:2
b	b	i	H	Me	OEt	Cl	H	45	> 98:2
c	e	j	H	Et	OEt	Br	H	50	> 98:2
b	g	k	H	Me	OEt	CO ₂ Et	H	54	> 98:2
d	b	l	Me	H	OMe	Me	H	46	> 98:2
e	b	m	Et	H	OMe	Me	H	50	> 98:2
f	b	n	H	H	Ph	Me	H	62	> 98:2
f	d	o	H	H	Ph	Cl	H	51	> 98:2
f	e	p	H	H	Ph	Br	H	44	> 98:2
f	f	q	H	H	Ph	CO ₂ Et	H	41	> 98:2
g	g	r	H	H	CH ₂ OMe	Me	H	51	> 98:2
g	d	s	H	H	CH ₂ OMe	Cl	H	45	> 98:2

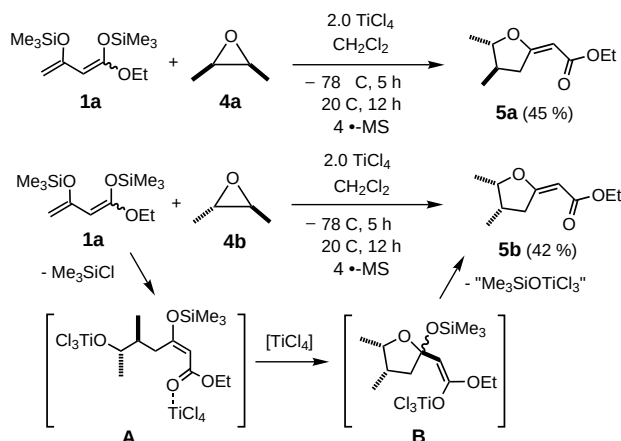
[a] Yield of isolated product. [b] The diastereoselectivity observed for **3f** was > 98:2 in favor of the isomer drawn in Scheme 1. The diastereoselectivity observed for **3l** and **3m** was 4:1.

furnished the 2-alkylidenetetrahydrofurans **3g** and **3h** containing ester groups in the side chain. It is noteworthy that reaction of the dianion of ethyl acetoacetate with epoxides **2d–f** resulted in attack of the dianion on the carbon attached to the halogen atom rather than at the epoxide.^[9] Reaction of the dianion with the ester-substituted epoxides **2g** and **2h** gave complex mixtures only. Reaction of 1-chloro-2,3-propenoxide with 1,3-bis(trimethylsilyloxy)-1,3-diene **1b**, containing a methyl group at the central carbon atom, afforded the 2-alkylidenetetrahydrofuran **3i** with very good chemo-, regio-, and *E*-selectivity. Reaction of dienes **1b** and **1c** with 1-bromo-2,3-propenoxide (**2e**) and ethyl 3,4-epoxy-3-butenate (**2g**), both containing base-labile functional groups, afforded the bromo- and ester-substituted 2-alkylidenetetrahydrofurans **3j** and **3k**, respectively, in good yields and with very good regio- and *E*-selectivities.

The reaction of butenoxide **2b** with the dienes **1d** and **1e**, containing a methyl and an ethyl group at the terminal carbon atom, respectively, afforded the *Z*-configured 2-alkylidenetetrahydrofurans **3l** and **3m**, respectively, in good yields and with good 1,3-diastereoselectivities. The change of the geometry of the exocyclic double bonds from *E*- to *Z*-configuration can be explained by the steric influence of the substituents R¹. Reaction of the benzoylacetone derived diene **1f** with 1,2-butenoxide (**2b**) afforded the 2-alkylidenetetrahydrofuran **3n** in good yield and with very good chemo-, regio-, and *E*-selectivity. Reaction of diene **1f** with epoxides **2d–e** and **2g** afforded the chloro-, bromo-, and ester-substituted 2-alkylidenetetrahydrofurans **3o–q** in good yields and with very good regio- and *E*-selectivities. Reaction of 1,2-butenoxide and 1-chloro-2,3-propenoxide with 1,3-bis(trimethylsilyloxy)-1,3-diene **1g**, derived from 5-methoxy-2,4-pentanedione, afforded the 2-alkylidenetetrahydrofurans **3r** and **3s**, respectively, with very good chemo-, regio-, and *E*-selectivity.

To obtain some insight into the mechanism of the reaction, the reactions of 1,3-bis(trimethylsilyloxy)-1,3-diene **1a** with

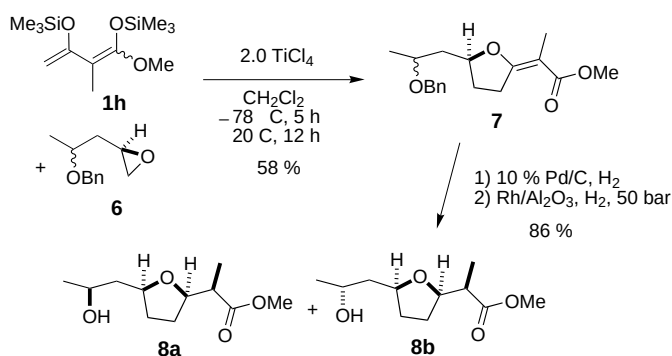
the 1,2-disubstituted epoxides **4a** and **4b** were studied (Scheme 2): reaction of **1a** with *cis*-2,3-butenoxide **4a** afforded the *E*-configured 2-alkylidenetetrahydrofuran **5a** containing two *trans*-configured methyl groups in good yield. In contrast, reaction of **1a** with *trans*-2,3-butenoxide **4b** gave the 2-alkylidenetetrahydrofuran **5b** containing two *cis*-configured methyl groups. The observed stereoselectivity suggests that formation of 2-alkylidenetetrahydrofurans **3a–s** and **5a, b** can be explained by the following working hypothesis:



Scheme 2. Cyclization of epoxides **4a, b** with 1,3-bis(trimethylsilyloxy)-1,3-diene **1a**.

regioselective attack of the terminal carbon atom of the diene on the epoxide affords intermediate **A** with inversion of the configuration. The retention of the configuration of the carbon atom attached to the oxygen atom suggests that the cyclization proceeds by TiCl_4 -mediated attack of the epoxide-derived hydroxy group on the α,β -unsaturated ester to give intermediate **B**.^[10] In contrast, attack of the silyl enol ether derived oxygen atom of intermediate **A** on the hydroxy group would have resulted in inversion of the configuration. Elimination of silanolate subsequently leads to the final product. The presence of the Lewis acid seems to be important for both the initial condensation step and the subsequent cyclization. The stereoselectivity in favor of the products containing *E*-configured exocyclic double bonds can be explained by the W-shaped configuration of intermediate **A** which allows a minimization of the dipole–dipole repulsion of the oxygen atoms.^[11]

To demonstrate the preparative usefulness of our new cyclization we have studied its application to the synthesis of methyl nonactate, a precursor to the natural product nonactin.^[7, 8a, 12] This compound belongs to the class of macro-tetrolide antibiotics (nactins) isolated from a variety of *Streptomyces* cultures.^[13] Reaction of the 1,3-bis(trimethylsilyloxy)-1,3-butadiene **1h** with epoxide **6** (1:1 diastereomeric mixture) afforded the 2-alkylidenetetrahydrofuran **7** in 58% yield (Scheme 3). Deprotection and subsequent stereoselective hydrogenation^[12c] afforded, following a known protocol,^[7c] methyl nonactate **8a** and methyl 8-*epi*-nonactate **8b** as a diastereomeric mixture (1:1) in 86% yield.



Scheme 3. Synthesis of methyl nonactate and methyl 8-*epi*-nonactate.

The first cyclization reactions of 1,3-bis(trimethylsilyloxy)-1,3-butadienes—electroneutral equivalents of 1,3-dicarbonyl dianions—with epoxides reported herein provide an efficient, chemo-, regio-, and *E*-selective synthesis of a variety of functionalized 2-alkylidenetetrahydrofurans that are useful precursors for the synthesis of pharmacologically relevant tetrahydrofuran derivatives and natural products.

Experimental Section

3f: TiCl_4 (2 mmol, 0.38 g) in CH_2Cl_2 (5 mL) was added at -78°C to a solution of *threo*-3-bromo-1,2-epoxybutane (1.5 mmol, 0.23 g), 1,3-bis(trimethylsilyloxy)-1,3-diene **1a** (1.5 mmol, 0.40 g), and molecular sieves (4 Å) in CH_2Cl_2 (30 mL). The temperature of the reaction mixture was allowed to rise to 20°C over 5 h. After the mixture had been stirred for 12 h at 20°C , a saturated solution of NaCl was added, the organic layer was separated, and the aqueous layer was repeatedly extracted with diethyl ether. The combined organic extracts were dried (MgSO_4), filtered, and the solvent of the filtrate was removed in vacuo. The residue was purified by column chromatography (silica gel, diethyl ether/petroleum ether 1/10 $\rightarrow$ 1/3) to give **3f** as a colorless oil. ^1H NMR (CDCl_3 , 200 MHz): δ = 1.18 (t, 3 H, J = 7 Hz; CH_2CH_3), 1.70 (d, 3 H, J = 8 Hz; CHCH_3), 1.97, 2.30 (2 $\times$ m, 2 $\times$ 1 H; CH_2), 2.98, 3.30 (2 $\times$ m, 2 $\times$ 1 H; CH_2), 4.05 (q, 2 H, J = 7 Hz; OCH_2), 4.35, 4.58 (2 $\times$ m, 2 $\times$ 1 H; 2 $\times$ CH), 5.21 (t, 1 H, J = 1 Hz; =CH); ^{13}C NMR (CDCl_3 , 50 MHz): δ_c = 13.94, 21.42, 26.80, 30.29, 51.06, 58.58, 86.43, 89.04, 167.44, 176.18; MS (70 eV): m/z : 262 (100, $[M^+]$); elemental analysis (%): calcd for $\text{C}_{10}\text{H}_{15}\text{O}_3\text{Br}$: C 45.65, H 5.75; found: C 45.46, H 5.87. The geometry of the exocyclic double bond of all products and the relative configuration of **5a, b** were determined by NOE measurements and based on analogy of chemical shifts and coupling constants to compounds with known configuration.^[14] The relative configuration of the *Z*-configured products **3l, m** could not be unambiguously determined. All compounds were characterized by spectroscopic methods and gave correct elemental analyses and/or high-resolution mass spectra.

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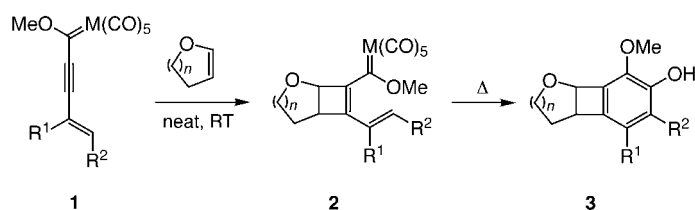
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Eight-Membered Carbocycles from a Dötz-Like Reaction**

José Barluenga,* Fernando Aznar, and M. Angel Palomero

The reaction of Fischer carbene complexes with alkynes can lead, under appropriate conditions, to a diverse array of structures.^[1] The most important and widely used among these reactions is the well-known Dötz benzannulation,^[2] which affords *p*-alkoxyphenol derivatives by successive insertion of one molecule of alkyne and one CO ligand in an α,β -unsaturated carbene, and subsequent electrocyclic ring closure.^[1] Although reaction of chromium arylcarbene complexes with alkynes can produce more than fifteen different types of organic compounds as side products,^[1a,c] alkenylcarbenes yield benzannulated products with greater fidelity.^[3] The formation of cyclopentadiene derivatives from the coupling of acetylenes and vinylcarbenes has been observed in a few cases.^[4] On the other hand, compounds such as cyclopentenones,^[5] heterocycles,^[6] or spirocycles,^[7] have been achieved when (2-amino-1-vinyl)carbenes were used.^[8] The presence of additional unsaturation, either in the starting carbene or in the alkyne, allows entry to new types of structures, usually polycycles.^[9] However, as far as we know, the reaction of dienyl carbenes and alkynes has never been reported.^[10]

One of the current interests in our research group focuses on the preparation and synthetic applications of 1-metalla-1,3,5-hexatrienes. In this context, we have recently described the synthesis of stable dienyl complexes **2** by [2+2] cycloaddition of alkynyl carbenes **1** and enol ethers (Scheme 1).^[11] The complexes **2** were unreactive at room temperature, but



Scheme 1. M = Cr, W; n = 1, 2.

gave benzannulation products when heated. Cyclopentadiene derivatives, which usually result from 1-metalla-1,3,5-hexatrienes, were not observed, not even as side products. We assumed that this different behavior was due to the presence of the cyclobutene ring. In order to ascertain whether the unusual reactivity of **2** could be extended to other processes,

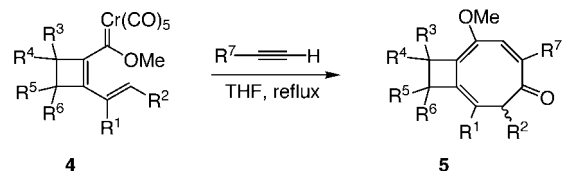
[*] Prof. J. Barluenga, F. Aznar, M. A. Palomero
Instituto Universitario de Química Organometálica "Enrique Moles"
Unidad Asociada al C.S.I.C.
Julián Clavería 8, 33071 Oviedo (Spain)
Fax: (+34) 98-510-3446
E-mail: barluenga@sauron.quimica.uniovi.es

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we decided to check their reaction with alkynes. Herein we report preliminary results of the study of the coupling of cyclobutene-containing metallatrienes and alkynes.

When dienylyl carbene complexes **4** were treated with three equivalents of a terminal alkyne in THF and warmed to reflux, the corresponding cyclooctatrienones **5** (Scheme 2) were

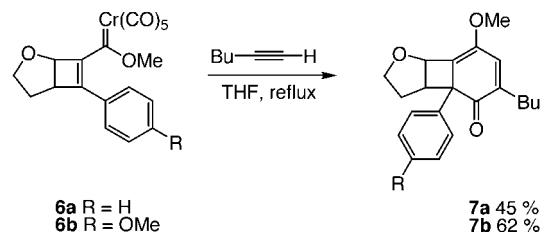


Scheme 2.

obtained in moderate yields (Table 1). The reaction proceeded in a regioselective fashion and led, owing to the newly created stereogenic center, to a mixture of diastereoisomers. This process can be viewed as a variation of the Dötz reaction,

obtained in slightly lower yields compared to those of the stepwise reactions.^[13]

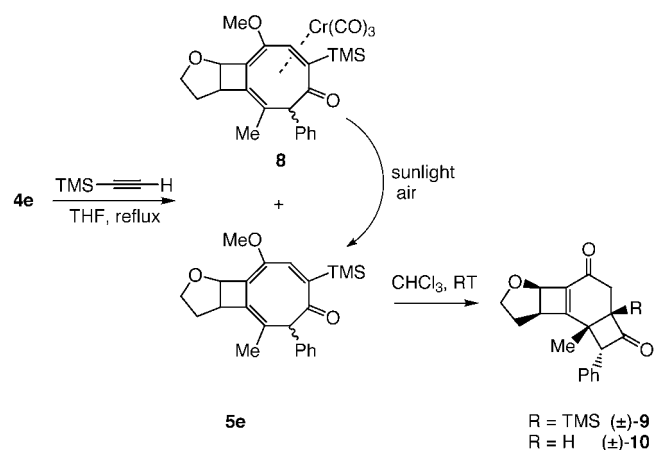
With these data in hand, we decided that it would be interesting to extend this novel reaction to carbenes **6**, in which the terminal double bond is part of an aromatic ring. As shown in Scheme 3, these systems reacted in the fashion expected for single alkenyl complexes, affording the corresponding benzannulation products **7** in moderate yields.



Scheme 3.

The formation of **5** can be explained by means of a mechanism in which the first steps are identical to those of the Dötz reaction. The sequential insertion of an alkyne and a CO molecule gives rise to a trienylketene complex intermediate which undergoes an eight-electron electrocyclic ring closure, instead of the expected six-electron electrocyclization, to afford the $\text{Cr}(\text{CO})_3$ -complexed carbocycles. The decomplexation of the metal–tricarbonyl moiety provides the final products. We believe that the presence of a four-membered ring introduces geometric restraints in the intermediate, which promote the eight-electron ring closure. However, when the terminal double bond is contained within an aromatic ring, as in compounds **6**, the geometrically disfavored six-electron electrocyclization of the intermediate is preferred, yielding the cyclohexadienones **7**. This is presumably due to the high energetic cost that would be required to break the aromaticity of the ring.

This mechanistic proposal is partially supported by the isolation of one of the reaction intermediates, the $\text{Cr}(\text{CO})_3$ -complexed cyclooctatrienone **8** (Scheme 4).^[14] Treatment of **4e** with trimethylsilylacetylene affords **8**, together with a mixture of both diastereoisomers of **5e**. The former com-



Scheme 4.

Table 1. Preparation of eight-membered carbocycles **5**.

Com- pound	R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶	R ⁷	d.r. ^[a]	Yield [%] ^[b]
5a	-(CH ₂) ₃ -		H	-O(CH ₂) ₂ -		H	Ph	1.2:1	71
5b	-(CH ₂) ₃ -		H	-O(CH ₂) ₂ -		H	Bu	3:1	70
5c	-(CH ₂) ₃ -		H	-O(CH ₂) ₂ -		H	CH ₂ OTBS ^[f]	1.3:1	59
5d	Me	Ph	H	-O(CH ₂) ₂ -		H	Bu	5:1	57
5e	Me	Ph	H	-O(CH ₂) ₂ -		H	TMS ^[g]	1.4:1	63 ^[c,d]
5f	-(CH ₂) ₄ -		MeO	Me	H	H	Bu	15:1	53 ^[e]
5g	-(CH ₂) ₄ -		MeO	MeO	MeO	MeO	Bu	–	52

[a] Determined by integration of the ¹H NMR signals in the crude reaction mixture. [b] Yield of isolated products by flash chromatography. [c] Diastereoisomers could not be separated by chromatography. [d] 20% of the product was isolated as the $\text{Cr}(\text{CO})_3$ complex. [e] Only the major diastereoisomer was isolated. [f] TBS = *tert*-butyldimethylsilyl. [g] TMS = trimethylsilyl.

since both an alkyne and CO are inserted. However, the additional double bond present in the starting complex participates in the subsequent electrocyclic ring closure, giving rise to eight-membered carbocycles. Although Fischer carbene complexes have proven to be useful starting materials for the preparation of up to seven-membered carbocycles,^[1a,c] this is, as far as we know, the first time that reactions of these complexes have resulted in the formation of an eight-membered carbocyclic ring.

The photochemically driven reaction of metallatriene **4a** and 1-hexyne was also tested. We found that both the yield and the diastereoselectivity obtained were comparable to those of the thermal process. The structure of the new compounds was elucidated on the basis of their COSY and HMBC NMR spectra. To date, it has not been possible to determine the relative stereochemistry between the created stereocenter and the ones preexisting in the starting dienylyl complex.

Carbocycles **5** could also be prepared in a tandem one-pot process from the vinyl-substituted alkynyl chromium carbenes **1** (Scheme 1).^[12] Upon refluxing the starting complexes with three equivalents of 2,3-dihydrofuran and three equivalents of the corresponding alkyne in THF, the desired products were

pound was purified by flash chromatography, and its structure was assigned by COSY and HMBC experiments. This compound could be quantitatively converted into the metal-free carbocycle **5e** by exposure to air and sunlight.

Most of the compounds **5** are unstable in solution leading in a few hours to complex and unidentifiable mixtures of products. However, compound **5e** gave a single diastereoisomer of polycyclic system **9**, in 90% yield, upon standing in CHCl₃ for ten days (Scheme 4). Longer reaction times led to the release of the trimethylsilyl (TMS) group, and thus to the formation of **10** (92% yield). These products are evidently derived from the six-electron electrocyclization of the starting trienyl system **5e** and the hydrolysis of the resulting enol ether. The single diastereoisomer of **9** can best be explained as arising from epimerization of the α -carbonyl stereocenter in **5e** and the stereoselective cyclization of one of the epimeric ketones.

In conclusion, we have reported the first Dötz-like reaction between conjugated dienyl carbene chromium complexes and terminal alkynes to afford eight-membered carbocycles. We have also studied the ring closure of one of the mentioned systems to yield new polycyclic compounds.

Experimental Section

A solution of dienyl carbene **4** (1 mmol) and of the alkyne (3 mmol) in THF (15 mL) was refluxed, under a nitrogen atmosphere, until analysis by thin-layer chromatography revealed total consumption of the starting complex. The reaction mixture was diluted with hexane (20 mL) and exposed to sunlight and air in order to oxidize the metallic species to the corresponding organic compounds. Filtration through a pad of Celite and flash chromatography provided carbocycles **5**.

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Combinatorial Solid-Phase Synthesis of Multivalent Cyclic Neoglycopeptides**

Valentin Wittmann* and Sonja Seeberger

Dedicated to Professor Horst Kessler

The molecular recognition of carbohydrates by carbohydrate-binding proteins (lectins) is the basis of numerous intercellular recognition processes.^[1] High-affinity lectin ligands are of considerable medicinal interest in the diagnosis and manipulation of such processes.^[2] Individual carbohydrate epitopes (normally mono- to pentasaccharides) are, however, mostly bound by lectins with only low affinity (dissociation constants in the milli- to micromolar range) and in part broad specificity.^[3] Since many membrane-associated lectins have several binding sites or occur in oligomeric or clustered form,^[4] the creation of multivalent carbohydrate derivatives is a promising approach to arrive at effective lectin ligands.^[5, 6]

Several strategies have been described to achieve the formation of a sufficient number of individual interactions necessary for high avidity of a multivalent ligand. Glycopolymers,^[7] for example, are able to cover large areas of cell surfaces and bridge several membrane-located lectins ("statistical" multivalency). Small oligovalent carbohydrate derivatives (miniclusters)^[6] on the other hand bind preferentially to several binding sites of a single (oligomeric) lectin proximate

[*] Dr. V. Wittmann, Dipl.-Chem. S. Seeberger
Institut für Organische Chemie
Johann Wolfgang Goethe-Universität
Marie-Curie-Strasse 11, 60439 Frankfurt (Germany)
Fax: (+49) 69-798-29148
E-mail: wittmann@chemie.uni-frankfurt.de

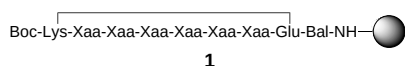
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in space and may be tailored to lectins with known three-dimensional (3D) structure ("directed" multivalency).^[8] With the carbohydrates connected by an inflexible scaffold, particularly affine ligands are obtained—provided that the sugars are oriented in a way required for multidentate binding.^[5] Moreover, conformationally restricted miniclusters are in principle able to differentiate between various multivalent lectins with the same carbohydrate specificity but varying orientation of their binding sites. If the 3D structure of the targeted lectin is unknown and especially if conformationally restricted linkers are used, many potential ligands have to be synthesized and screened in order to "hit" the required orientation of the sugar residues.

We now report a synthetic concept to generate libraries of conformationally restricted miniclusters, comprising the following steps: a) "split–mix" synthesis^[9] of a library of scaffold molecules with side chain amino groups in varying amounts and spatial orientation, b) attachment of several identical copies of a carbohydrate ligand to the amino groups.

As scaffolds for the multivalent presentation of carbohydrate ligands, we have chosen cyclic peptides of general type **1** (Bal = β -alanine) in which the residues Xaa are varied



combinatorially.^[10] Using commercially available D- and L-configured amino acids, a high degree of conformational diversity may be easily generated.^[11] Diamino acids with Ddv-protected^[12] side chains of varying length serve as sugar attachment points. To this end, a new urethane-type linker based on the Alloc protecting group^[13] has been developed. In contrast to glycosylation reactions employing solid-phase-bound peptides,^[14] the formation of an urethane bond proceeds in virtually quantitative yield. Furthermore, after the binding assay the carbohydrates may be cleaved off again under Pd catalysis regenerating the unmodified side chain cyclized peptides. Thus, the analysis of compounds bound to single beads which has to be carried out when using the split–mix method is confined to an automated microsequencing (Edman degradation) under standard conditions.

Scheme 1 illustrates the synthesis of carbohydrate-linker derivative **5** activated as *p*-nitrophenyl carbonate which was applied to attach *N*-acetylglucosamine residues to the cyclo-

peptides. Reaction of literature-known glycosyl donor **2**^[15] with mono TBDPS-protected *cis*-but-2-ene-1,4-diol under methyl triflate activation gave β -configured O-glycoside **3** in high yield. Substitution of the Troc protecting group with an acetyl group followed by removal of the TBDPS group with HF·pyridine complex led to *N*-acetylglucosamine derivative **4**. Condensation with 4-nitrophenyl chloroformate finally gave active carbonate **5** as a crystalline substance that could be stored at room temperature (Table 1).

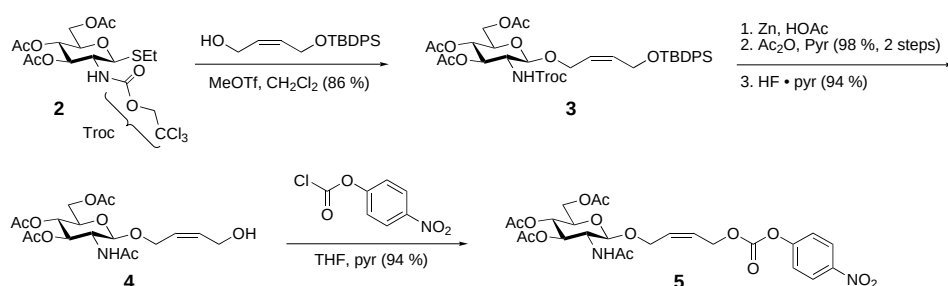
Table 1. Selected physical data of compounds **5** and **12**.

5: R_f = 0.43 (silica, EtOAc); m.p. 133 °C (EtOAc/*n*-hexane); ¹H NMR (400 MHz, CDCl₃, 300 K, TMS): δ = 8.29–8.25 (m, 2H; arenes), 7.40–7.36 (m, 2H; arenes), 5.85–5.75 (m, 2H; vinyl-H), 5.64 (d, J = 8.7 Hz, 1H; NH), 5.28 (dd, J = 9.3, 10.6 Hz, 1H; H-3), 5.05 (dd, J = 9.3, 10.0 Hz, 1H; H-4), 4.90–4.79 (m, 2H; allyl-H), 4.75 (d, J = 8.3 Hz, 1H; H-1), 4.44–4.29 (m, 2H; allyl-H), 4.22 (dd, J = 4.7, 12.3 Hz, 1H; H-6a), 4.13 (dd, J = 2.5, 12.3 Hz, 1H; H-6b), 4.83 (ddd, J = 8.3, 8.7, 10.6 Hz, 1H; H-2), 3.69 (ddd, J = 2.5, 4.7, 10.0 Hz, 1H; H-5), 2.06 (s, 3H; C(O)CH₃), 2.01 (s, 3H; C(O)CH₃), 2.00 (s, 3H; C(O)CH₃), 1.93 (s, 3H; C(O)CH₃); ¹³C NMR (100 MHz, CDCl₃, 300 K, TMS): δ = 170.8, 170.6, 170.2, 169.3, 155.4, 152.4, 145.4, 131.0 and 125.8 (olefins), 125.3 and 121.8 (arene CH), 99.5 (C-1), 72.2 (C-3), 71.8 (C-5), 68.6 (C-4), 64.6 and 64.2 (CH₂^{allyl}), 62.0 (C-6), 54.7 (C-2), 23.3 (CH₃), 20.67 (CH₃), 20.61 (CH₃), 20.55 (CH₃); elemental analysis calcd for C₂₅H₃₀N₂O₁₄ (%): C 51.55, H 5.19, N 4.81; found: C 51.52, H 5.28, N 4.79.

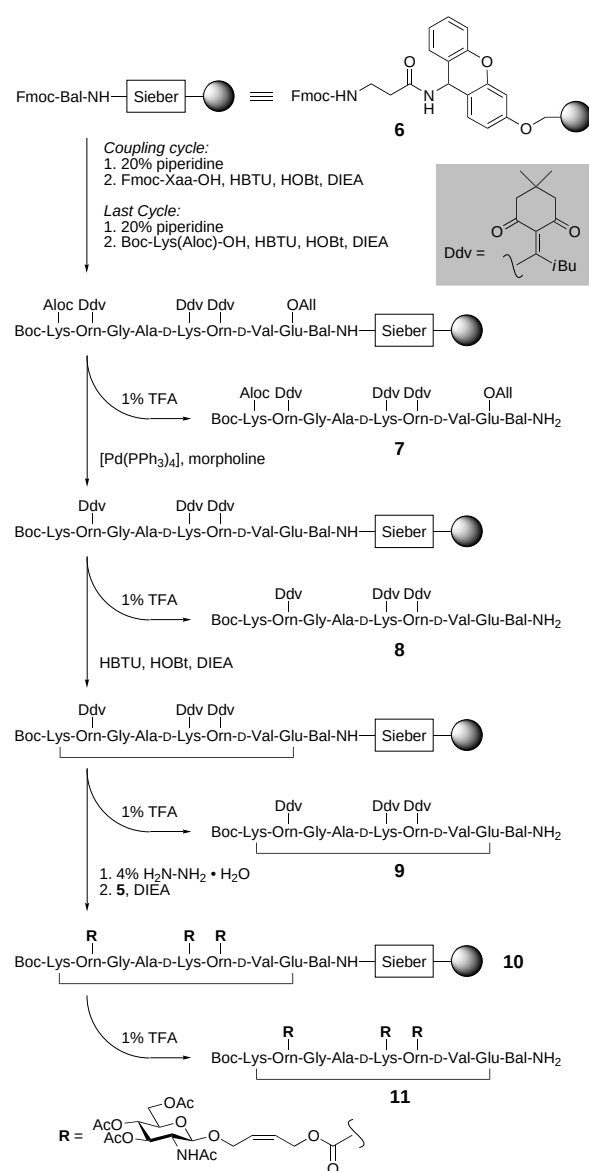
12: ¹H NMR (600 MHz, H₂O/D₂O 9:1, 298 K): Boc-Lys¹: δ = 6.767 (α NH), 3.917 (α H), 1.565 (β H₂), 1.27–1.17 (γ H₂), 1.379 (δ H₂), 3.083 and 3.003 (ϵ H₂), 7.688 (ϵ NH), 1.304 (Boc); Orn: δ = 8.262 (α NH), 4.254 (α H), 1.722 and 1.633 (β H₂), 1.47–1.38 (γ H₂), 3.023 (δ H₂), 6.791 (δ NH); Gly³: 8.201 (NH), 3.855 and 3.788 (α H₂); Ala⁴: 7.999 (NH), 4.203 (α H), 1.277 (β H₃); D-Lys⁵: 8.148 (α NH), 4.185 (α H), 1.690 and 1.615 (β H₂), 1.248 and 1.215 (γ H₂), 1.382 (δ H₂), 2.998 (ϵ H₂), 6.733 (ϵ NH); Orn: δ = 8.316 (α NH), 4.254 (α H), 1.731 and 1.633 (β H₂), 1.478 and 1.420 (γ H₂), 3.032 (δ H₂), 6.767 (δ NH); D-Val⁷: δ = 8.136 (NH), 3.982 (α H), 2.041 (β H), 0.820 and 0.786 (2 γ H₃); Glu⁸: δ = 8.201 (NH), 4.107 (α H), 1.831 and 1.999 (β H₂), 2.201 and 2.136 (γ H₂); Bal⁹: δ = 8.049 (NH), 2.41–2.33 (α H₂), 3.40–3.30 (β H₂); 3 equivalent GlcNAc: δ = 8.103 (NH), 4.427 (H-1), 3.588 (H-2), 3.429 (H-3), 3.38–3.31 (H-4, H-5), 3.810 and 3.651 (2 H-6), 1.927 (Ac); 5.72–5.60 (6 vinyl-H), 4.52–4.48 and 4.30–4.18 (each 6 allyl-H); ESI-MS [$M+H$]⁺: calcd. 1963.0, found 1963.7.

The assembly of the cyclic neoglycopeptides was optimized by using model compound **12** as an example (Schemes 2 and 3, Table 1). For attachment of the first amino acid to the solid support (TentaGel) the Sieber linker was applied.^[16] Thus it was possible to release the product of each reaction step by treatment with a 1 % solution of TFA in dichloromethane ($\rightarrow$ **7–9**, **11**, **12**) and monitor the course of the reaction by analytical HPLC (Figure 1) and subsequent ESI-MS. Syn-

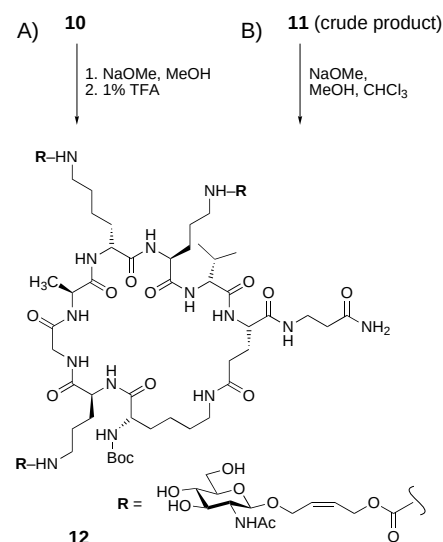
thesis of the linear peptide followed the Fmoc strategy;^[17] solely in the last coupling step an *N* ^{α} -Boc-protected amino acid was applied. For protection of the ω -NH₂ groups of lysine and ornithine we preferred the Ddv group^[12] which distinguishes itself from the frequently used Dde group^[18] by a higher stability under the conditions of Fmoc cleavage. Preparatory for the



Scheme 1. Synthesis of carbohydrate derivative **5** activated as its *p*-nitrophenyl carbonate.



Scheme 2. Solid-phase synthesis of cyclic neoglycopeptide **11**.



Scheme 3. Synthesis of deprotected neoglycopeptide **12**.

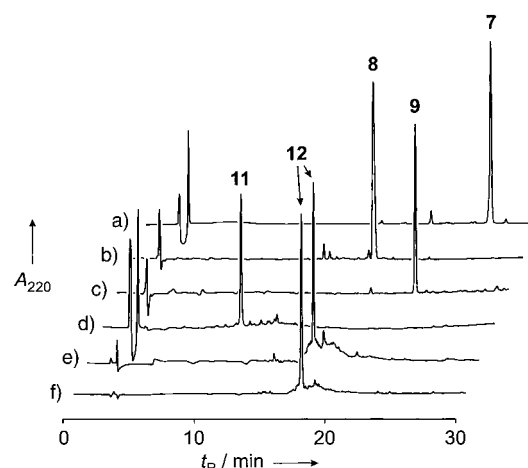


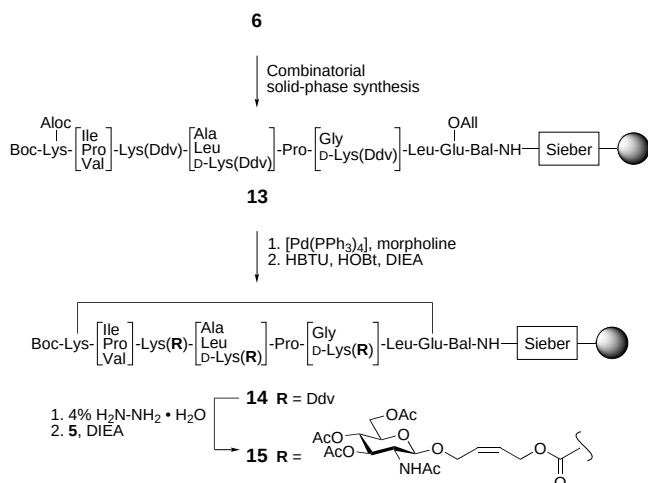
Figure 1. RP-HPLC analysis of the crude peptides **7–9**, **11**, and **12** obtained according to Scheme 2 (a–d), Scheme 3, route A (e), and Scheme 3, route B (f).

cyclization step the allyl ester and the Aloc group were simultaneously removed by palladium(0)-catalyzed allyl transfer to morpholine.^[13]

Addition of a mixture of HBTU, HOBT, and Hünig's base (DIEA) led to the desired cyclopeptide which after cleavage from the resin was obtained in excellent purity (Figure 1c). Side products due to reaction of coupling reagent with the free amino group^[19] (guanylation) was not observed. Other cyclization methods (*N,N'*-diisopropyl carbodiimide/HOBT or activation of the carboxyl group as a Pfp ester^[20]) were clearly less effective. Subsequently, the Ddv groups were removed by batchwise treatment with a 4% solution of hydrazine hydrate which turned out to be advantageous over the literature-recommended^[12] 2% solution.^[21]

Attachment of the sugars was effected by addition of excess (5 equiv per free NH₂ group) of **5** in the presence of DIEA. After complete reaction, the absence of free amino groups was verified by Kaiser test^[22] and by addition of bromophenol blue.^[23] In order to deacetylate the sugars, **10** was treated with a solution of sodium methoxide in methanol (Scheme 3, route A). After cleavage from the resin, the HPL-chromatogram shown in Figure 1e was obtained in which beside the desired glycopeptide **12** noticeable amounts of side products are visible. Since their appearance is possibly due to the acid lability of **12**^[24] and therefore the chromatogram might not reflect the realities on the solid support, we carried out the deacetylation after cleavage from the resin for comparison (Scheme 3, route B). The obtained chromatogram of the crude product (Figure 1f) now indicates only slight amounts of side products demonstrating the high efficiency of the whole synthesis.

In order to verify that the described reaction conditions, especially those of the critical cyclization step, are transferable to other peptide sequences, we synthesized the neoglycopeptide library **15** comprising 18 compounds by using the split–mix method (Scheme 4). Again, the course of the synthesis was monitored by withdrawal of small resin samples and analysis of the cleavage products by HPLC in combination with ESI-MS (Figure 2, Table 2). The expected



Scheme 4. Split-mix synthesis of neoglycopeptide library **15**. At the positions denoted by square brackets the resin was distributed at two and three reaction vessels, respectively, and each coupled with one of the given amino acids.

18 products were identified in all chromatograms. It turned out that all peptides underwent cyclization without forming noteworthy amounts of side products. Particularly, after mass spectrometric analysis of **14** we were not able to detect any starting materials or linear or cyclic peptide dimers.

The introduced synthetic strategy is suitable for efficient construction of large libraries of neoglycopeptides presenting any desired carbohydrate ligands in variable quantity and varying distances to each other. With the convergent approach it is easily possible to apply a once prepared cyclo-peptide library in studying different lectins by simply attaching appropriate carbohydrate ligands to it.

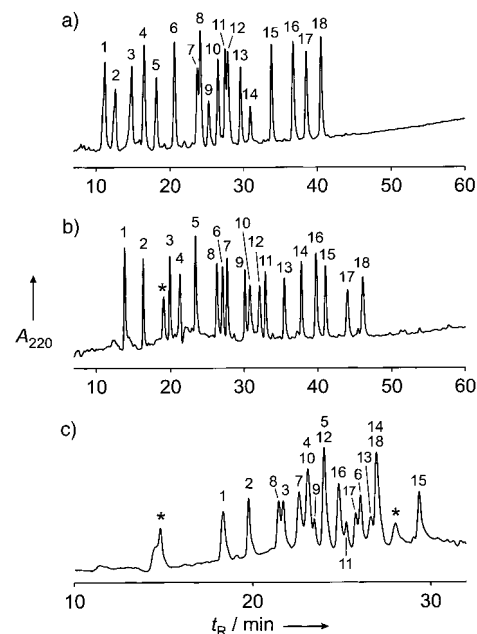


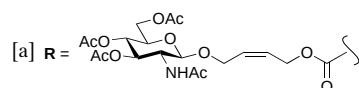
Figure 2. RP-HPLC analysis of the compound libraries obtained from treatment of a) **13**, b) **14**, and c) **15** with a 1% solution of TFA. The assignment of peaks resulted from mass spectrometric analysis and is given in Table 2. Peaks of corresponding peptide derivatives are marked with identical numbers. Those marked with asterisks stem from non-peptide impurities.

Experimental Section

The solid-phase peptide synthesis was carried out on NovaSyn TG Sieber resin (Novabiochem) (abbreviation: Sieber-TG) following standard protocols^[17] (loading after immobilization of the first amino acid: 0.17 mmol g⁻¹). Couplings were performed in NMP. For cleavage solid-phase aliquots (ca. 5 mg) were repeatedly treated with TFA/*i*Pr₃SiH/CH₂Cl₂ (1:1:98). Products

Table 2. Calculated and experimentally found masses of the library of cyclic neoglycopeptides obtained by treatment of **15** with a 1% solution of TFA (peak numbers correspond to the assignment given in Figure 2c).

Peak	Compound ^[a]	[M+H] ⁺	
		calcd	found
1	<i>cyclo</i> [Boc-Lys-Pro-Lys(R)-Ala-Pro-Gly-Leu-Glu]-Bal-NH ₂	1434.7	1435.4
2	<i>cyclo</i> [Boc-Lys-Val-Lys(R)-Ala-Pro-Gly-Leu-Glu]-Bal-NH ₂	1436.7	1437.3
8	<i>cyclo</i> [Boc-Lys-Pro-Lys(R)-D-Lys(R)-Pro-Gly-Leu-Glu]-Bal-NH ₂	1934.9	1935.7
3	<i>cyclo</i> [Boc-Lys-Ile-Lys(R)-Ala-Pro-Gly-Leu-Glu]-Bal-NH ₂	1450.8	1451.2
7	<i>cyclo</i> [Boc-Lys-Pro-Lys(R)-Ala-Pro-D-Lys(R)-Leu-Glu]-Bal-NH ₂	1949.0	1950.0
4	<i>cyclo</i> [Boc-Lys-Pro-Lys(R)-Leu-Pro-Gly-Leu-Glu]-Bal-NH ₂	1476.8	1477.4
10	<i>cyclo</i> [Boc-Lys-Val-Lys(R)-D-Lys(R)-Pro-Gly-Leu-Glu]-Bal-NH ₂	1937.0	1938.0
9	<i>cyclo</i> [Boc-Lys-Val-Lys(R)-Ala-Pro-D-Lys(R)-Leu-Glu]-Bal-NH ₂ or <i>cyclo</i> [Boc-Lys-Ile-Lys(R)-D-Lys(R)-Pro-Gly-Leu-Glu]-Bal-NH ₂	1951.0	1952.0
5	<i>cyclo</i> [Boc-Lys-Ile-Lys(R)-D-Lys(R)-Pro-Gly-Leu-Glu]-Bal-NH ₂	1478.8	1479.4
12	<i>cyclo</i> [Boc-Lys-Ile-Lys(R)-D-Lys(R)-Pro-Gly-Leu-Glu]-Bal-NH ₂ or <i>cyclo</i> [Boc-Lys-Val-Lys(R)-Ala-Pro-D-Lys(R)-Leu-Glu]-Bal-NH ₂	1951.0	1952.0
16	<i>cyclo</i> [Boc-Lys-Pro-Lys(R)-D-Lys(R)-Pro-D-Lys(R)-Leu-Glu]-Bal-NH ₂	2449.2	2450.1
11	<i>cyclo</i> [Boc-Lys-Ile-Lys(R)-Ala-Pro-D-Lys(R)-Leu-Glu]-Bal-NH ₂	1965.0	1966.2
17	<i>cyclo</i> [Boc-Lys-Val-Lys(R)-D-Lys(R)-Pro-D-Lys(R)-Leu-Glu]-Bal-NH ₂	2451.2	2452.8
6	<i>cyclo</i> [Boc-Lys-Ile-Lys(R)-Leu-Pro-Gly-Leu-Glu]-Bal-NH ₂	1492.8	1493.3
13	<i>cyclo</i> [Boc-Lys-Pro-Lys(R)-Leu-Pro-D-Lys(R)-Leu-Glu]-Bal-NH ₂	1991.0	1991.8
14	<i>cyclo</i> [Boc-Lys-Val-Lys(R)-Leu-Pro-D-Lys(R)-Leu-Glu]-Bal-NH ₂	1993.0	1993.6
18	<i>cyclo</i> [Boc-Lys-Ile-Lys(R)-D-Lys(R)-Pro-D-Lys(R)-Leu-Glu]-Bal-NH ₂	2465.2	2465.9
15	<i>cyclo</i> [Boc-Lys-Ile-Lys(R)-Leu-Pro-D-Lys(R)-Leu-Glu]-Bal-NH ₂	2007.0	2008.0



that were hardly soluble in the cleavage cocktail were washed off the solid support with a suitable solvent. Before concentration cleavage solutions were neutralized with pyridine. Analytical HPLC was carried out on C18 reversed-phase columns (250 × 4 mm) with linear gradients of acetonitrile in water/0.1% TFA and a flow of 1 mL min⁻¹. Product peaks were characterized by ESI-MS. Experimental details for the synthesis of **8**, **9**, **11**, and **12** are found in the Supporting Information.

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Wound-Activated Chemical Defense in Unicellular Planktonic Algae**

Georg Pohnert*

Diatoms are highly successful unicellular algae occurring in ocean and fresh water phytoplankton, as well as in biofilms on solid substrates. They are exceedingly abundant and are among the most important primary sources sustaining the marine food chain. Despite this, little is known about the chemical defense of these micro algae. Two of the few reported examples are the aldehydes decadienal **5** and decatrienal **6** (see Scheme 1) from the diatom *Thalassiosira rotula*, which reduce the hatching success from eggs of copepods (zooplankton grazers).^[1] This observed activity explains the paradox that herbivorous copepods are less successful feeding on diatoms, although these algae are considered as high-quality food.

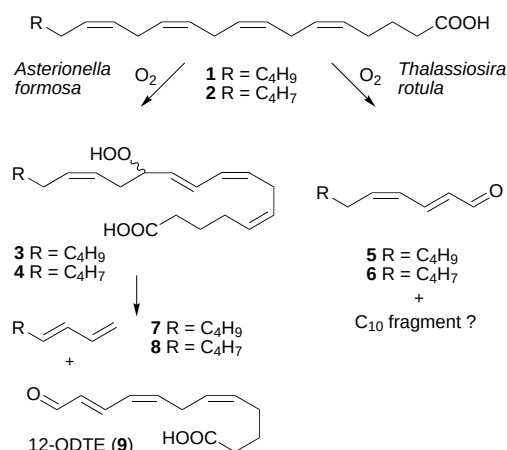
Here I provide biosynthetic and kinetic data on the formation of fatty acid derived metabolites in planktonic diatoms, demonstrating that the release of α,β,γ,δ-unsaturated dienals is widespread among this class of algae. The enzymatic mechanism to produce these metabolites is efficiently activated seconds after cell disruption and leads to high local concentrations of the defensive metabolites **5** and **6** or of structurally related potentially active aldehydes like **9**.

The simultaneous production of C₁₁ hydrocarbons and 9-oxonona-5*Z*,7*E*-dienoic acid from C₂₀ fatty acids was demonstrated with the benthic diatom *Gomphonema parvulum*.^[2, 3] The polar diennoic acid contains the same aldehydic

[*] Dr. G. Pohnert
Max-Planck-Institut für Chemische Ökologie
Carl-Zeiss-Promenade 10, 07745 Jena (Germany)
Fax: (+49) 3641-643665
E-mail: pohnert@ice.mpg.de

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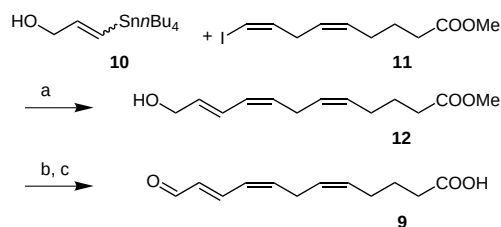
structural element as **5** and **6** and is likely to play an important role in the chemical defense of *G. parvulum*.^[2] To establish if the production of frequently detected volatile hydrocarbons is generally indicative of the release of $\alpha,\beta,\gamma,\delta$ -unsaturated dienals, I investigated the biosynthesis of C_8 hydrocarbons from the planktonic freshwater diatom *Asterionella formosa*. (3*E*,5*Z*)-Octa-1,3,5-triene (**8**) and (3*E*)-octa-(1,3)-diene (**7**) were detected in suspensions of broken cells of *A. formosa* or under osmotic stress conditions.^[4, 5] In analogy to the biosynthesis of C_{11} hydrocarbons,^[2] we reasoned that (5*Z*,8*Z*,10*E*)-12-oxododeca-5,8,10-trienoic acid (**9**; 12-ODTE) could be released as a second product, besides **7** or **8**, after oxidative cleavage of the fatty acids **1** or **2** (Scheme 1).



Scheme 1. Suggested mechanism for the oxidative transformation of eicosanoic fatty acids by damaged diatom cells.

Direct investigation of cell-free *A. formosa* extracts, without extra extraction or enrichment steps, by high pressure liquid chromatography/mass spectrometry (HPLC/MS) revealed the rapid production of a dominant medium-polarity compound.^[6] The molecular species (m/z 209 [$M+H^+$]), the main fragment ion (m/z 191 [$M+H^+ - H_2O$]), and the UV absorption ($\lambda_{\max} = 279$ nm) were consistent with the structure of 12-ODTE (**9**). To facilitate identification of this metabolite and to provide material for bioassays, an authentic sample of 12-ODTE (**9**) was prepared according to Scheme 2.

Addition of [5,6,8,9,11,12,14,15-²H₈]arachidonic acid (**1**) to the cell-free solution of *A. formosa* resulted in 12-ODTE (**9**)



Scheme 2. Synthesis of 12-ODTE (**9**): a) **11**,^[17] **10** (1.5 equiv; $E/Z = 75/25$), [(CH₃CN)₂PdCl₂] (0.1 mol %), DMF, RT, 86 % (5*Z*/*E* > 97/3, 8*Z*/*E* > 97/3, 10*Z*/*E* = 5/95); b) MnO₂, CH₂Cl₂, 5 min, RT, 84 %; c) PPL, phosphate buffer (pH 7), reverse-phase MPLC, 81 % (5*Z*/*E* > 97/3, 8*Z*/*E* > 97/3, 10*Z*/*E* = 5/95). For spectroscopic data, see ref. [18]. DMF = dimethylformamide, RT = room temperature, PPL = porcine pancreatic lipase.

labeled with six deuterium atoms (m/z 215 [$M+H^+$]), which indicates that the intact C1–C12 segment of the substrate was transformed into **9**. The second fragment, comprising the aliphatic part of [²H₈]-**1**, [²H₂]- (1,3)-octa-(3*E*)-diene (**7**), was detected as a major volatile product by GC/MS analysis.

Since the production of **7** and **8** is oxygen dependant^[4] and 12-hydroperoxyeicosatetraenoic acid (**3**) could be trapped as a biosynthetic intermediate,^[7] the formation of C_8 hydrocarbons in *A. formosa* is most likely to be catalyzed by a lipoxygenase/hydroperoxide lyase. Thus, the hydrocarbons frequently detected during algal blooms may indeed be considered as indicators for the simultaneous production of reactive Michael acceptors like 12-ODTE (**9**) or 9-oxonona-5*Z*,7*E*-dienoic acid.^[2]

Interestingly, decadienal **5** and decatrienal **6** from *T. rotula* were also found to be derived from eicosanoic (C_{20}) fatty acids. Addition of [²H₈]arachidonic acid (**1**) to broken cells of *T. rotula* led to rapid formation of [²H₄]decadienal **5**.^[8] In contrast, linoleic acid (C_{18}) did not serve as precursor for **5**. Unlike terrestrial plants, where volatile aldehydes are derived from C_{18} fatty acids,^[9] the alga uses the pool of eicosanoids for the production of **5** and **6**. Therefore *T. rotula* follows the general observed substrate preference of eicosanoic fatty acids for production of oxylipins in other algae.^[10]

Despite the fact that both unsaturated aldehydes and hydrocarbons are reported from numerous diatoms, quantification experiments with carefully isolated intact algae gave no detectable oxygenated fatty acid degradation products.^[4, 11] This situation changes dramatically seconds after mechanical damage of the cells. A rapid onset of aldehyde production is observed and levels of 49 fmol of **9** per cell of *A. formosa* (Figure 1 a) and 4.1 fmol of **6** per cell of *T. rotula* (Figure 1 b) are produced after only 1–3 minutes.^[11] The rapid enzymatic fatty acid degradation to **5** and **6**, therefore, represents an exceptionally fast and efficient mechanism of wound-activated chemical defense in *T. rotula*.

Although the role of **5** and **6** in the defense chemistry of *T. rotula* has already been described,^[1] the ecological role of 12-ODTE (**9**) and related products remains to be established in more detail. Preliminary studies show that (5*Z*,7*E*)-9-oxonona-5,7-dienoic acid and **9** are active fungicides against *Schizophyllum commune* and *Aspergillus nidulans*.^[12] This observation still has to be validated in field experiments with parasitic fungi that are known to control the spring increase of *A. formosa*.^[13] Encouraging results from bioassays with herbivorous marine amphipods have already been obtained with (5*Z*,7*E*)-9-oxonona-5,7-dienoic acid, which proved highly active as a deterrent against *Amphitoe longimana*.^[14]

The strategy of rapid transformation of eicosanoic fatty acids after cell damage allows *A. formosa* and *T. rotula* to build up a remarkably high local concentration of the defensive metabolites **5** or **6** and other related Michael acceptors, like **9**. These reactive aldehydes are targeted very efficiently against the herbivores that activate their production and they might even be formed in the digestive tract of those grazers.^[15] Such mechanisms could account for the formation the high local concentrations (approximately 10 μ M of **5** or **6**) which are required for the reduction of copepod hatching success.^[1]

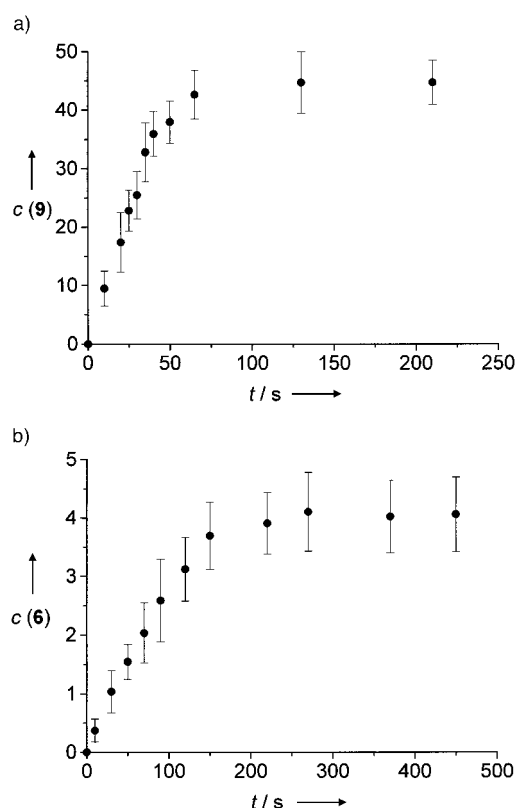


Figure 1. Kinetic data for $\alpha,\beta,\gamma,\delta$ -unsaturated aldehyde production after mechanical damage of planktonic diatoms. a) 12-ODTE (9) production [fmol per cell] after mechanical damage of *A. formosa*. b) Decatrienal 6 production [fmol per cell] after mechanical damage of *T. rotula*. Control experiments with undamaged cells showed no detectable 9 or 6.

In the diatoms investigated, cellular resources are invested in the production of C_{20} fatty acids that are activated only upon demand for chemical defense. The production of costly constitutive defensive secondary metabolites can thus be minimized and the risk of self-toxicity is simultaneously reduced. The exploration of chemical defense in planktonic microbes is still in its infancy, and the discovery of this highly dynamic defensive strategy might contribute to a more detailed understanding of the complex connections in this community.

Experimental section

General: *A. formosa* (SAG, University of Göttingen, Germany) was grown as previously described;^[5] *T. rotula* (obtained as a gift from S. Poulet, Roscoff, France) was grown in artificial sea water,^[16] as standing cultures at 19 °C (day:night 14:10). Cells were harvested through centrifugation and experiments were carried out with the concentrated cell suspensions in the culture medium. Preparation of cell-free extracts was performed as previously described.^[2]

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- [7] Trapping experiments were performed as previously described^[2] and the identity of the intermediate was confirmed through comparison of the HPLC/MS properties with authentic 12-hydroxyicosatetraenoic acid (SIGMA, Deisenhofen, Germany).
- [8] Compounds 5 and 6 were extracted from cell-free preparations of *T. rotula* in the presence and absence of 6 μ M of [^{2} H₈]arachidonic acid (1) by solid-phase microextraction (SPME) and were analyzed by GC/MS according to: D. Spiteller, G. Spiteller, *Angew. Chem.* **2000**, 112, 595–597; *Angew. Chem. Int. Ed.* **2000**, 39, 583–585.
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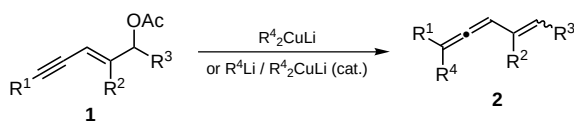
“Remote Stereocontrol” in Organocopper Chemistry: Highly Enantioselective Synthesis of Vinylallenes by 1,5-Substitution of Enyne Acetates**

Norbert Krause* and Martin Purpura

Dedicated to Professor Henning Hopf
on the occasion of his 60th birthday

Vinylallenes, as a special class of allenes, are highly interesting substrates, for example in Diels–Alder reactions, because of their high reactivity and stereoselectivity.^[1, 2] In contrast to ordinary 1,3-dienes, they can transfer their inherent axial chirality with high stereoselectivity to the new stereogenic centers formed during the cycloaddition;^[3, 4] furthermore, due to the more favorable equilibrium between the *s-trans* and the *s-cis* conformer, they are also more reactive.^[3, 5] Particularly with regard to the chirality transfer, the enantioselective synthesis of substituted vinylallenes if of high current interest.

Recently, we reported a new synthesis of vinylallenes by 1,5-(*S_N'*)-substitution of enyne acetates (**1**) and oxiranes with organocuprates.^[6] Irrespective of the substitution pattern, these substrates react regioselectively at the triple bond. Normally, the vinylallenes **2** were formed as a mixture of the *E/Z* isomers; in some cases, however, one geometric isomer was obtained exclusively.



In order to study the enantioselectivity of copper-promoted 1,5-substitutions, the enyne acetates **1** were required in enantiomerically enriched or pure form. We obtained these with enantiomeric excesses of 92–99 % *ee*^[7] by lipase-catalyzed kinetic resolution of the racemic acetates and subsequent acylation of the alcohols (*R*)-**3** (Table 1). The absolute configuration of enyne acetate (*S*)-**1a** (*R*¹ = *R*³ = Me, *R*² = H) was determined by correlation with mit (*S*)-ethyl lactate.^[8]

The first experiments with (*R*)-**1a** (96 % *ee*) were unsatisfactory. Reaction with the cyano-Gilman reagent *t*Bu₂CuLi · LiCN at –80 °C furnished vinylallene **2a** as a 25:75 mixture of the *E/Z* isomers with enantiomeric excesses of 8 and 68 %, respectively; these could be improved slightly to 20 and 74 % *ee* by lowering the reaction temperature to –90 °C. (Table 2, entries 1, 2).^[6] A possible reason for these insufficient enantioselectivities is a racemization of the vinylallene by the cuprate or other reactive copper species formed in the reaction mixture. Such racemizations (which probably occur

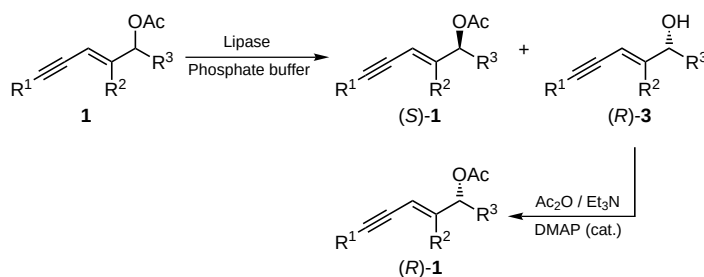


Table 1. Preparation of nonracemic enyne acetates **1** by lipase-catalyzed kinetic resolution.

Acetate	<i>R</i> ¹	<i>R</i> ²	<i>R</i> ³	Lipase	(<i>S</i>)- 1		(<i>R</i>)- 1	
					Yield [%]	<i>ee</i> [%]	Yield [%]	<i>ee</i> [%]
1a	Me	H	Me	<i>Ps. fluorescens</i>	53	94	37	96
1b	<i>n</i> Bu	H	Me	<i>Ps. fluorescens</i>	48	98	39	94
1c	Ph	H	Me	<i>Ps. cepacia</i>	51	99	42	92
1d	Me ₃ Si	H	Me	<i>Ps. fluorescens</i>	51	99	43	99
1e	<i>n</i> Bu	–(CH ₂) ₄ –		<i>Ps. fluorescens</i>	51	98	47	99

via one-electron transfer steps) have already been observed several times in copper-promoted *S_N'*-substitutions of propargyl electrophiles.^[9] According to Alexakis,^[9a] it is possible to improve the enantioselectivity of these transformations considerably by addition of trialkylphosphanes; apparently, these ligands modify the copper intermediates which are responsible for the racemization. As a matter of fact, the reaction of (*R*)-**1a** with two equivalents of *t*Bu₂CuLi · LiCN at –80 °C in the presence of four equivalents of *n*Bu₃P provided the vinylallene **2a** with high enantiomeric excesses of 92 % *ee* for the *E* isomer and 93 % *ee* for the *Z* isomer (Table 2, entry 3). Consequently, the 1,5-substitution of chiral enyne acetates is one of the few examples of efficient “remote stereocontrol” in organocopper chemistry.^[10]

Further examples examined by us impressively prove the general applicability of this method. Thus, the moderate stereoselectivities found in the reaction of the *n*-butyl-substituted enyne acetate (*S*)-**1b** with lithium di-*tert*-butylcyanocuprate were again raised to high values of 94 (*E* isomer) and 96 % *ee* (*Z* isomer) by addition of *n*Bu₃P (Table 2, entries 4–6). The same holds for the 1,5-substitution of the aromatic substrate (*S*)-**1c** (92/95 % *ee*; Table 2, entry 7, 8). Moreover, the method is not limited to the *tert*-butyl cuprate, as is demonstrated by the reaction of (*R*)-**1d** with *n*Bu₂CuLi · LiCN in the presence of tri-*n*-butylphosphane (99/99 % *ee*; Table 2, entry 9, 10). The transformations of the cyclic enyne acetate (*S*)-**1e** with the methyl cuprates Me₂CuLi · LiX (X = CN, I) revealed that the enantioselectivity of the 1,5-substitution is hardly affected by the copper salt used for the preparation of the cuprate (76 and 74 % *ee*; Table 2, entry 11, 12); again, addition of the ligand *n*Bu₃P caused a pronounced improvement of the stereoselectivity to 95 % *ee* (Table 2, entry 13). In contrast to this, addition of the phosphane led to a decrease of the selectivity from 72 to 46 % *ee* in the reaction of (*S*)-**1e** with *t*Bu₂CuLi · LiCN (Table 2, entry 14, 15). This surprising effect could not be explained rationally, but it is of no practical relevance since a

[*] Prof. Dr. N. Krause, Dr. M. Purpura
Lehrstuhl für Organische Chemie II der Universität
44221 Dortmund (Germany)
Fax: (+49) 231-755-3884
E-mail: nkrause@pop.uni-dortmund.de

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Table 2. Synthesis of nonracemic vinylallenes **2** by 1,5-substitution of enyne acetates **1** with organocuprates.

Entry	Acetate	Cuprate	T [°C]	Additive	Product	R ¹	R ²	R ³	R ⁴	E:Z	ee(E) [%]	ee(Z) [%]
1	(R)- 1a	<i>t</i> Bu ₂ CuLi · LiCN	−80	—	2a	Me	H	Me	<i>t</i> Bu	25:75	8	68
2	(R)- 1a	<i>t</i> Bu ₂ CuLi · LiCN	−90	—	2a	Me	H	Me	<i>t</i> Bu	25:75	20	74
3	(R)- 1a	<i>t</i> Bu ₂ CuLi · LiCN	−80	<i>n</i> Bu ₃ P	2a	Me	H	Me	<i>t</i> Bu	25:75	92	93
4	(S)- 1b	<i>t</i> Bu ₂ CuLi · LiCN	−80	—	2b	<i>n</i> Bu	H	Me	<i>t</i> Bu	33:67	6	72
5	(S)- 1b	<i>t</i> Bu ₂ CuLi · LiCN	−90	—	2b	<i>n</i> Bu	H	Me	<i>t</i> Bu	33:67	36	86
6	(S)- 1b	<i>t</i> Bu ₂ CuLi · LiCN	−80	<i>n</i> Bu ₃ P	2b	<i>n</i> Bu	H	Me	<i>t</i> Bu	33:67	94	96
7	(S)- 1c	<i>t</i> Bu ₂ CuLi · LiCN	−80	—	2c	Ph	H	Me	<i>t</i> Bu	40:60	74	95
8	(S)- 1c	<i>t</i> Bu ₂ CuLi · LiCN	−80	<i>n</i> Bu ₃ P	2c	Ph	H	Me	<i>t</i> Bu	40:60	92	95
9	(R)- 1d	<i>n</i> Bu ₂ CuLi · LiCN	−80	—	2d	Me ₃ Si	H	Me	<i>n</i> Bu	60:40	8	28
10	(R)- 1d	<i>n</i> Bu ₂ CuLi · LiCN	−80	<i>n</i> Bu ₃ P	2d	Me ₃ Si	H	Me	<i>n</i> Bu	60:40	99	99
11	(S)- 1e	Me ₂ CuLi · LiCN	−80	—	2e	<i>n</i> Bu	−(CH ₂) ₄ −	Me	—	—	76	—
12	(S)- 1e	Me ₂ CuLi · LiI	−80	—	2e	<i>n</i> Bu	−(CH ₂) ₄ −	Me	—	—	74	—
13	(S)- 1e	Me ₂ CuLi · LiI	−80	<i>n</i> Bu ₃ P	2e	<i>n</i> Bu	−(CH ₂) ₄ −	Me	—	—	95	—
14	(S)- 1e	<i>t</i> Bu ₂ CuLi · LiCN	−80	—	2f	<i>n</i> Bu	−(CH ₂) ₄ −	<i>t</i> Bu	—	—	72	—
15	(S)- 1e	<i>t</i> Bu ₂ CuLi · LiCN	−80	<i>n</i> Bu ₃ P	2f	<i>n</i> Bu	−(CH ₂) ₄ −	<i>t</i> Bu	—	—	46	—
16	(R)- 1e	<i>t</i> Bu ₂ CuLi · LiCN	−80	(EtO) ₃ P	2f	<i>n</i> Bu	−(CH ₂) ₄ −	<i>t</i> Bu	—	—	91	—

value of 91 % *ee* could be obtained in this case by addition of triethylphosphite.

We have already demonstrated that the 1,5-substitution can also be achieved efficiently with catalytic amounts of copper salts.^[6] These reaction conditions could also be applied to chiral, nonracemic substrates without problems. For example, slow addition of one equivalent of (R)-**1b** (94 % *ee*) and one equivalent of *t*BuLi to 10 mol % of *t*Bu₂CuLi · LiCN · 2 *n*Bu₃P at −50 °C gave vinylallene **2b** as a 33:67 *E/Z* mixture with 90 % *ee* for both isomers (80 % chemical yield). Thus, also this catalytic reaction takes place with efficient “remote stereocontrol”. We are currently examining the absolute configuration of the vinylallenes formed in enantioselective 1,5-substitutions and their application in organic synthesis.

Experimental Section

(EtO)₃P (2.0 g, 12.0 mmol) was added to a suspension of CuCN (537 mg, 6.0 mmol) in diethyl ether (30 mL) at 0 °C, and the mixture was allowed to warm to room temperature. After complete dissolution of the copper salt, the solution was cooled to −30 °C, and *t*BuLi (7.1 mL of a 1.7 M solution in pentane) was added dropwise. The cuprate solution was stirred for 15 min at −30 °C, then cooled to −80 °C, and (R)-**1e** (703 mg, 3.0 mmol; 99 % *ee*) in diethyl ether (30 mL) was added dropwise. After the mixture had been stirred for 1 h at −80 °C, saturated NH₄Cl solution (15 mL) was added and the mixture was filtered through Celite. In order to remove the phosphite, the filtrate was washed several times with 1 N sulfuric acid and then dried with MgSO₄. Removal of the solvent by distillation in vacuum and chromatography with silica gel (cyclohexane) furnished vinylallene **2f** (577 mg; 80 %) with $[\alpha]_D^{20} = +26$ (*c* = 0.1, Et₂O). An enantiomeric excess of 91 % *ee* was determined by gas chromatography with octakis(2,6-di-*O*-methyl-3-*O*-pentyl)- γ -cyclodextrin.

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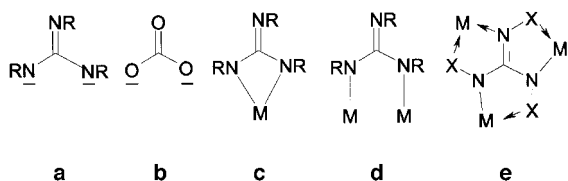
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A New Class of Easily Obtained Carbonate-Related μ_3 -Ligands and a Protein-Sized Doughnut-Shaped Coordination Oligomer**

Iris M. Müller and Richard Robson*

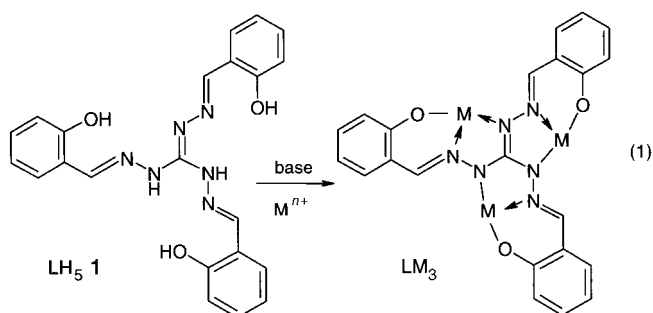
A possible way to achieve more robust coordination polymer frameworks is to put the powerfully stabilizing chelate effect to use in the bridging ligands.^[1] Presented herein is a strategy for the synthesis of a new class of easily accessible and widely variable ligands based on a central dianionic, carbonate-like CN_3 core that can bind three metal centers at chelating sites. Dianionic guanidates **a** ($\text{R} = \text{H}$, *i*Pr, *t*Bu, cyclohexyl, and phenyl) which are isoelectronic with



carbonate ions **b**, have been shown to form 4-membered chelate rings (**c**)^[2] or to bridge two metal ions (**d**).^[3] What we are proposing is that if appropriate donor centers are introduced into the three substituents on the CN_3 core, then it may be possible to chelate three metal centers strongly and symmetrically (**e**). With regard to optimizing metal–metal communication it is difficult to imagine any other system capable of simultaneously chelating three metal centers in a roughly planar fashion to provide a closer metal–metal contact than this.

Herein we report on the metal-binding properties of tris(2-hydroxybenzylidene)triaminoguanidine LH_5 (**1**), which is very easily made from triaminoguanidine and salicylaldehyde. The structure of **1** was determined by X-ray crystallography.^[4] Before exploring the coordination polymers afforded by this ligand, it appeared desirable and worthwhile to attempt to synthesize discrete molecular trimetallic derivatives to determine how easily, if at all, the intended central dianionic carbonate-like unit could be generated and whether or not three metal centers could be bound, as we hoped, in the manner LM_3 [Eq (1)].

Reaction of LH_6Cl with $(\text{Et}_4\text{N})_2[\text{PdCl}_4]$ in acetonitrile in the presence of Et_4NOH yielded dark red crystals of $(\text{Et}_4\text{N})_2[\text{LPd}_3\text{Cl}_3] \cdot \text{CH}_3\text{CN}$ (**2**). The chiral propeller-like structure of the $[\text{LPd}_3\text{Cl}_3]^{2-}$ ion, determined by X-ray crystallog-



raphy,^[5] is shown in Figure 1a. Diffusion of ammonia into an aqueous ethanolic solution containing LH_6Cl and ZnCl_2 gave yellow crystals of $[\text{LZn}_3(\text{NH}_3)_3(\text{H}_2\text{O})_3]\text{Cl} \cdot 3\text{H}_2\text{O}$ (**3**)^[6] the cationic component of which was found to have the structure shown in Figure 1b. Like the Pd derivative, it also adopts a

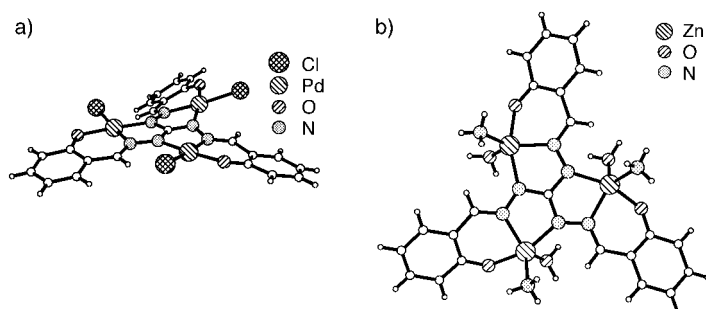


Figure 1. a) The structure of the $[\text{LPd}_3\text{Cl}_3]^{2-}$ ion in **2**. b) The structure of the $[\text{LZn}_3(\text{NH}_3)_3(\text{H}_2\text{O})_3]^+$ ion in **3**.

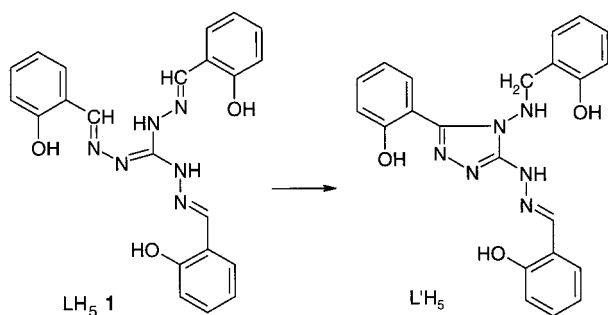
propeller-like conformation. The three NH_3 ligands are found on one face of the ion and the three H_2O ligands on the other; these ligands participate in an extensive hydrogen bonded network involving the lattice H_2O molecules and Cl^- counterions.

The wide scope offered by these types of LM_3 species as 3-connecting building blocks in the construction of networks is immediately apparent.^[7] One possibility would be to substitute the ligands external to the LM_3 core (such as the Cl ligands in the LPd_3 system or the NH_3 and H_2O ligands in the LZn_3 system) by appropriate bridging ligands linking one LM_3 unit to another. Another possibility would be to link two L^{5-} units directly to a single shared metal center. With regard to future attempts to obtain coordination networks it is reassuring to know that (as was revealed in the preparation of the zinc complex **3** above) a base as weak as ammonia is all that is required to generate the central carbonate-like dianionic CN_3 core.

Although the L^{5-} unit remains intact during the formation of the trimetallic complexes **2** and **3**, it does undergo secondary reactions under certain circumstances. Thus, upon heating with base, the azine of salicylaldehyde $((\text{HOC}_6\text{H}_4\text{CH}=\text{N})_2)$ is formed, which indicates fission of a C–N bond within the central guanidine CN_3 unit. In the presence of Cd^{II} and triethylamine cycloisomerization of the ligand occurs to produce a new triazole-containing ligand $\text{L}'\text{H}_5$. This ligand appears, in deprotonated form, as one

[*] Dr. R. Robson, Dr. I. M. Müller
School of Chemistry
University of Melbourne
Parkville, Victoria 3052 (Australia)
Fax: (+61) 3-9347-5180
E-mail: r.robson@chemistry.unimelb.edu.au

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component of a remarkable and very large coordinative oligomer (**4**), which resembles a doughnut in shape. The isomerization of LH_5 to $L'H_5$ involves oxidative cyclization to form a triazole ring accompanied by the reduction of one $CH=N$ link to CH_2-NH . A related cyclization has been reported.^[8] The oligomer **4** contains 24 Cd centers, 12 LH_2^{3-} , and 6 $L'H_2^{3-}$ ligands and has the composition $[[Cd_4(LH_2)_2(L'H_2)(H_2O)(DMF)](Et_3NH)]_6$.^[9] The asymmetric unit containing four different cadmium centers, two different LH_2^{3-} ligands and one $L'H_2^{3-}$ ligand is shown in Figure 2, together with a schematic representation to aid identification of the various components. The complete assemblage consists of six such asymmetric units arranged around a $\bar{3}$ axis (Figure 3); the resemblance to a doughnut is clear. The six Et_3NH^+ ions are buried inside the doughnut in crevices within the anionic oligomer, hydrogen bonded to an oxygen atom of the LH_2^{3-} ligand ($N \cdots O$, 2.73(1) Å). The



Figure 3. The structure of **4**.

large size of the oligomer ($M_w > 11\,000\text{ g mol}^{-1}$, diameter $> 30\text{ Å}$), external shape and internal folding of the assemblage are reminiscent of a protein; the system also has features in common with the large annular metal-oxo species reported recently by Müller et al.^[10] and with the coordinate “molecular loop” reported recently by Beer et al.^[11]

In conclusion, it is clear that these very easily obtained guanidine-based bridging ligands promise a rich coordination chemistry and access to a wide range of oligomers and polymers.

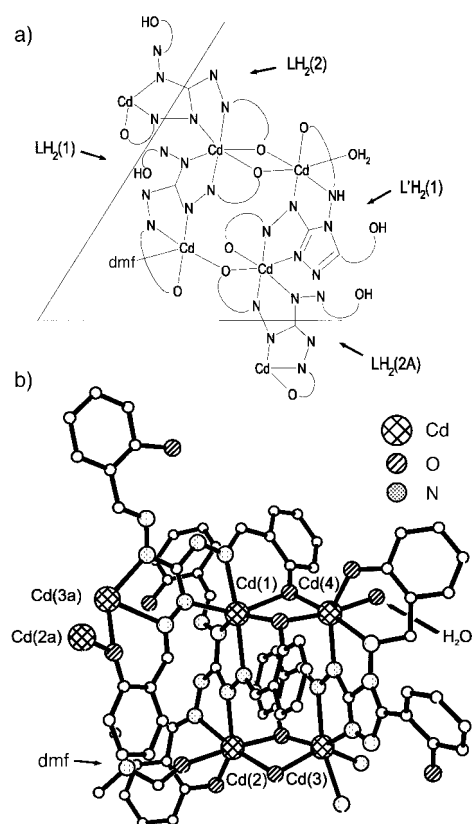


Figure 2. a) A schematic representation of the components present in the asymmetric unit of **4**. b) The structure of the asymmetric unit in **4**. The triethylammonium ion and the protons have been omitted for clarity.

Experimental Section

$[C_{22}H_{21}N_6O_3]Cl$ (**1**·HCl): Triaminoguanidinium chloride (4.215 g; 30 mmol), prepared as in ref[12] was dissolved in a hot mixture of H_2O (30 mL) and ethanol (60 mL). HCl was added until the pH = 3. The mixture was allowed to cool to room temperature and a solution of salicylaldehyde (11.1 mL, 0.10 mol) in ethanol (15 mL) was slowly added. The yellow mixture was warmed until the first precipitate formed. The mixture was stirred over night at room temperature and the crude product was recrystallized from methanol (180 mL) and water (70 mL). Yield: 10.406 g (23 mmol, 77 %); elemental analysis found (%): C 58.05, H 4.63, N 18.74; calcd C 58.34, H 4.67, N 18.56; ^1H NMR (400 MHz, CD_3OD): δ = 8.884, 8.015, 7.311, 6.934, 6.901, 3.339; ^{13}C NMR (100 MHz, CD_3OD): δ = 158.7, 150.8, 150.3, 133.9, 129.2, 120.9, 120.6, 117.2. Crystals suitable for X-ray crystallography were grown by slow diffusion of HCl gas into a solution of **1** in aqueous $NaHCO_3$ (pH 8).

$[(C_2H_5)_4N]_2[(PdCl)_3(C_{22}H_{15}N_6O_3)] \cdot CH_3CN$ (**2**): $PdCl_2$ (266.7 mg, 1.50 mmol) and Et_4NCl (527.3 mg, 3.19 mmol) were dissolved in acetonitrile (36 mL). A solution of **1**·HCl (226.3 mg, 0.50 mmol) in acetonitrile (30 mL) and Et_4NOH (1.7 mL, 25 % in water) was slowly added dropwise yielding a deep red solution. Na_2SO_4 (2 g) was added and the mixture stirred for 5 min to remove most of the water. The Na_2SO_4 was removed by filtration and the filtrate left to stand open to the air for two days resulting in the formation of dark red crystals of **2**. Yield: 384.1 mg (0.35 mmol, 70 %); elemental analysis found (%): C 42.26, H 5.25, N 10.89; calcd: C 42.20, H 5.13, N 11.07.

$[Zn(OH_2)(NH_3)]_3(C_{22}H_{15}N_6O_3)]Cl \cdot 3H_2O$ (**3**): $ZnCl_2 \cdot H_2O$ (61.2 mg, ~ 0.35 mmol) and **1**·HCl (48.8 mg, 0.11 mmol) were dissolved in water (4 mL) and ethanol (8 mL). After filtration ethanol (2 mL) was added. Slow diffusion of NH_3 into the reaction mixture resulted in the formation of yellow crystals of **3** within 3 days. Yield: 45.8 mg (0.057 mmol, 52 %); elemental analysis found (%): C 32.89, H 4.70, N 15.71; calcd: C 32.94, H 4.52, N 15.72.

[C₄₅₀H₄₅₆Cd₂₄N₁₂₀O₆₆] · x H₂O · y DMF (**4**): Cd(NO₃)₂ · 4 H₂O (48.3 mg, 0.16 mmol) and **1** · HCl (46.0 mg, 0.10 mmol) were dissolved in DMF (10 mL). Water (10 mL) followed by triethylamine (2 mL) in methanol (8 mL) were carefully layered on top. Over a period of two months orange crystals of **4** grew. Yield: 6.2 mg (0.55 × 10⁻³ mmol, 8.2 %); elemental analysis found (%): C 45.51, H 3.85, N 14.46; calcd (for x = 30, y = 10): C 45.82, H 4.66, N 14.48.

Crystallography: Intensity data were collected on an Enraf-Nonius CAD4 diffractometer employing the ω -2 θ scan method, the data were corrected for Lorentz and polarization effects. An empirical absorption correction was applied for **2** and **4**.^[13] All structures were solved using direct methods (SHELXS-97)^[14] and refined using a full-matrix least-squares refinement procedure (SHELXL-97).^[15] The data of **4** were corrected using the "SQUEEZE" routine in PLATON.^[16] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-146818–146821 for **1**, **2**, **3**, and **4**, respectively. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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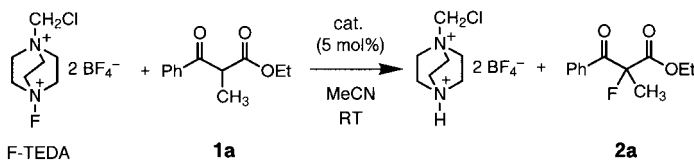
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- [4] 0.10 × 0.10 × 0.10 mm³, triclinic, *P* $\bar{1}$, *a* = 8.905(2), *b* = 9.807(2), *c* = 13.086(3) Å, α = 88.10(3), β = 78.50(3), γ = 83.89(3)°, *V* = 1113.5(4) Å³, ρ_{calcd} = 1.378 g cm⁻³, $2\theta_{\text{max}}$ = 120°, λ = 1.54178 Å, *T* = 293 K, 3774 measured reflections, 3278 independent reflections (*R*_{int} = 0.0359), μ = 1.856 mm⁻¹, 340 parameters, *R*₁ = 0.0678, *wR*₂ = 0.1880, max. residual electron density 0.354 e Å⁻³.
- [5] 0.33 × 0.14 × 0.03 mm³, triclinic, *P* $\bar{1}$, *a* = 12.848(1), *b* = 13.0504(6), *c* = 15.158(2) Å, α = 92.667(7), β = 102.81(1), γ = 110.512(6)°, *V* = 2299.6(5) Å³, ρ_{calcd} = 1.644 g cm⁻³, $2\theta_{\text{max}}$ = 120°, λ = 1.54178 Å, *T* = 293 K, 7858 measured reflections, 6818 independent reflections (*R*_{int} = 0.0568), μ = 11.343 mm⁻¹, min./max. transmission factors 0.141/0.698, 591 parameters, *R*₁ = 0.0669, *wR*₂ = 0.1424, max. residual electron density 1.219 e Å⁻³.
- [6] 0.54 × 0.46 × 0.34 mm³, trigonal (hexagonal settings), *R* $\bar{3}$, *a* = 16.310(2), *c* = 21.552(3) Å, *V* = 4965(1) Å³, ρ_{calcd} = 1.610 g cm⁻³, $2\theta_{\text{max}}$ = 54.9°, λ = 0.71073 Å, *T* = 140 K, 3173 measured reflections, 2537 independent reflections (*R*_{int} = 0.0273), numerical absorption correction, μ = 2.296 mm⁻¹, min./max. transmission factors 0.314/0.490, 181 parameters, *R*₁ = 0.0391, *wR*₂ = 0.1204, max. residual electron density 1.141 e Å⁻³.
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- [9] 0.46 × 0.43 × 0.34 mm³, trigonal (hexagonal settings), *R* $\bar{3}$, *a* = 33.838(7), *c* = 46.23(1) Å, *V* = 45845(18) Å³, ρ_{calcd} = 1.227 g cm⁻³, $2\theta_{\text{max}}$ = 100°, λ = 1.54178 Å, *T* = 130 K, 11987 measured reflections, 10394 independent reflections (*R*_{int} = 0.1104), μ = 7.051 mm⁻¹, min./max. transmission factors 0.120/0.264, 473 parameters, *R*₁ = 0.1064, *wR*₂ = 0.3105, max. residual electron density 0.385 e Å⁻³.
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Catalytic Enantioselective Fluorination of β -Ketoesters**

Lukas Hintermann and Antonio Togni*

Although in nature organofluorine metabolites are extremely rare, bioactive fluoroorganic compounds are eminently important in medicinal chemistry.^[1] Therefore, it is not surprising that research in the field of synthetic organofluorine chemistry is flourishing more than ever.^[2, 3] Protocols for the introduction of the fluorine atom into organic molecules rely upon the use of dozens of different reagents,^[4] the most successful of which contain the N–F fragment.^[5, 6] Methods for the enantioselective fluorination (C–F bond-forming reactions) of organic molecules are quite rare, and catalytic methods are not known. An important breakthrough occurred when Differding and Lang reported the first example of enantioselective electrophilic fluorination of a β -ketoester enolate in up to 70 % *ee* by using an *N*-fluorosultam derived from camphor.^[7] This strategy, requiring the intermediate generation of the enolate from an activated methylene compound, has been further developed more recently.^[8–13] Another impetus in this field came also from the discovery and commercial availability of new N–F reagents, such as 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis[tetrafluoroborate] (F-TEDA; also called Selectfluor, see Scheme 1; TEDA = triethylenediamine).^[14, 15] However, the total absence of an efficient catalytic reaction for stereoselective fluorination prompted us to embark in a study aimed at developing such a reaction. Recent reports had indicated



Scheme 1. The catalytic fluorination of **1a** with F-TEDA.

[*] Prof. Dr. A. Togni, L. Hintermann
Laboratory of Inorganic Chemistry
Swiss Federal Institute of Technology, ETH Zentrum
8092 Zürich (Switzerland)
Fax: (+41) 1-632-1090
E-mail: togni@inorg.chem.ethz.ch

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that N–F reagents were capable of fluorinating unactivated ketones in the α -position in refluxing acetonitrile, whereas activated ketones, notably β -ketoesters and β -diketones, were already fluorinated at room temperature.^[16] As these reactions proceed via the enol form of the substrate, we reasoned that substoichiometric amounts of a Lewis acid might accelerate the reaction by catalyzing the enolization process.^[17] Thus, we started a systematic study with model compound **1a** (Scheme 1), a monosubstituted β -ketoester, and found that it does not react with a saturated solution of F-TEDA in acetonitrile at room temperature, in accordance with its low enol content (<0.5% in CD₃CN by ¹H NMR spectroscopy). However, the addition of a range of Lewis acids^[18, 19] (5 mol % level) effectively catalyzed the reaction (Table 1).

Table 1. Qualitative ordering of catalytic activity of several Lewis acids for the reaction shown in Scheme 1.^[a]

Very fast (<1 h)	Fast (<1 d)	Slow (≤2 w)	Very slow or no reaction (>2 w)
TiCl ₄	[CpTiCl ₃]	[Cp ₂ Ti(OTf) ₂]	[Cp ₂ TiCl ₂]
AlCl ₃	[TiCl ₂ (diolato)]	HBF ₄	HCl
		BF ₃	ZnCl ₂
		Me ₃ SiOTf	Cu(ClO ₄) ₂
			[Cp ₂ Zr(OTf) ₂]
			TiF ₄
			TaCl ₅
			Yb(OTf) ₃

[a] OTf = trifluoromethanesulfonate.

It is evident that titanium-based Lewis acids constitute the most potent catalysts, with TiCl₄ driving the reaction to completion in less than 1 h at room temperature. Since many chiral, enantiopure Lewis acidic Ti complexes are known, we were in the comfortable situation of being able to screen a large number of known catalysts^[20] for the enantioselective version of this reaction. We found that with 5 mol % of in situ prepared [TiCl₂(*R,R*-TADDOLato)] ((*R*)-**3a**)^[21] as catalyst, a quite fast reaction of racemic β -ketoester **1a** with F-TEDA took place (quantitative conversion in less than 5 h), yielding the fluoro- β -ketoester **2a** in good yield and with 28% *ee*. Notably, [TiCl₂(TADDOLato)] catalysts have been extensively used by Seebach and others^[22] but these were typically prepared in situ and not used in isolated form. We found that the two prototypical catalysts (*R*)-**3a** and (*R*)-**3b**,^[23] that have been mainly exploited for this study as catalysts, may be isolated in high yields (85–90%) as air-stable highly crystalline materials as dimethoxyethane (DME) or acetonitrile adducts, respectively, in the latter case with a varying number of acetonitrile molecules per titanium. Furthermore, these isolated materials gave more reliable results than the corresponding forms generated in situ, in terms of both reproducibility and enantioselectivity of catalytic experiments (see below). Compound [(*R*)-**3a**(DME)] and the bis(acetonitrile) adduct [(*R*)-**3b**(NCMe)₂] were characterized by X-ray crystallography^[24] and ORTEP views of the octahedral complexes are shown in Figure 1.

The results obtained by using the isolated forms of (*R*)-**3a** or (*R*)-**3b** as catalyst precursors for the fluorination of

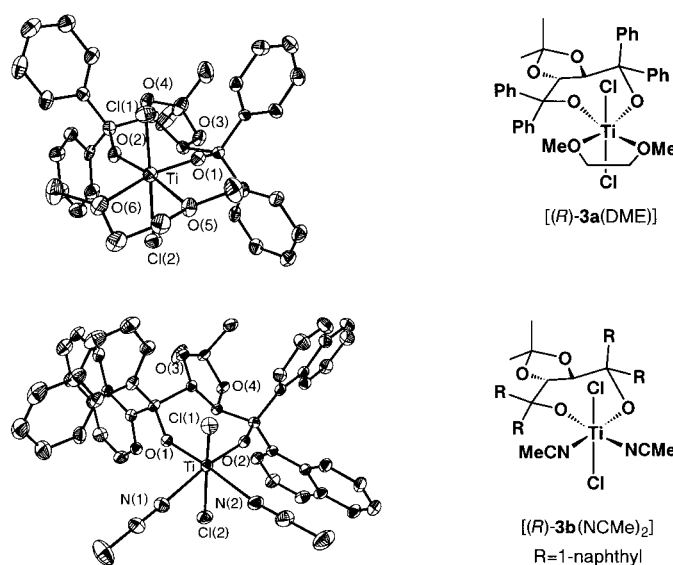


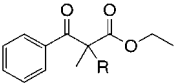
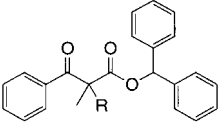
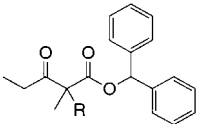
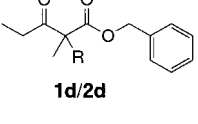
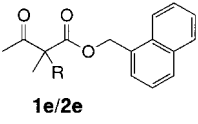
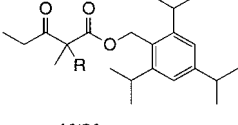
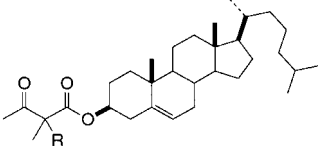
Figure 1. Structures of the complexes [(*R*)-**3a**(DME)] and [(*R*)-**3b**(NCMe)₂] (ORTEP views; hydrogen atoms are omitted for clarity). Selected bond lengths [Å] and angles [°]: [(*R*)-**3a**(DME)]: Ti–O(1) 1.752(5), Ti–O(2) 1.765(5), Ti–O(5) 2.224(5), Ti–O(6) 2.164(6), Ti–Cl(1) 2.337(2), Ti–Cl(2) 2.342(3), O(1)–Ti–O(2) 98.6(2), O(1)–Ti–O(5) 90.9(2), O(2)–Ti–O(6) 96.8(2), O(5)–Ti–O(6) 73.7(2), Cl(1)–Ti–Cl(2) 164.97(10); [(*R*)-**3b**(NCMe)₂]: Ti–O(1) 1.780(3), Ti–O(2) 1.773(3), Ti–N(1) 2.283(4), Ti–N(2) 2.264(4), Ti–Cl(1) 2.3343(13), Ti–Cl(2) 2.3622(13), O(1)–Ti–O(2) 97.18(12), O(1)–Ti–N(1) 90.74(13), O(2)–Ti–N(2) 88.86(13), N(1)–Ti–N(2) 83.24(14), Cl(1)–Ti–Cl(2) 162.11(5).

different β -ketoesters are presented in Table 2. We so far restricted this reaction to substrates bearing an α -methyl group, thus leading to products having a quaternary stereogenic center. The reactions have been typically conducted at room temperature in a closed vessel and with a slight excess of a saturated F-TEDA solution in acetonitrile (this proved to be more reactive than, e.g., *N*-fluorobenzenesulfonimide or *N*-fluoropyridinium tetrafluoroborate). Products were isolated in analytically pure form after column chromatography, in all cases in yields between 80% and more than 95%.^[25] It is apparent from Table 2 that the steric bulk of the catalyst is important with regard to the stereoselectivity. Thus, the better enantioselectivities for all substrates under study were obtained by using catalysts **3b**, bearing 1-naphthyl groups. Also the nature of the ester group clearly influences enantioselectivity; the best results have been obtained with the substituted benzyl ester of substrate **2f** (90% *ee*; using the in situ prepared catalyst a maximum of 85% *ee* was obtained). This level of stereoselectivity compares favorably with the highest enantioselectivity obtained by using chiral enantio-pure fluorinating agents.

The fluorination of cholesteryl ester **2g** using [CpTiCl₃] as catalyst gave a diastereoisomeric product ratio of 55:45. This poor selectivity was gradually improved in the reactions catalyzed by the complexes (*R*)-**3a** (60:40), (*R*)-**3b** (80:20), and (*S*)-**3b** (16:84), indicating that for this doubly stereodifferentiating reaction both the sense and the extent of asymmetric induction are dominated by the catalyst.

We assume that the interaction of the β -ketoester with the catalyst triggers an enolization reaction, and that the coordinated enol or enolate is the reactive form of the substrate,

Table 2. Selected results of catalytic enantioselective fluorination reactions using isolated $[\text{TiCl}_2(\text{TADDOLato})]$ complexes.

1 (R = H) (racemic) 2 (R = F)	Selectivity (Reaction time)		$[\alpha]_D^{20}(\mathbf{2})^{[a]}$ [<i>ee</i> [%]]	$\delta(^{19}\text{F})(\mathbf{2})^{[b]}$	HPLC analysis ^[c]	
	cat. = (<i>R</i>)- 3a	cat. = (<i>R</i>)- 3b				
 1a/2a	28% <i>ee</i> (<i>S</i>) (4 h)	62% <i>ee</i> (40 min)	+ 53.8 (<i>c</i> = 0.545) [61.7]	− 152.3	OB 0.5	96/4 13.5/16.0
 1b/2b	59% <i>ee</i> (1 d)	82% <i>ee</i> (1 d)	+ 19.0 (<i>c</i> = 1.83) [56]	− 152.5	OJ 1	95/5 14.1/18.9
 1c/2c	58% <i>ee</i> (2 h)	81% <i>ee</i> (20 min)	+ 38.2 (<i>c</i> = 1.02) [81.2]	− 159.5	OJ 0.25	70/30 36.1/39.4
 1d/2d	48% <i>ee</i> (15 min)	71% <i>ee</i> (< 7 min)	+ 32.5 (<i>c</i> = 0.905) [70.8]	− 159.5	OJ 1	96/4 11.7/15.3
 1e/2e	51% <i>ee</i> (< 15 min)	68% <i>ee</i> (< 15 min)	+ 19.6 (<i>c</i> = 1.14) [68.8]	− 157.4	OJ 1	96/4 24.0/27.7
 1f/2f	55% <i>ee</i> (1 h)	90% <i>ee</i> (< 15 min)	+ 24.1 (<i>c</i> = 1.11) [85.6]	− 159.2	OD-H 0.3	99.8/0.2 27.8/29.8
 1g/2g	d.r. = 60:40	d.r. = 80:20 ^[d]	n.d. ^[e]	− 157.3 ^[f]	–	–

[a] Measured in MeOH at room temperature on a sample of given *ee* [value in square brackets]. [b] Measured in CDCl_3 , relative to CFCl_3 . [c] Daicel Chiralcel 25 cm column type; solvent mixture: hexane/*i*PrOH (v/v); flow rate in mL min^{-1} . Retention times (HPLC) in min of *minor/major* enantiomer (UV detection at $\lambda = 210$ nm and 254 nm). [d] (*S*)-**3b**: d.r. = 16:84. [e] Not determined. [f] The difference of the shifts between the two diastereomers is $\Delta\delta = 0.01$.

susceptible to external electrophilic attack by the fluorinating agent. Thus, the role of the Lewis acid consists in activating the nucleophile and not, as it is more common in reactions involving carbonyl compounds, in enhancing the electrophilicity of the coordinated carbonyl group. We are currently exploring these mechanistic aspects, in order to better understand this quite unique and novel catalytic reaction. Furthermore, the extension of this convenient and expeditious fluorination process to other enolizable substrates, as well as to the corresponding asymmetric chlorination reaction^[26] using *N*-chlorosuccinimide (NCS) are the object of current and future work.^[27]

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- [24] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-145414 ([(R)-**3a**(DME)]) and CCDC-145415 ([(R)-**3b**(NCME)₂]). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [25] A representative experimental procedure is as follows: Complex **3b** (10.3 mg, 0.0125 mmol) was added to a solution of β -ketoester **1d** in acetonitrile (0.25 mmol in 1 mL), and the mixture was stirred until a clear yellow solution was obtained (5 min). To this was added a saturated F-TEDA solution (2.0 mL; 0.145 mol L⁻¹; 0.29 mmol) in MeCN. After 7 min, TLC (TBME/hexane 1/5, *R_f*(starting material) = 0.36, *R_f*(product) = 0.50; detection by UV and/or phosphomolybdic acid; TBME = *tert*-butyl methyl ether) indicated complete conversion. The reaction mixture was extracted with water (50 mL) and TBME (30 mL), the organic phase was filtered through Al₂O₃ (1 g; washed with additional TBME) and evaporated. Liquid chromatography (TBME/hexane 1/20) gave **2d** (49 mg; 82 %) of as colorless oil (70.8 % ee by HPLC).
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Octahedral SeO₆⁶⁻ and Square-Pyramidal SeO₅⁴⁻, Two New Oxoselenate Anions**

Helmut Haas and Martin Jansen*

For kinetic as well as thermodynamic reasons cations in tetrahedral and octahedral complexes usually show a lower Lewis acidity than in three- or fivefold coordination. We have therefore considered the main driving force for the surprisingly smooth acid–base reaction of the addition of O²⁻ to SeO₄²⁻ in the synthesis of Li₄SeO₅^[1] to be the formation of a collective solid-state structure A₄A'B₅, thus the order variant of an AB structure type,^[2] and the associated gain in lattice energy. Also the unexpected coordination polyhedron (CN = 5, trigonal-bipyramidal) was attributed to this overriding structure-chemical argument. That such explanations are not applicable, is confirmed by the preparation of two other oxoselenates, for which at first glance there are no structural features apparent that stabilize a hexaoxo- or pentaoxo anion.

For the Na₂O/Na₂SeO₄ system the solid-state reaction at a molar ratio of the starting materials of 1:2^[3] under standard pressure gave Na₆Se₂O₉, which contains hexaoxoselenate(vi) and tetraoxoselenate(vi) anions, whereas at a ratio of 1:1 under hydrostatic pressure Na₄SeO₅ was formed. The single-crystal structure analysis^[4] confirmed in the first case that according to the formulation Na₁₂(SeO₆)(SeO₄)₃ an octahedral orthoselenate anion had been obtained for the first time (Figure 1). This displays the point symmetry *O_h* within the

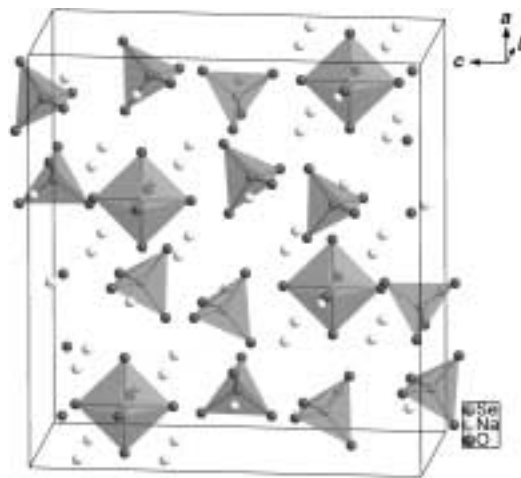


Figure 1. Crystal structure of Na₆Se₂O₉ (perspective representation).

margins of error of the structure determination. The dimensions of the tetrahedral SeO₄²⁻ ions ($\bar{d}_{\text{Se-O}} = 163.6$ pm, O–Se–O = 109.53°) are comparable with those of already known oxoselenates(vi) with tetrahedral anions (cf. Na₂SeO₄^[6] with $\bar{d}_{\text{Se-O}} = 164.8$ pm). The increase of the average Se–O bond

[*] Prof. Dr. M. Jansen, Dipl.-Chem. H. Haas
Max-Planck-Institut für Festkörperforschung
Heisenbergstrasse 1, 70569 Stuttgart (Germany)
Fax: (+49) 711-689-1502
E-mail: martin@jansen.mpi-stuttgart.mpg.de

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lengths with the increase of the coordination number from 4 (SeO_4^{2-}) to 6 (SeO_6^{6-}) by 11.8% corresponds to that for the transition from WO_4^{2-} to WO_6^{6-} (12.2%). The highly charged SeO_6^{6-} ion is stabilized by eight sodium ions, one located above each face, and by an additional six sodium ions, one located at each corner of the octahedron. The resulting unit $\text{SeO}_6\text{Na}_8\text{Na}_6'$ (Figure 2) resembles a $\text{M}_6\text{X}_8\text{X}_6'$ building block

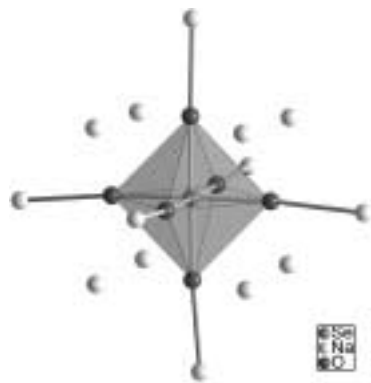


Figure 2. $(\text{SeO}_6)\text{Na}_8\text{Na}_6'$ building block in $\text{Na}_6\text{Se}_2\text{O}_9$. Bond lengths [pm] and angles [°]: Se1–O3 180.8(2) $2 \times$, Se1–O4 181.4(2), Se1–O6 182.4(2), Se1–O9 183.8(2), Se1–O7 184.3(2); O3–Se1–O3 179.16(11), O3–Se1–O4 90.27(6) $2 \times$, O3–Se1–O6 89.73(6) $2 \times$, O4–Se1–O6 179.69(11), O3–Se1–O9 90.32(5) $2 \times$, O4–Se1–O9 89.49(11), O6–Se1–O9 90.20(11), O3–Se1–O7 89.68(5) $2 \times$, O4–Se1–O7 90.29(12), O6–Se1–O7 90.02(12), O9–Se1–O7 179.77(11).

from transition metal cluster chemistry.^[7] In analogy to MoCl_2 these are linked through four common sodium atoms (Na') to give layers (Figure 3). In contrast to the Cl bridges in $\text{Mo}_6\text{Cl}_8\text{Cl}_6'$, the Na bridges here are almost linear. The SeO_4^{2-} ions are located in the holes between four $(\text{SeO}_6)\text{O}_8$ cubes and above and below the bridging sodium ions.

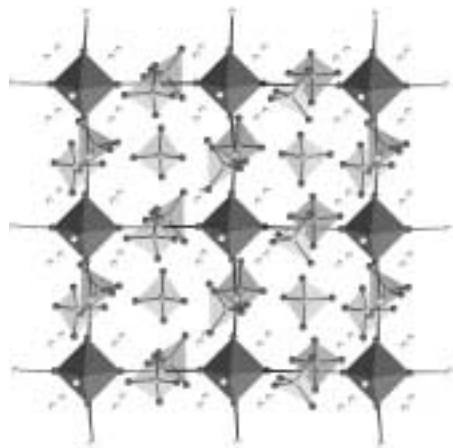


Figure 3. Layer of $\text{Na}_6\text{Se}_2\text{O}_9$.

Na_4SeO_5 only forms if a hydrostatic pressure of at least 2.5 GPa is used in the solid-state reaction. It precipitates in the form of a microcrystalline powder, thus the structure was determined by X-ray powder diffraction methods^[8,9] (Figure 4). According to the structure analysis a new type of structure is present in which square-pyramidal SeO_5^{4-} ions are observed for the first time (Figure 5). These are, however, so

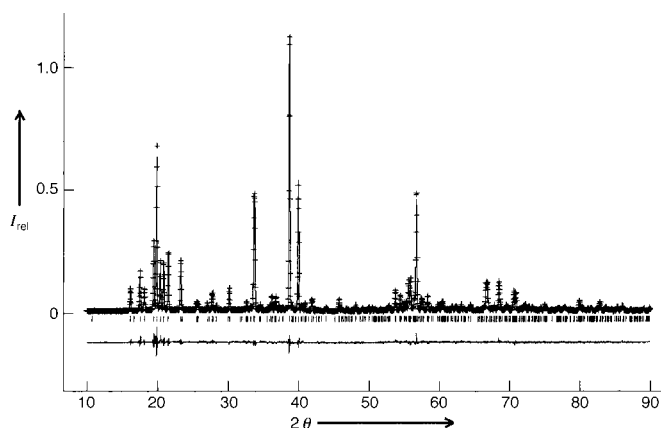


Figure 4. Powder diffraction diagram of Na_4SeO_5 . Top: Measured (data points) and calculated intensities (solid line), bottom: difference plot.

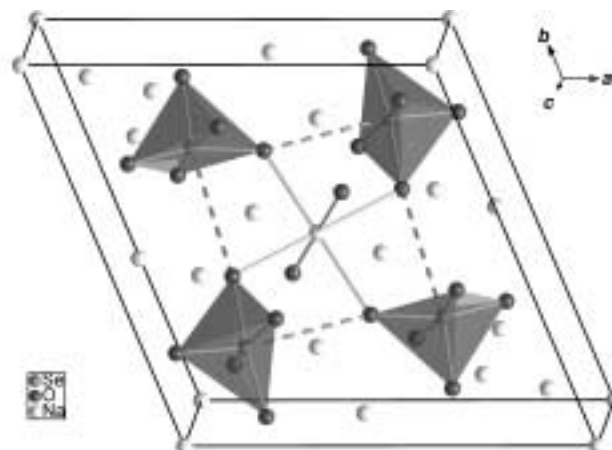


Figure 5. Crystal structure of Na_4SeO_5 . Bond lengths [pm]: Se1–O1 172.8(8), Se1–O2 173.2(8), Se1–O6 181.3(7), Se1–O8 176.5(7), Se1–O10 176.6(7), Se2–O3 181.5(7), Se2–O4 177.2(8), Se2–O5 172.9(8), Se2–O7 172.9(8), Se2–O9 178.0(8).

distorted that they can hardly be described with the ideal point symmetry C_{4v} . The increase of the average Se–O bond lengths with the increase of the coordination number from 4 (SeO_4^{2-}) to 5 (SeO_5^{4-}) by 8.0% lies significantly below the value for the octahedral SeO_6^{6-} ion and in the same range as for the trigonal-bipyramidal SeO_5^{4-} ion (6.7%). The SeO_5^{4-} ions are arranged in the structure such that four selenium atoms span a near ideal square with an edge length of 510.1 pm, and the polyhedra are directed so that an oxygen atom from the basal plane of one square pyramid is directed toward the selenium atom of a neighboring polyhedron (Figure 5). This appears to indicate a 5+1 coordination at selenium, however, the shortest Se–O distance between two polyhedra (330.2 pm) lies above that of the van der Waals distance. That, nevertheless, a bonding interaction is present, is evident from the fact that this oxygen atom displays the longest Se–O distance in the SeO_5 polyhedron. The polyhedral arrangement can be derived from $(\text{NbF}_5)_4$,^[10] in which the four corner-shared NbF_6 octahedra likewise span a square. If this square is stretched isotropically and it is assumed that each polyhedron retains one of the two bridging atoms in its coordination sphere, a polyhedral arrangement is achieved such as that found in Na_4SeO_5 . The positions of the selenium,

sodium, and four of the oxygen atoms can be described in a monoclinic space group, and the unit cell can be transformed accordingly into a monoclinic one. Only one oxygen position is not consistent with this crystallographic description. The knowledge of the structure of the isotopic compound Na_4MoO_5 ,^[11] however, substantiates the structure analysis and clarifies the observed pseudosymmetry in favor of the low symmetry space group in the triclinic system.

Experimental Section

$\text{Na}_6\text{Se}_2\text{O}_9$ was prepared by the solid-state reaction of Na_2O with Na_2SeO_4 in a molar ratio of 1:2 in a closed silver crucible sealed in a glass ampule. Na_4SeO_5 was prepared by the solid-state reaction of Na_2O with Na_2SeO_4 in a molar ratio 1:1 in a sealed silver tube under a pressure of 2.5 GPa (piston-cylinder-press^[12]). In both cases, the reaction time was two days and the reaction temperature was 500 °C. Na_2O was prepared according to reference [13], and Na_2SeO_4 (purum p.a. > 99%, Fluka) was dried under vacuum prior to reaction (10^{-3} mbar, 400 °C, 24 h).

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Total Synthesis of (+)-Ratjadone**

Mathias Christmann, Ulhas Bhatt, Monika Quitschalle, Eckhard Claus, and Markus Kalesse*

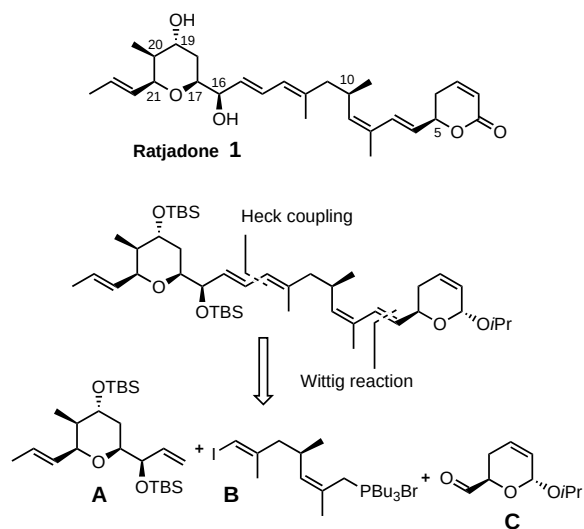
Ratjadone (**1**) was isolated in 1994 by Höfle et al. from *Sorangium cellulosum* (So ce360) collected as a soil sample at Cala Ratjada (Mallorca, Spain).^[1] It is similar in both structure and its biological profile to polyketides like leptomycin,^[2] callistatin A,^[3] and other related compounds.^[4] Ratjadone shows high cytotoxicity in cultured mouse cell lines (L929) with an IC_{50} value of 50 pg mL⁻¹ and it inhibits the growth of the HeLa cell line (KB3.1) at remarkably low concentrations (40 pg mL⁻¹). In addition, it shows other interesting biological effects (e.g. changes in the cell morphology and growth of yeast) and a narrow spectrum of antifungal activity with MIC values (MIC = minimal inhibiting concentration) ranging from 0.04 to 0.6 µg mL⁻¹ for *Mucor hiemalis*, *Phytophthora drechsleri*, *Ceratocystis ulmi*, and *Monila brunnea*.^[5] Despite these promising biological properties, neither the exact mode of action nor the molecular targets are known.

Here we report the first total synthesis of ratjadone.^[6] Our highly convergent approach allows the rapid assembly of various ratjadone derivatives for the identification of biologically active substructures. Since only the relative stereochemistry of the tetrahydropyran moiety (C17–C21) was known at the outset of this synthesis, it was necessary to design a flexible strategy that would allow the formation of all possible stereoisomers.^[7] The structure of ratjadone reveals the diene systems as attractive sites for the retrosynthetic disassembly (Scheme 1). We dissected ratjadone into three fragments, the tetrahydropyran subunit **A**, the phosphonium salt **B**, and the aldehyde **C**. In the synthesis, fragments **B** and **C** were joined by a Wittig reaction followed directly by a Heck coupling for the connection with the **A** fragment.

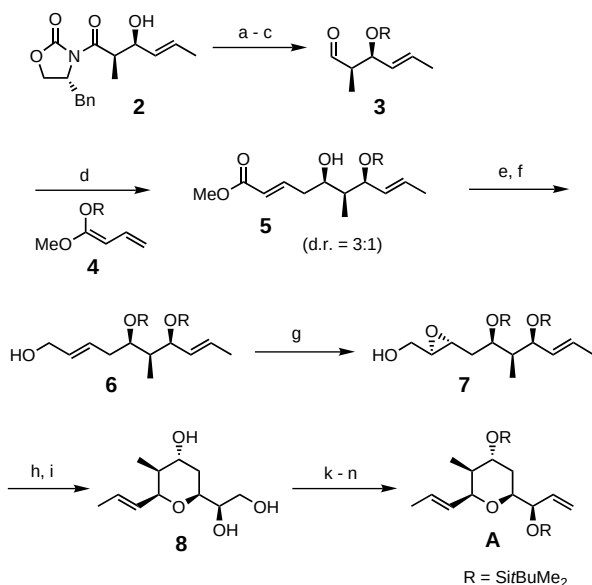
The synthesis of **A** began with the transformation of the known aldol product **2**^[8] into the Weinreb amide^[9] followed by protection of the hydroxy group (TBS) and subsequent reduction with Dibal-H to generate aldehyde **3** (Scheme 2). The all-*syn* stereo triad (C19, C20, C21) of the **A** fragment was generated by a vinylogous Mukaiyama aldol reaction.^[10] The reaction of **3** with the ketene acetal **4** and $\text{BF}_3 \cdot \text{OEt}_2$ as the Lewis acid gave the α,β -unsaturated ester **5** with a selectivity of 3:1 in favor of the desired Felkin–Ahn product. After silylation of the hydroxy group and reduction of the ester functionality, the allylic alcohol **6** was diastereoselectively epoxidized ($de > 95\%$ in favor of the *anti* isomer) with *m*CPBA.^[11] Removal of the TBS protecting groups with TBAF was followed by treatment with amberlyst-15 to induce the intramolecular 6-*exo* ring closure.^[12] This key step generates

[*] Priv.-Doz. Dr. M. Kalesse, M. Christmann, Dr. U. Bhatt, M. Quitschalle, Dr. E. Claus
Institut für Organische Chemie der Universität Hannover
Schneiderberg 1B, 30167 Hannover (Germany)
Fax: (+49) 511-762-3011
E-mail: Kalesse@mbox.oci.uni-hannover.de

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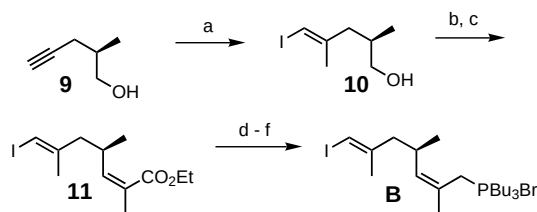
Scheme 1. Retrosynthetic analysis of ratjadone. TBS = *tert*-butyldimethylsilyl.



Scheme 2. Synthesis of the **A** fragment. a) MeONHMe·HCl, Me₃Al, CH₂Cl₂, –20 → 25 °C; b) TBSOTf, 2,6-lutidine, CH₂Cl₂, –78 °C; c) Dibal-H, THF, –78 °C, 83 % (over three steps); d) BF₃·Et₂O, CH₂Cl₂, Et₂O, –78 °C, 67 %; e) TBSOTf, 2,6-lutidine, CH₂Cl₂, –78 °C; f) Dibal-H, THF, –78 °C; g) *m*CPBA, NaHCO₃, CH₂Cl₂, 0 °C, 85 % (over three steps); h) TBAF, THF, 88 %; i) amberlyst-15, THF, 93 %; k) TBSOTf, 2,6-lutidine, CH₂Cl₂, –78 °C, 87 %; l) CHCl₃·HCl, 97 %; m) Dess–Martin periodinane, CH₂Cl₂, 0 °C, 92 %; n) Tebbe reagent, THF, 0 °C, 95 %. Dibal-H = diisobutylaluminum hydride; *m*CPBA = *m*-chloroperoxybenzoic acid; TBAF = tetra-*n*-butylammonium fluoride; TBSOTf = *tert*-butyldimethylsilyltri-fluoromethanesulfonate; Tebbe reagent = [Cp₂Ti(μ -CH₃)(μ -Cl)AlMe₂].

the tetrahydropyran moiety in fragment **A** and also establishes the C16-OH in *R* configuration. Protection of all three hydroxy groups (TBS) and treatment with acidic CHCl₃^[13] liberated the primary hydroxy group selectively, whose oxidation with Dess–Martin periodinane^[14] followed by olefination with the Tebbe reagent^[15] provided the **A** fragment.

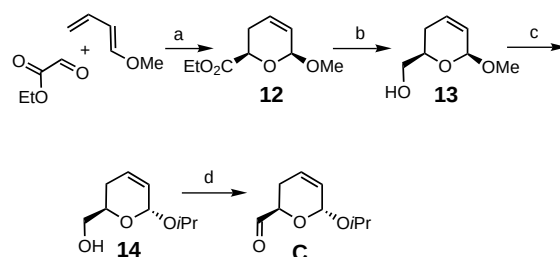
The synthesis of the **B** fragment commences with alkyne **9**,^[16] derived from (*S*)-3-hydroxyisobutyric acid (Scheme 3). Carbometalation of **9** and subsequent quenching with iodine



Scheme 3. Synthesis of the **B** fragment. a) [Cp₂ZrCl₂], AlMe₃, I₂, CH₂Cl₂, THF, –15 °C → 25 °C, 83 %; b) Dess–Martin periodinane, CH₂Cl₂, 0 °C, 81 %; c) (CF₃CH₂O)₂P(O)CHMeCO₂Et, KHMDS, [18]crown-6, THF, –78 °C, 85 %; d) Dibal-H, CH₂Cl₂, –78 °C, 77 %; e) CBr₄, Ph₃P, CH₃CN; f) Bu₃P, CH₃CN, 87 % (over two steps). KHMDS = potassium hexamethyldisilazane.

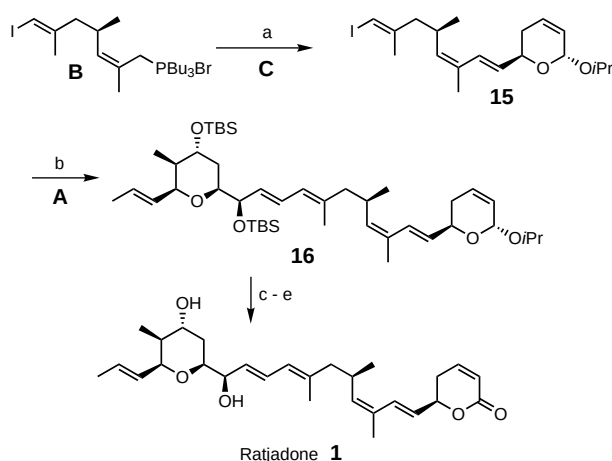
according to the protocol developed by Negishi et al.^[17] generated vinyl iodide **10**. The trisubstituted *Z*-double bond was introduced by Dess–Martin oxidation, followed by Still–Gennari olefination.^[18] After the reduction of the ester **11** with Dibal-H, the allyl alcohol was initially transformed into a bromide (CBr₄, PPh₃) and then converted to the corresponding phosphonium salt **B** with tributylphosphane.^[3b]

The key step in the construction of fragment **C** is a hetero-Diels–Alder reaction^[19] between 1-methoxy butadiene and ethyl glyoxalate in which the chiral Lewis acid generated from (+)-BINOL and Ti(O*i*Pr)₄ was used as catalyst (10 mol %) (Scheme 4). The hetero-Diels–Alder reaction generated compound **12** as a mixture of *endo* and *exo* adducts (56 % *de* for the *endo* adduct) in high enantioselectivity (>90 % *ee*); the desired *R* configuration at C5 was favored in both diastereomers. Reduction of the resulting ester with LiAlH₄ furnished alcohol **13**. During the course of our investigations it became clear that the β -anomer led to substantial epimerization at C5 in the Wittig reaction of aldehyde **C**. We therefore converted the β -anomer into the thermodynamically more stable α -anomer (**14**) by substituting the methoxy group for an isopropoxy group.^[20] Swern oxidation^[21] of **14** then established fragment **C** in 50 % overall yield.



Scheme 4. Synthesis of the **C** fragment. a) Ti(O*i*Pr)₄, (+)-BINOL, 4-Å molecular sieves, CH₂Cl₂, 65 %; b) LiAlH₄, Et₂O, 0 °C; c) *i*PrOH, PPTS; d) Swern oxidation, 77 % (over three steps). BINOL = 2,2'-dihydroxy-1,1'-binaphthyl; PPTS = pyridinium *p*-toluenesulfonate.

Now the stage was set for coupling the individual fragments (Scheme 5). The Wittig^[3c,d] reaction between the aldehyde **C** and the phosphonium salt **B** in toluene using *t*BuOK as base gave the vinyl iodide **15** in 76 % yield. Compound **15** was then coupled to the **A** fragment by a Heck reaction.^[22] In this transformation the Jeffery conditions^[23] (phosphane-free) were superior to a variety of other Pd⁰ sources such as



Scheme 5. Coupling of the three fragments. a) *t*BuOK, toluene, 0 °C, 76 %; b) Pd(OAc)₂, Bu₄NBr, Cs₂CO₃, Et₃N, DMF, 65 %; c) acetone/H₂O, PPTS, 83 %; d) TPAP, NMO, MS 4 Å, CH₂Cl₂, 77 %; e) HF · py, THF, py, 76 %. NMO = *N*-methylmorpholine-*N*-oxide; Py = pyridine; TPAP = tetra-*n*-propylammonium perruthenate.

[Pd(PPh₃)₄] and [Pd(dba)₂] (dba = dibenzylideneacetone) and provided **16** selectively. Acetal **16** was hydrolyzed under mild acidic conditions (PPTS, acetone, H₂O), and the resulting hemiacetal was oxidized to the α,β -unsaturated lactone by using TPAP/NMO.^[24] Attempts with standard TBS deprotection reagents (TBAF, tris(dimethylamino)sulfur–trimethylsilyl difluoride (TASF), and HF · pyridine) were unsuccessful. Only HF · pyridine in THF/pyridine^[24] as the solvent gave clean conversion to ratjadone.

Comparison of the spectroscopic data (¹H NMR, circular dichroism (CD), optical rotation) of the synthetic compound with that of the natural product^[25] showed that both compounds were identical. It can be concluded that the previously unknown configurations at C5, C10, and C16 have the *R* configuration.^[26] The highly convergent total synthesis of ratjadone described herein has 18 linear steps and gives 6.8 % overall yield. In addition, the intermolecular Heck reaction has been demonstrated to be an attractive alternative to Stille and Suzuki couplings in the total syntheses of complex natural products. With this reliable synthetic route we are now in a position to synthesize structural analogues with which the various cellular processes mediated by ratjadone can be probed.^[27]

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Combinatorial Testing of Supported Catalysts for the Heterogeneous Polymerization of Olefins**

Martin Stork, Andreas Herrmann, Tanja Nemnich, Markus Klapper, and Klaus Müllen*

Combinatorial chemistry, which has had a high impact on pharmaceutical and biotechnological research and development, has recently also been applied to catalysis research and is strongly connected with the development of high-throughput methods.^[1] However, few efforts have been directed towards taking advantage of this approach in polymer science.^[2] Herein we present a novel approach using tagged supports for the testing and direct comparison of silica-^[3] or polymer-supported^[4] heterogeneous catalysts for industrial olefin polymerizations.^[5] In contrast to a process described in the literature^[2c] which requires a chemical transformation, here direct detection of the different product beads obtained by different catalysts is available through fluorescence. This process accelerates the optimization and standardization of supported catalyst composition, polymerization conditions (e.g. temperature, pressure, and comonomer concentration), and product profiles because standard equipment can be applied.

This truly combinatorial approach starts with the tagging of different catalysts with fluorescent dyes, which exhibit different emission wavelengths. These labeled catalysts are then mixed and introduced into a single polymerization vessel. During the olefin polymerization each catalyst particle forms only one product granule through a particle growth process and can be considered as a microreactor.^[6]

To assign the different components of the product mixture to the employed catalysts, the polymer products are exposed to UV light. Owing to the different emissions of the labels incorporated in the beads, the resulting products can be directly identified which permits manual separation and characterization. In this way the properties of each of the polymer products can be assigned to the characteristics of the employed catalyst. This protocol is illustrated in Figure 1.

For tagging the catalysts, we chose rylene dyes (see Figure 2) because of their chemical stability, their high tendency to physisorb on silica, and their high fluorescence quantum yields.^[7] It had to be shown that these dyes do not influence the polymerization and are stable under different polymerization conditions (Table 1).

Catalyst **A**, consisting of metallocene **3** (dimethylsilylbis(2-methylbenzindenyl) zirconocene dichloride; Figure 2), silica-supported and activated with MAO (methylaluminoxane), was tagged with dye **2** (perylene monoimide) and compared with the corresponding untagged catalyst at a range of pressures and temperatures (Table 1). In all cases similar

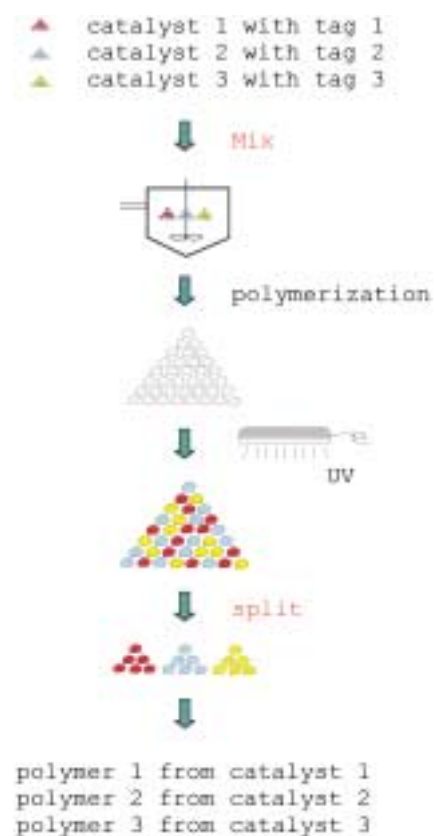


Figure 1. The combinatorial approach.

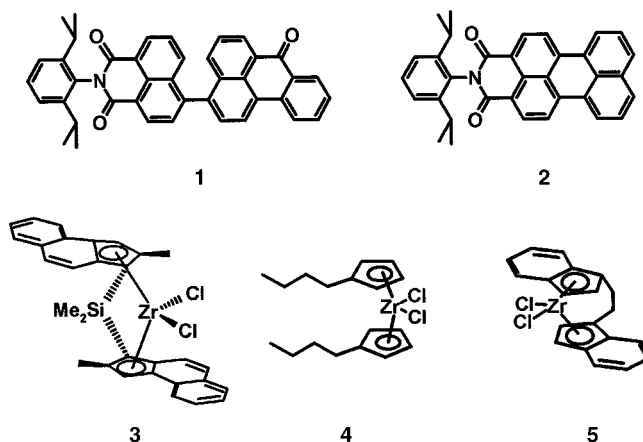


Figure 2. Dyes and catalysts.

Table 1. Comparison of the polymerization behavior of tagged and untagged catalyst **A**.^[a]

Run	Dye	<i>P</i> [bar]	<i>T</i> [°C]	Productivity [g(PE)g(cat) ⁻¹ h ⁻¹]	<i>M_n</i>
1	–	40	70	1180	180 000
2	2	40	70	1220	177 000
3	–	40	50	320	188 000
4	2	40	50	320	194 000
5	–	40	30	60	277 000
6	2	40	30	60	318 000
7	–	20	70	200	78 000
8	2	20	70	200	82 000

[a] *P*: pressure, *T*: polymerization temperature, 5 mL triisobutylaluminum (TIBA).

[*] K. Müllen, M. Stork, A. Herrmann, T. Nemnich, M. Klapper

Max-Planck-Institut für Polymerforschung
Ackermannweg 10, 55128 Mainz (Germany)
Fax: (+49) 6131-379-350
E-mail: muellen@mpip-mainz.mpg.de

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productivities from the corresponding experiments with and without dye were obtained and the polyethylene samples possessed the same molecular weight and polydispersity as well as the same particle morphology.

The preparation of the catalysts was performed by a facile impregnation method described in the Experimental Section (Figure 3).^[8]

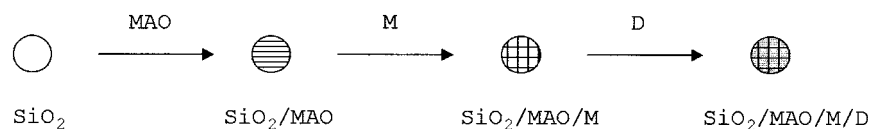


Figure 3. Preparation of labeled catalysts. M = metallocene, D = dye, MAO = methylaluminoxane.

To prove our concept of a combinatorial approach a set of two catalysts was used simultaneously. Catalyst **B** contained bis(*n*-butylcyclopentadienyl)zirconocene dichloride (**4**) with benzanthroneynaphthaleneimide **1** as a tag (Figure 2). Catalyst **C** consisted of ethylene-bis(indenyl)zirconocene dichloride (**5**) and perylenemonoimide **2** (Figure 2). Zirconocene **4** and ansa-zirconocene **5** were selected due to their similar activities. They gave product particles of nearly the same size and thus demonstrated the efficiency of our detection method. On the other hand, the two zirconocenes polymerize ethylene with different molecular weight and polydispersity.

By using catalysts **B** and **C** simultaneously, polyethylene products were obtained (see Experimental Section) as spherical granules within a range of 0.5 to 2 mm diameter. Upon exposure to UV light, particles prepared from the two different catalysts fluoresce with different colors. A fluorescence microscope image (Figure 4) clearly revealed two

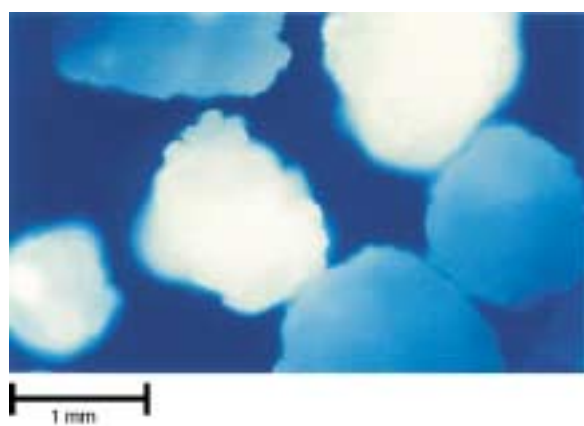


Figure 4. Fluorescence microscope image of the polymer products obtained from the labeled catalysts **B** and **C**.

different kinds of particles: fluorescent and nonfluorescent ones. Owing to the different positions of the absorption maxima and the selection of suitable optical filters, each of the dyes could be excited separately: light with a wavelength of 436 nm only excited **2**, while dye **1** remained dark. The different fluorescence behavior of the dyes allowed an exact assignment of the particles resulting from catalysts **B** and **C**.

For separation, the particles were exposed to UV light of 354 nm and 15 particles (ca. 15 mg) were collected. The amount of polymer collected was sufficient to allow a standard polymer characterization; thus we were able to determine copolymer composition (by NMR spectroscopy), melting temperatures (T_m) (by differential scanning calorimetry (DSC) measurements), molecular weights and distributions (by gel permeation chromatography (GPC)) and even crystallinity (by powder diffractometry).

The emission spectra as well as the fluorescence microscope images of the polyethylene samples showed that no migration of labels had occurred. Furthermore, the stability of the dyes under the aggressive reaction conditions with strong Lewis acids and alkylating reagents could be demonstrated.

For each sample the shape of the GPC curves showed a monomodal distribution. Catalyst **B** produced polyethylene with a molecular weight M_n of 207 000 Da and a molecular weight distribution D of 2.24; polyethylene from catalyst **C** exhibited almost half the molecular weight M_n of 124 000 Da and a broad polydispersity D of 3.47. These results agreed exactly with GPC data obtained from single runs with each catalyst. Additionally, the shape of the GPC curves as well as the values indicated that no exchange of catalyst between the particles took place.

To prove that there is no exchange either of catalyst or of the dyes on the product beads, two different catalysts were used known for having strongly different behavior in the copolymerization of ethylene/hexene. By means of DSC measurements the melting temperatures were used as a probe to determine the amount of incorporated hexene. A migration of the metallocenes should easily be detectable by any deviation from the expected melting temperatures.

Catalyst **D** contained **4** and **2** as a tag. Catalyst **E** consisted of metallocene **3** and was labeled with **1**. Each catalyst system was run separately with and without dye, and then as a mixture. Table 2 indicates that the dye had no influence on T_m , and that the M_n values (GPC measurements) were

Table 2. Copolymerization of ethylene/hexene (40 bar, 70 °C, 5 mL TIBA).

	Dye	Zirconocene	T_m	M_n
D	–	4	129.3	113 000
	1	4	129.9	107 000
E	–	3	119.5	227 000
	2	3	119.0	234 000
mixture of D + E				
particles of D	1	4	129.3	117 000
particles of E	2	3	120.3	221 000

essentially unaffected. This clearly demonstrates that the copolymerization behavior was independent of the dye. More importantly, however, the polymers obtained in the isolated mixture of different tagged product beads have the same properties as in the single experiment.

The concept described herein can be implemented in present polymerization and analytical facilities. The sets of

two catalysts can be easily enlarged because a great variety of rylene dyes covering the whole visible spectra is available.^[9] Our rapid testing and comparison of supported metallocene catalysts under identical experimental conditions is suitable for other heterogeneous polymerization processes. In order to create larger libraries of heterogeneous catalysts, on-line methods for the detection and characterization of the particles will have to be developed and applied.

Experimental Section

Procedure for supporting and labeling zirconocenes: All experiments were performed under argon by using standard Schlenk techniques. Catalyst **B**: SiO₂/MAO (2.50 g, 25 % MAO on silica, TA 02794/HL, Witco GmbH, Bergkamen, Germany) was suspended in toluene (100 mL) followed by addition of **4** (7 mg) in toluene (50 mL) at room temperature and stirred for 2 h. Subsequently, a solution of dye **1** (60 mg) in toluene (50 mL) was added dropwise and the mixture was stirred for 1 h. The almost colorless solution was removed by filtration and the resulting silica/MAO/zirconocene/dye catalyst system was washed twice with toluene and dried in vacuum. Catalysts **C–E** were prepared in a similar way.

Polymerization: General procedure: Two catalysts were mixed and used simultaneously in a single vessel for the polymerization of ethylene. Isobutane (400 mL) and triisobutylaluminum (TIBA) (5 mL; 10 % in n-hexane) were introduced into a Büchi stainless steel reactor (1 L) equipped with a magnetic stirrer and saturated with ethylene (standard conditions: 40 bar, 70 °C). The mixture of catalysts was introduced into the reactor using a pressure gate. After 90 min the polymerization was stopped by releasing the pressure.^[10]

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An Allosteric Ribozyme Regulated by Doxycycline**

Nicolas Piganeau, Andreas Jenne, Vincent Thuillier,* and Michael Famulok*

Specific ligand-binding nucleic acid molecules (aptamers) can be isolated by searching the “shape-space” of vast combinatorial nucleic acid libraries for functional sequences in vitro.^[1–3] Selection strategies for the isolation of aptamers usually involve incubation of a complex library of nucleic acids with the immobilized target molecule or the immobilization of a preformed target/nucleic acid complex.

A considerable number of RNA aptamers that bind a variety of targets with high affinity and specificity have been isolated and extensively characterized. Detailed structural investigations of many aptamer/ligand complexes have established that complex formation in most cases is accompanied by extensive conformational changes only in the presence of the cognate ligand by adaptive binding events.^[4–11] This property of small-molecule-binding aptamers allowed the rational design of artificial allosteric ribozymes inhibited or activated by a ligand that is bound to an aptamer which has been positioned in immediate proximity to a ribozyme sequence.^[12–15]

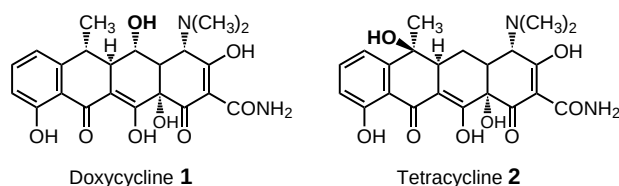
We have applied a novel in vitro selection strategy based on allosteric inhibition of a hammerhead ribozyme fused to a

[*] Prof. Dr. M. Famulok, N. Piganeau,^[+] Dr. A. Jenne
Kekulé-Institut für Organische Chemie und Biochemie
Gerhard Domagk-Strasse 1, 53121 Bonn (Germany)
Fax: (+49) 228-735388
E-mail: m.famulok@uni-bonn.de
Dr. V. Thuillier
Aventis Gencell
3825 Bay Center Place
Hayward, CA 94545 (USA)
Fax: (+1) 510-887-1680
E-mail: vincent.thuillier@aventis.com

[+] New address: Aventis Gencell, 3825 Bay Center Place, Hayward, CA 94545 (USA)

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randomized RNA library by low concentrations of the antibiotic doxycycline **1**. After 16 rounds of selection, ribozymes were isolated with inhibition constants as low as 20 nM and a remarkable specificity for **1**. Doxycycline **1** was



chosen as the “switch molecule” because it represents a cell-permeable small molecule with low toxicity for higher eucaryotes. Thereby, allosteric ribozymes that respond to **1** may potentially be used for the development of conditional gene expression systems based on the cleavage of mRNAs by the inserted hammerhead ribozyme depending on whether or not **1** is present in the cell.

The design of the randomized library is shown in Figure 1 a. Previously, allosteric hammerhead ribozymes have been rationally designed by fusing an aptamer RNA onto helix II.^[16, 17] Because helix II is important for the activity of the hammerhead ribozyme,^[18] we have chosen this site for positioning the randomized sequence. To obtain a library of active ribozymes the first two base pairs of this helix were maintained. The course of the selection is shown in Figure 1 c. After five cycles of selection an enrichment of the pool for doxycycline-inhibited ribozymes was detected. However, a control selection cycle in the absence of **1** revealed that the selected pool was only poorly responding to the ligand; in the presence of doxycycline **1**, 14% of the RNA was eluted in selection step 4 (Figure 1 b), whereas 10% of the RNA was eluted in the negative control without **1**.

Therefore, we hypothesized that parasitic ribozymes were coselected in the course of the selection. These fold into different conformations, some of which are cleavage-competent and others are inactive. During the first incubation (step 2, Figure 1 b), these ribozymes would only partly be eluted because a fraction of them cannot be cleaved due to misfolding. During denaturation/renaturation between steps 2 and 3 (Figure 1 b) some of these ribozymes would refold, again only partly in an active conformation, which would unintentionally be eluted together with the doxycycline-inhibited species after the second incubation (step 3, Figure 1 b). As a consequence, doxycycline-independent (“parasitic”) ribozymes would be coselected. To eliminate these species, we modified the selection procedure from cycle 6 onwards by interrupting the first incubation (step 2, Figure 1 b) by repetitive washing steps under denaturation/renaturation conditions. Indeed, after cycle 7 the enriched pool showed much lower parasitic activity (10% elution in step 4 in presence of **1**, 0.5% without **1**). After this cycle the selection pressure was gradually increased by reducing the ligand concentration and the incubation time in step 2 (Figure 1 b,c). After cycles 10 and 13, the complexity of the enriched pool was increased by mutagenic PCR.^[19, 20] In parallel, the selection stringency was also increased to

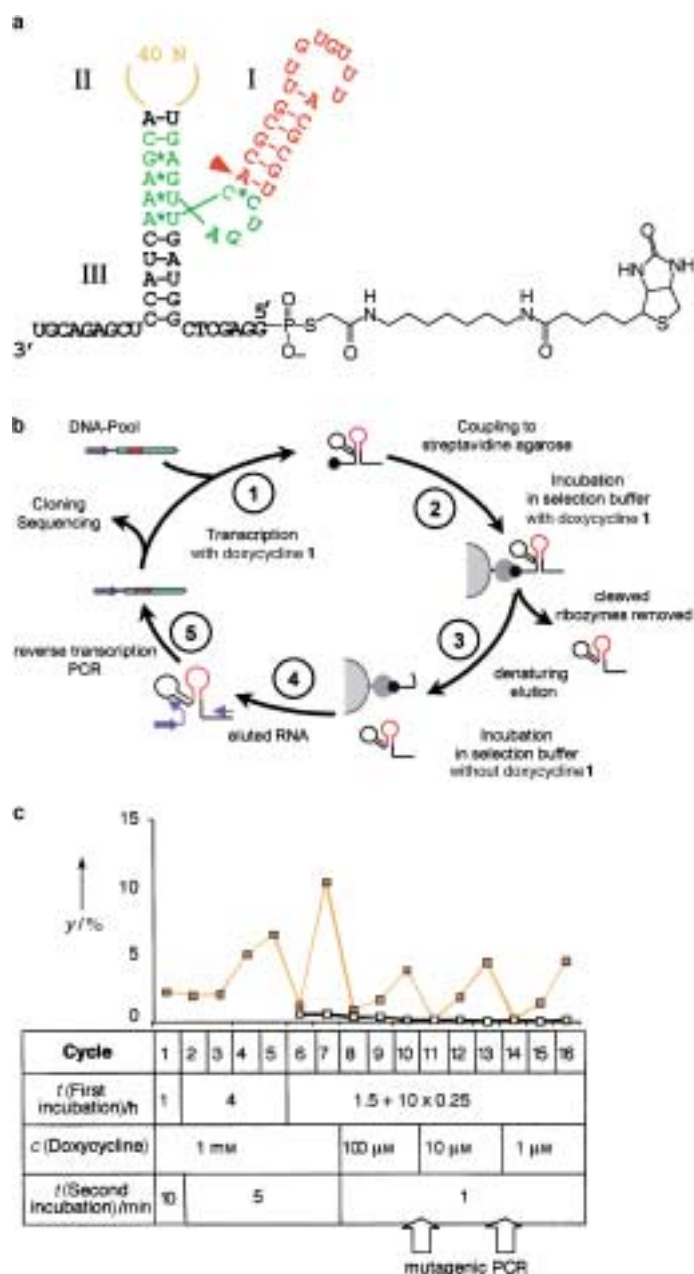


Figure 1. Selection of allosteric hammerhead ribozymes that are inhibited by doxycycline **1**. a) Secondary structure of the transcripts from the initial pool. Helix II was shortened to two base pairs, and the loop II was replaced with a 40 nucleotide random region. Green: nucleotides of the catalytic center of the hammerhead ribozyme (HHR). Red arrow: cleavage site. b) Schematic representation of the selection procedure. For details see Experimental Section. Step 1: Transcription of the DNA library by using T7 RNA polymerase in the presence of guanosine monophosphothioate (GMPS) and 1.0 mM **1** (red: randomized region; blue arrow: T7 promoter); separation of uncleaved from cleaved RNA transcripts by denaturing polyacrylamide gel electrophoresis and chemical biotinylation of the 5'-sulfanyl group (black filled circle). Step 2: Coupling to streptavidine agarose and incubation with **1**. Step 3: Washing under denaturing conditions to remove **1** and cleaved ribozyme products; incubation of the remaining immobilized RNA without **1** in the selection buffer. Step 4: Elution of the cleaved ribozymes. Step 5: Reverse transcription and PCR amplification; blue arrows: primer 3 and 4. c) Percentages of eluted radioactivity y after the second incubation step. Yellow: in the presence of **1**; black: control experiment. The table below the graph relates each selection cycle to the respective selection conditions. Arrows indicate selection cycles amplified under mutagenic PCR conditions. For details see text.

promote the enrichment of allosteric ribozymes with improved values of K_i and catalytic rate.

The activity of the selected pools from cycles 10, 13, and 16 was monitored by time-course experiments with different concentrations of the inhibitor. The sensitivity of the pool ribozymes for doxycycline **1** increased continuously (Figure 2); from cycle 16 the pool was completely inhibited in the presence of $1\ \mu\text{M}$ **1**. The selected pools were therefore cloned and sequenced after cycles 10, 13, and 16 (Figure 2b). We obtained eight different sequence classes from which one representative member from each was chosen for further analysis. Kinetic analyses were performed in the presence or absence of $1\ \mu\text{M}$ **1** (Figure 2b). The values of k_{obs} and the factor of inhibition with respect to the unselected pool are also shown in Figure 2b. At this concentration, no allosteric inhibitory effect was found for a sequence member from cycle 10 which is not surprising as this cycle was performed at $100\ \mu\text{M}$ of **1**. Clones selected under higher stringency exhibited clear allosteric effects with differences in the k_{obs} values ranging from threefold to nearly 50-fold. Interestingly, selected ribozymes showed a decreased catalytic activity in the absence of the regulatory drug. Their mean k_{obs} values are around $0.3\ \text{min}^{-1}$ which is roughly 10-fold slower than a corresponding “wild-type” hammerhead ribozyme that does not contain additional sequences fused to helix II and for which a value of k_{obs} around $3.0\ \text{min}^{-1}$ was determined (data not shown).

The four clones showing the best inhibition values were chosen for a determination of their inhibition constants (K_i)

(Table 1). The clones exhibited impressive sensitivity for **1** with values of K_i ranging from 20 to 70 nM. These values belong to the highest affinities found for RNA/small molecule interactions until now.^[21] To investigate the specificity of these

Table 1. K_i values for **1** and **2** as well as rate constants of the uninhibited reaction, and maximal levels of inhibition.

Clone	k_{obs} (uninhibited) [min^{-1}]	$K_i(\mathbf{1})$ [nM]	Maximal inhibition factor	$K_i(\mathbf{2})$ [nM]
1D13-01	0.13 ± 0.02	70 ± 20	11	> 1000000
1D16-05	0.40 ± 0.02	20 ± 5	49	100 ± 20
1D16-06	0.13 ± 0.02	25 ± 3	12	100 ± 20
1D16-13	0.09 ± 0.01	50 ± 30	12	> 1000000

clones we determined the K_i value in the presence of tetracycline **2**, which differs from **1** only in the substitution pattern of a single OH group (Table 1). At $1.0\ \text{mM}$ **2**, no allosteric effect was observed for clones D13-01 and D16-13, corresponding to at least a 10000-fold discrimination between **1** and **2** (Table 1). The two other clones tested showed moderate selectivity (four- to fivefold). These data suggest that clones D13-01 and D16-13 might form a different binding pocket for **1** than clones D16-05 and D16-06.

The most active clone D16-05 was chosen to determine a minimal motif for doxycycline binding. For that, we synthesized 16 truncated versions of a cleavage-inactive mutant of this clone and carried out cleavage reactions in the presence of a tenfold molar excess of these constructs with respect to

the inhibitor **1**. If truncated constructs act as aptamers they would be able to compete with the allosteric ribozyme for binding to the inhibitor, thereby restoring ribozyme activity. Truncated constructs without aptameric activity cannot act as decoys for **1**. Therefore, in this case, the allosteric ribozyme D16-05 remains inhibited. The result of this experiment is shown in Figure 3a. According to these data, the randomized region alone is not capable of binding to **1**. For an effective competition the presence of helix I of the original hammerhead motif is required in addition to the randomized region. Experiments 9–11 in Figure 3a show that not only the sequence of the loop region in this helix but also that of the paired region appear to be important. Thus, the sequence shown in Figure 3b is likely to represent the minimal motif for the aptameric binding of **1**. According to the algorithm of Turner and Zuker^[22] it folds into the secondary structure shown.

The requirement of helix I for inhibition suggests that this helix is an integral part of the doxycycline-

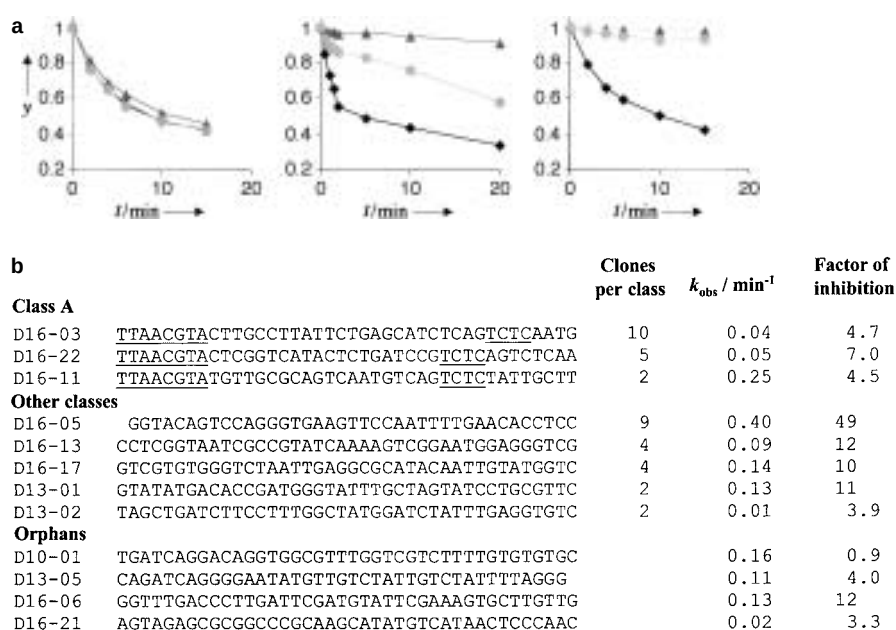


Figure 2. Allosteric inhibition of pools and individual selected clones. a) With the number of selection cycles (10, 13, 16, from left to right), the sensitivity of the library for **1** increases. Cleavage occurred in the selection buffer (SB; 40 mM Tris-HCl, pH 8, 50 mM NaCl, 10 mM spermidine, 8 mM MgCl_2) at 37°C with or without **1** [$c(\mathbf{1})=0$ ($\bullet$), $1\ \mu\text{M}$ ($\circ$), $10\ \mu\text{M}$ (Δ)]. Cleavage reactions were initiated by addition of Mg^{2+} . b) Sequences of the clones analyzed after the selection. The label (D10, D13, or D16) refers to the selection cycle from which the clone stems. Some clones could be grouped into classes based on sequence homologies. Family A represents a class that contains a short common motif (underlined). The k_{obs} values in the first 3 min of the reaction were used to determine the ratio of the k_{obs} values of the inhibited and the uninhibited reaction, designated as “factor of inhibition”.

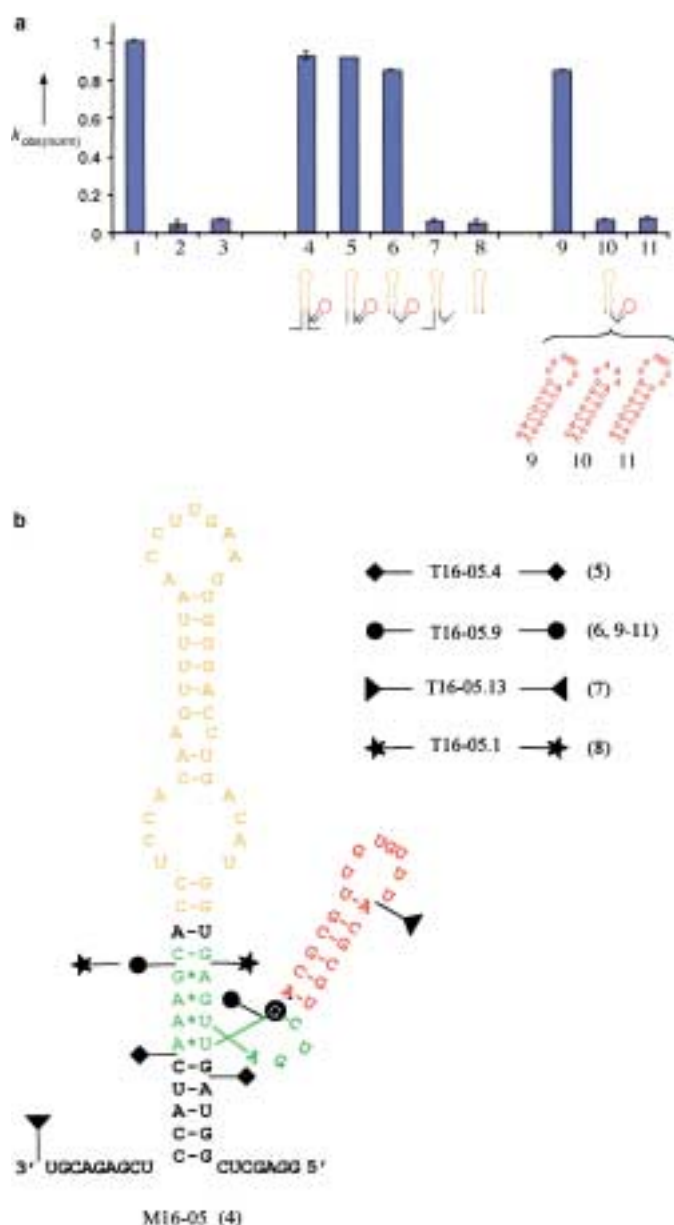


Figure 3. Determination of the minimal sequence of clone D16-05. a) Relative activity $k_{\text{obs(norm)}}$ of the cleavage reaction under various conditions (always in the presence of $1 \mu\text{M}$ tRNA as an unspecific competitor). 1: Cleavage activity of D16-05 (1 nM). 2: Same in the presence of 200 nM **1**. 3: Same as in 2 in the presence of $2.0 \mu\text{M}$ unselected pool as an unspecific competitor. 4–8: Same as in 2 in the presence of $2.0 \mu\text{M}$ cleavage inactive point mutant M16-05 (4), construct T16-05.4 (5), construct T16-05.9 (6), construct T16-05.13 (7), construct T16-05.1 (8). 9–11: Same as in 2 in the presence of $2.0 \mu\text{M}$ T16-05.9 with the depicted variations in helix I. b) Computer-generated secondary structure of the point mutant D16-05. The various marker symbols flank the sequences of the truncated versions tested. To enable T7-transcription two guanosine residues, which are not shown here, were inserted into the 5'-position of the truncated constructs. Orange: The original randomized region; red: constant region from the HHR (helix I); green: catalytic core. The guanosine residue shaded in black shows the point-mutation C→G. The numbers in parentheses refer to the numbering in (a).

binding site. If binding-site formation is accompanied by a conformational change of the ribozyme tertiary structure, this would explain the inhibition induced by **1**. Another possible explanation for the requirement of helix I is that the antibiotic

is locking the structure of the ribozyme in a defined conformation thereby reducing the entropic advantage of product formation on the ribozyme which in turn shifts the equilibrium of the reaction towards ligation.^[23]

In conclusion, we have applied an in vitro selection scheme based on allosteric regulation, which led to ribozymes that respond to strikingly low concentrations of the target molecule. Some allosteric ribozymes exhibit unexpected specificity for the regulator molecule. Previously, Breaker et al. have applied an analogous selection principle in which allosteric ribozymes that respond to cAMP and cGMP as *effector* molecules were obtained de novo.^[24] While being inactive in the absence, ribozyme cleavage only took place in the presence of cAMP or cGMP at $100 \mu\text{M}$ concentrations. Thereby, a more than 1000-fold allosteric activation of hammerhead cleavage activity was observed. In the present study, selection for allosteric inhibition led to 10–50-fold responses, at nanomolar concentrations of a nontoxic, cell-permeable molecule of low molecular weight. When inserted into the mRNA of a certain target gene, allosteric molecular switches of this type may serve as a valuable tool for the development of tailored conditional gene expression systems that can be controlled by the presence or absence of any kind of small molecule.

Experimental Section

Random pool synthesis: DNA oligonucleotides were synthesized on an Expedite nucleic acid synthesizer and purified on denaturing polyacrylamide (PA) gels. The pool-primer (5'-CGCGTTGTGTTTACGCGTCTGATGAGT-N₄₀-ACGAACTACCTCGAGACGT) was initially amplified by using primers 1 (5'-CGCGTTGTGTTTACGCGTCTGATG) and 4 (5'-ACGTCTCGAGGTAGTTTCGT).^[25] Further amplification was performed under the same conditions by using primers 3 (5'-AGCTGGTACCTAATACGACTCCTATAGGAGCTCGGTAGT-CACGCGTTGTGTTTACGCGTCTGATG) and 4. The resulting DNA library with a complexity of 10^{15} different sequences was used as the template for the in vitro transcription with T7-RNA polymerase for the initial selection cycle.

In vitro selection: Standard transcription reactions contained the following components: 250 U T7-RNA-polymerase (Stratagene), T7-buffer, 2.5 mM dNTPs, 20 mM GMPS, 20 μCi $\alpha^{32}\text{P}$ GTP, RNasin (50 U), **1** (1 mM), template DNA (2 μM). Transcription was performed for 16 h at 37°C . The unclevaged RNA transcript was purified by 8% denaturing polyacrylamide gel electrophoresis (PAGE) and the 5'-sulfanyl group was subsequently derivatized by treatment with *N*-biotin-*N'*-iodoacetylhexamethylenediamine (200-fold excess) for 90 min at 25°C . The biotinylation was achieved with an efficiency of 40%. 5'-Modified RNA was again purified by PAGE and incubated with streptavidine agarose (Pierce) for 30 min at 25°C . Immobilized RNA was then washed six times alternatively with 1 mL of washing buffer A (WA; 25 mM HEPES pH 7.4, 1 M NaCl, 5 mM EDTA), washing buffer B (WB; 3 M urea, 5 mM EDTA), and water. It was then incubated in the selection buffer (SB; 40 mM Tris HCl pH 8, 50 mM NaCl, 10 mM spermidine, 8 mM MgCl_2) at 37°C for different times (see Figure 1 c) and in the presence of the respective concentrations of **1**. Cleavage reactions were started by addition of Mg^{2+} . After cycle 5 incubation during the first 2.5 h was interrupted ten times by alternate washes with WB, WA, and water. The second incubation in SB was performed under the same conditions without **1**. The RNA was eluted, extracted with phenol/ CHCl_3 , precipitated with EtOH, and reverse transcribed by using Tth-DNA-polymerase (Boehringer); the resulting cDNA was PCR-amplified with primers 3 and 4. The PCR primers were designed in such a way that the cleaved 5'-end of HHR and the T7 promoter were reintroduced. This DNA was used as a template for T7 transcription for the next round of selection.

Kinetic analysis of the 12 clones: The clones were labeled at the 5'-end with γ - ^{32} P-ATP and incubated at 1.0 nM in SB+8 mM MgCl₂ at 37 °C. Aliquots were taken at different times, quenched with urea and EDTA on ice, and loaded onto a denaturing PA gel. Bands corresponding to the uncleaved and cleaved fractions were quantified by phosphorImaging (Molecular Dynamics). The k_{obs} values were then determined by using an exponential curve fitting of the measured values of the uncleaved ribozyme fraction during the first 3 min.

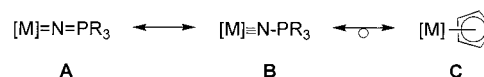
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Homoleptic Phosphoraneiminato Complexes of Rare Earth Elements as Initiators for Ring-Opening Polymerization of Lactones**

Thorsten Gröb, Gert Seybert, Werner Massa, Frank Weller, Ravi Palaniswami, Andreas Greiner, and Kurt Dehnicke*

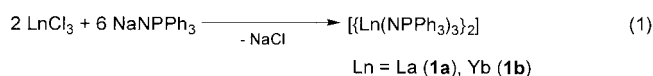
Phosphoraneiminato complexes of transition metals containing the NPR_3^- group are known in many coordination-chemical variations^[1] as a result of varying bonding modes. In complexes with metal ions in low oxidation states (+I, +II) the NPR_3^- group acts as μ_3 -N bridging ligand, in medium oxidation states (+III, +IV) preferably as μ_2 -N bridging ligand, and in high oxidation states (+V to +VIII) exclusively as terminal ligand. In the latter mode short metal–nitrogen distances and linear or almost stretched M–N–P axes are observed, which can be described with the resonance structures **A** and **B**. As first deduced for imido complexes (NR^{2-})^[2]



style **B** requires a σ , 2π orbital set for the $\text{M} \equiv \text{N}$ bond that proves the NPR_3^- ligand to be ψ -isobal to the η^5 -cyclopentadienide(Cp) ligand **C**^[3] with respect to equal charge. Recent ab initio calculations confirm this interpretation.^[4]

Since we surprisingly found the bonding mode **A/B** also in the mixed-ligand complexes $[\text{Ln}_2\text{Cp}_3(\text{NPPH}_3)_3]$ ($\text{Ln} = \text{Y}, \text{Dy}, \text{Er}, \text{Yb}$) containing Ln^{3+} ions,^[5] we examined the opportunity to synthesize homoleptic compounds $[\text{Ln}(\text{NPPH}_3)_3]$, too, and to compare them with the ψ -isobal complexes $[\text{LnCp}_3^*]$ ($\text{Cp}^* = \text{C}_5\text{Me}_5$) that are known as initiators for ring-opening polymerization (ROP) of lactones.^[6–8]

Compounds **1** are formed in almost quantitative yields by reacting the alkaline phosphoraneiminates MNPPH_3 ($\text{M} = \text{Na}, \text{K}, \text{Rb}, \text{Cs}$)^[9] with the anhydrous metal trichlorides or anhydrous metal triflates in THF [Eq. (1)]. Interestingly, only



the lanthanum compound crystallizes as a THF-solvate, that is **1a**·2THF, whereas the ytterbium compound **1b** forms a solvate-free complex due to the ionic radius of the Yb^{3+} ion which is 16 pm smaller than that of the La^{3+} ion.^[10]

[*] Prof. Dr. K. Dehnicke, Dr. T. Gröb, Dipl.-Chem. G. Seybert, Prof. Dr. W. Massa, Dr. F. Weller, Dr. R. Palaniswami
Fachbereich Chemie der Philipps-Universität
Hans-Meerwein-Strasse, 35032 Marburg (Germany)
Fax: (+49)6421-282-8917
Prof. Dr. A. Greiner
Institut für Organische Chemie
Johannes Gutenberg-Universität
Duesbergweg 10-14, 55099 Mainz (Germany)

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The X-ray crystal structure analysis of **1a**·2 THF (Figure 1) reveals that both the lanthanum atoms are linked through N atoms of two NPPh₃[−] groups to give a nonplanar La₂N₂ four-membered butterfly arrangement. This is a result of the

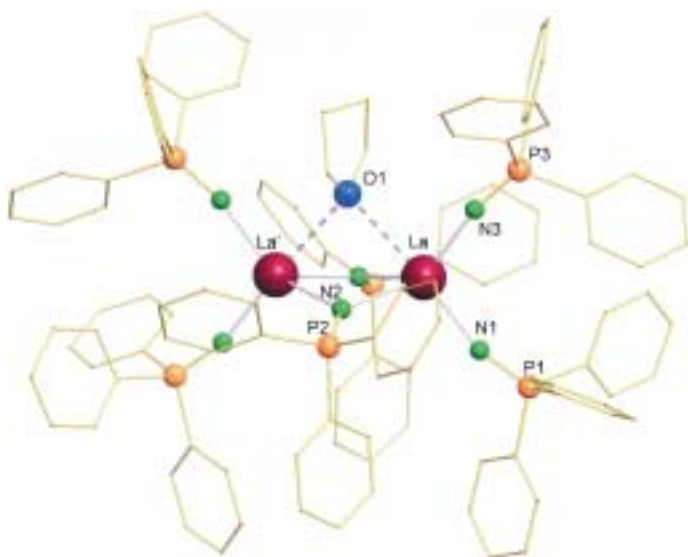


Figure 1. Structure of $[\text{La}(\text{NPPh}_3)_2] \cdot 2 \text{ THF}$ (**1a**·2 THF). Selected bond lengths [pm] and angles [°]: La–N1 228.3(6), La–N3 231.9(6), La–N2 245.4(7), La–N2A 252.3(7), La–O1 282.5(6), N1–La–O1 174.9(2), N1–La–N3 105.0(3), N1–La–N2 105.7(2), N2–La–N2A 79.9(3), N2–La–N3 120.5(3), N2A–La–N3 130.7(2), La–N1–P1 163.9(4), La–N3–P3 168.1(5).

additional μ -O function of the lanthanum-bridging THF molecule that leads to a trigonal-bipyramidal coordination around the lanthanum atoms with N1 (and N1A, respectively) and O1 at the axial positions. The μ -O-bridging function of THF is not unusual, it has occasionally been observed in organolithium compounds^[12] and in transition metal complexes.^[13] The La–N distances of the La₂N₂ ring (246.0 and 252.4 pm) are much longer than the La–N distances of the two terminally bound NPPh₃[−] groups which are among the shortest known La–N bonds (228.4 and 231.6 pm). Together with the large bonding angles at N1 (163.9°) and N3 (168.1°), this corresponds to mode A/B.^[14]

According to the X-ray crystal structure analysis^[11] the ytterbium complex **1b**, which crystallizes solvate-free from THF/toluene, has a centrosymmetric dimeric molecular structure with tetrahedrally coordinated Yb atoms (Figure 2). The Yb–N distances in the Yb₂N₂ planar four-membered ring differ only slightly (225.0 and 226.2 pm) and, as expected, are significantly longer than the Yb–N distances to the terminal NPPh₃[−] groups (210.7 and 213.7 pm). In relation to the La–N bonds in **1a**·2 THF both kinds of Yb–N bonds are still a little shorter than expected by ionic radii contraction according to Shannon,^[10] which is probably associated with the lower coordination number in **1b**.

If excess alkaline phosphoraneiminato is used in the reaction shown in Equation (1) homoleptic “ate-complexes” $[\text{Ln}(\text{NPPh}_3)_4]^-$ are obtained that are catalytically inactive although some other “ate-complexes” of rare earth elements with lower steric shielding of the metal atoms are effective initiators for the ring-opening polymerization of lactones.^[15]

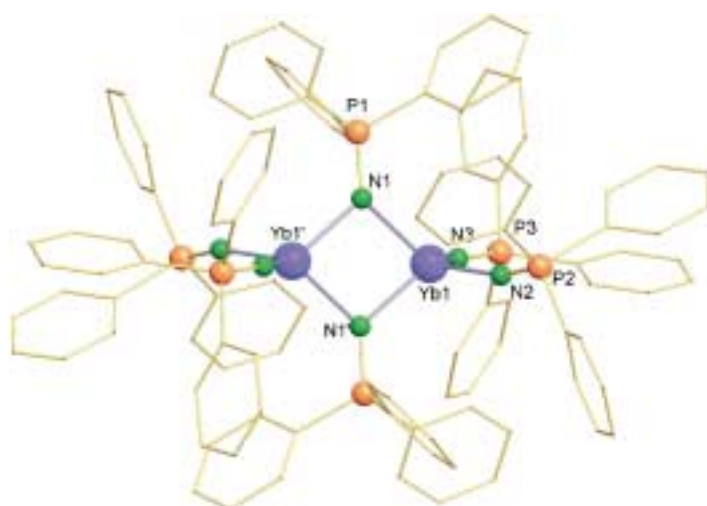


Figure 2. Structure of $[\text{Yb}(\text{NPPh}_3)_2]_2$ (**1b**). Selected bond lengths [pm] and angles [°]: Yb1–N1 225.0(4), Yb1–N1A 226.2(4), Yb1–N2 210.7(4), Yb1–N3 213.7(4), Yb1–N1–Yb1A 97.7(2), N1–Yb1–N1A 82.3(2), N1–Yb1–N2 108.4(2), N1–Yb1–N3 120.8(2), N2–Yb1–N1A 117.7(2), N2–Yb1–N3 110.2(2), N1A–Yb1–N3 115.3(2), Yb1–N2–P2 158.0(3), Yb1–N3–P3 158.5(3).

Despite the much smaller shape of phosphoraneiminato ligands compared to $\eta^5\text{-Cp}^-$ ligands, the rare-earth metal atoms in **1a** and **1b** preserve noticeably low coordination numbers (4 and 4 + 1, respectively). The special bonding modes of NPPh₃[−] groups in these structures show promise for use as initiators in polymerization reactions.

Indeed, **1a** and **1b** initiate the ring-opening polymerization of ϵ -caprolactone (CL) to poly- ϵ -caprolactone (PCL) within very short reaction times and in yields of >90%. The molecular weights of PCLs obtained from ratios of **1a**:CL = 1:150 at 20 °C in toluene are a little lower than those theoretically expected with reference to the dimeric complex **1a** (Table 1). Apparently, only the dimeric complex initiates the polymerization of CL at one active position, while the possible dissociation of **1a** plays a minor role.

Table 1. Ring-opening polymerization of ϵ -caprolactone (CL), initiated by **1a**, **1b**, **2**, and **3** at 20 °C.

	CL: 1 (molar)	<i>t</i> [min]	Yield PCL [%]	$M_n \times 10^{-4}$ (exp.)	M_w/M_n ^[a]
1a	150	< 1	91	1.303	1.77
1a	250	< 1	90	3.507	1.47
1b	150	3	98	1.266	1.32
1b	250	3	91	2.612	1.38
2	150	80	98	3.265	1.70
2	250	120	80	2.358	1.53
3	150	120	80	0.969	1.14
3	250	200	54	0.751	1.47

[a] M_n = Number-average molecular weight. M_w = Weight-average molecular weight. M_w/M_n = Polydispersity index.

By decreasing the ratio of **1b**:CL use of the ytterbium complex **1b** as catalyst leads to an increasingly better correlation between theoretical and experimental molecular weight, again related to the dimeric complex.

As a contribution to the study of the ψ -isolobal relationship between the Cp[−] and the NPPh₃[−] ligand we have also

investigated the polymerization activities of [LaCp₃] (**2**) and [YbCp₃] (**3**) under analogous conditions. Overall we found far worse and more nonuniform results with regard to yield, molecular weight, and polydispersity index of the polymers. However, the polymerizations with the initiators **1a** and **1b** did not produce the quality of polymers prepared by heteroleptic pentamethylcyclopentadienyl complexes [LnCp₂*X] of rare-earth elements.^[6, 16]

The future importance of catalysts based on phosphorane-iminato complexes lies in the almost unlimited possibilities to vary the phosphorus-bound residues R, including the introduction of chiral groups. This should provide fascinating new possibilities for controlling polymerizations of functionalized monomers.

Experimental Section

General: Standard Schlenk techniques; degassed and dried solvents under argon; IR: Bruker FT-IR IFS 88, nujol, CsI/PE plates; ³¹P NMR: Bruker AM-400, 161.975 MHz, 85% H₃PO₄; polymer analysis: Knauer GPC (polystyrene standard (PSS), 600 × 8 mm, 10 μm), Knauer differential refractometer K 200, polystyrene standard, THF solution.

1a: A solution of NaNPPH₃ (1.75 g, 5.60 mmol) in THF (15 mL) was added dropwise to LaCl₃ · 1.5 THF (0.66 g, 1.87 mmol) in THF (5 mL) and stirred for 24 h. After removal of THF in vacuo the solid was extracted by filtration with hot toluene. The filtrate was concentrated to about 5 mL and **1a** was precipitated as a beige solid (0.55 g, 31 %) by adding diethyl ether (20 mL). Single crystals of **1a** · 2 THF were obtained by cooling a saturated solution (hot) of toluene/THF (10/1). THF was removed under vacuum. Elemental analysis (%): calcd for C₁₀₈H₉₀La₂N₆P₆ (*M* = 1935.60 g mol⁻¹): C 67.02, H 4.69, N 4.34; found: C 62.49, H 4.57, N 4.36; IR: $\tilde{\nu}$ = 1195 (vs), 1163 (vs), 1141 (vs), 1101 (s), 1089 (m), 1067 (w), 1042 (vw), 1025 (w), 998 (w), 919 (vw), 875 (w), 748 (m), 706 (vs), 695 (vs), 552 (s), 534 (vs), 465 (m), 447 (m), 428 (w), 412 (w), 378 (m), 351 (vw), 321 (vw), 308 (m), 288 (m), 254 (m), 212 (m), 194 (m), 177 (m), 146 (m), 122 cm⁻¹ (vw); ³¹P NMR (THF, 25 °C): δ = -17.3 (s).

1b: A solution of NaNPPH₃ (1.79 g, 6.00 mmol) in THF (5 mL) was added to [Yb(O₃SCF₃)₃] (1.24 g, 2.00 mmol) in THF (35 mL). By stirring for 24 h **1b** (1.10 g, 55 %) was precipitated as a colorless powder of low solubility, whereas NaO₃SCF₃ remains dissolved. The resulting solution was reduced to a volume of 5 mL and extracted with toluene with accompanying precipitation of NaO₃SCF₃. After filtration colorless diamond-shaped crystals were formed within 12 h. The compounds are only sparingly soluble in toluene and THF both at room temperature as well as under higher temperatures. Elemental analysis (%): calcd for C₁₀₈H₉₀N₆P₆Yb₂ (*M* = 2003.87 g mol⁻¹): C 64.73, H 4.53, N 4.19; found: C 62.57, H 4.21, N 4.24; IR: $\tilde{\nu}$ = 1308 (w), 1221 (s), 1200 (vs), 1178 (s), 1155 (m), 1134 (s), 1103 (s), 1084 (s), 1069 (m), 1026 (m), 997 (m), 746 (m), 712 (s), 694 (vs), 568 (m), 542 (s), 534 (vs), 472 (m), 447 (m), 424 (m), 317 (w), 294 (w), 253 (vw), 215 (vw), 191 (vw), 156 (vw), 131 cm⁻¹ (vw); ³¹P NMR (THF, 25 °C): δ = 17.0 (s), -256.8 (s).

Polymerizations: Lactone was added to a suspension of the catalyst in toluene. This led to a spontaneous rise of solution viscosity. The product was precipitated with methanol/HCl, recrystallized twice from THF, and dried in vacuum for 24 h.

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Tailored Alkene Oligomerization with H-ZSM-57 Zeolite**

Johan A. Martens,* Raman Ravishankar, Igor E. Mishin, and Pierre A. Jacobs

Oligomerization reactions of short alkenes are used in the chemical industry to synthesize speciality C_6 – C_{12} alkenes as intermediates for plasticizer and surfactant alcohol synthesis through hydroformylation.^[1] Industrial alkene oligomerization processes in the liquid phase produce linear 1-alkenes with transition metal complexes as catalysts.^[1–3] To produce branched higher alkenes, the starting alkenes in the liquid, vapor, mixed liquid–vapor, or supercritical phase are treated with heterogeneous acid catalysts.^[1, 4, 5]

The phosphoric acid on kieselguhr catalyst, developed in 1935 by Ipatieff and Egloff,^[6] is applied for the oligomerization of dilute propene or butene streams at temperatures and pressures up to 520 K and 8.5 MPa, respectively.^[7] The catalyst is not selective and the operation conditions are critical. Acidic zeolites and, especially, H-ZSM-5 can be used for converting light alkenes into hydrocarbon fuels and lubricants.^[8] Reaction temperatures exceeding 473 K are required to prevent blocking of the channels by deposition of heavy oligomers.^[9] In ZSM-5, the oligomer chains undergo linearization before they can diffuse out of the channels.^[10] Besides oligomerization, the H-ZSM-5 zeolite catalyzes cyclization and hydrogen transfer reactions leading to complex product mixtures including arenes, cycloalkanes, and alkanes.^[11]

Acid-catalyzed alkene oligomerization can be rationalized with alkylcarbenium ion chemistry. The elementary reaction steps comprise 1) protonation of an alkene and formation of an alkylcarbenium ion, 2) addition of a second alkene to the alkylcarbenium ion, 3) eventual skeletal isomerization, and 4) deprotonation. When the approach of the positively charged carbon by the double bond in step 2) is hindered, an allylic hydrogen is transferred, leading to the formation of an alkane and an allylic cation. The latter is a coke precursor.^[12] The ideal zeolite catalyst should favor a crosswise arrangement of the alkene and the alkylcarbenium ion to suppress hydrogen transfer (Figure 1).

In the search among the known zeolite structures^[13] for suitable pore structures we discovered the ZSM-57 zeolite (MFS topology). It is a less studied zeolite,^[14, 15] in which 10-ring pores that run along the crystallographic *a* direction are provided with short wide lobes arising from the partial intersection with 8-ring pores that run along the crystallographic *b* direction (Figure 2). The elliptic 8-rings with

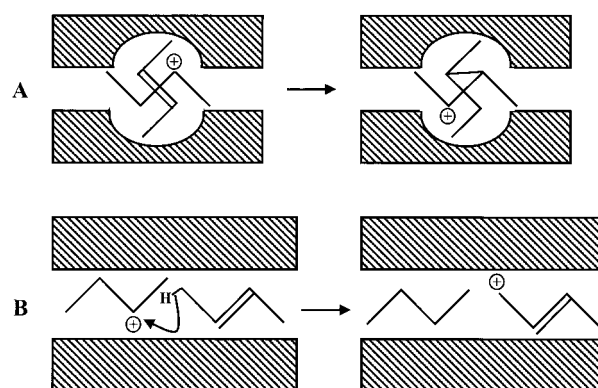


Figure 1. A) Crosswise arrangement of *trans*-but-2-ene and the 2-butyl cation in a lobed pore favoring addition; B) lining up of the molecules in a tubular pore leading to allylic hydride transfer.

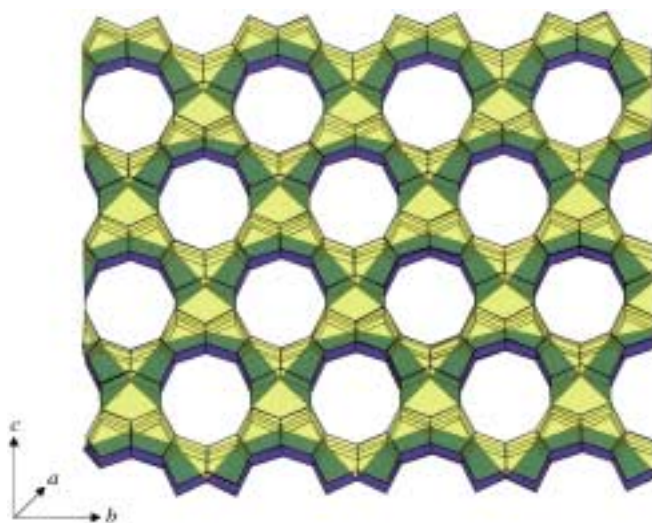


Figure 2. Representation of the MFS framework topology. The lobes along the 10-ring pores are colored in blue.

crystallographic free diameters of 0.33×0.48 nm terminating the lobes are impermeable by hydrocarbons. Two *trans*-but-2-ene molecules were docked in a ZSM-57 cluster and position-optimized with respect to their van der Waals interaction (Figure 3). The channel and lobe structure imposed the desirable crosswise arrangement of the two C_4 chains and favored the resulting formation of the dimer, *trans*-3,4-dimethylhex-2-ene (Figure 3).

ZSM-57 zeolite was synthesized by using N,N,N',N',N' -hexaethylpentamethylenediammonium ions as template.^[16] Molecular models reveal that during the crystallization process, these organic cations are trapped in the channels and impose positioning of balancing framework charges and, after calcination, of acid sites in the lobes. For comparison purposes, zeolite samples representative of the alternative pore types were selected: 10-ring channels without intersections (TON, ZSM-48, AEL topologies), 10-ring channels intersecting with 8-ring channels (FER topology), intersecting 10-ring channels (MFI, MEL), 10- and 12-ring channels without intersections (MWW topology), and intersecting 12-ring channels (BEA* topology). In the experimen-

[*] Prof. J. A. Martens, Dr. R. Ravishankar, Dr. I. E. Mishin, Prof. P. A. Jacobs
Centrum voor Oppervlaktechemie en Katalyse
Katholieke Universiteit Leuven
Kardinaal Mercierlaan 92, 3001 Heverlee (Belgium)
Fax: (+32)16-321998
E-mail: johan.martens@agr.kuleuven.ac.be

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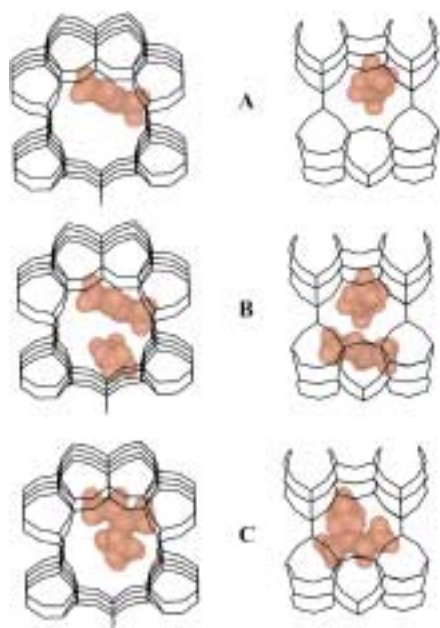


Figure 3. Crosswise arrangement of *trans*-but-2-ene molecules in the ZSM-57 pores giving rise to the formation *trans*-3,4-dimethylhex-2-ene (view along the 10-ring channels (left) and of the 8-ring channels (right) of the zeolite). A) A single *trans*-but-2-ene molecule preferentially adsorbed in the lobe; B) second *trans*-but-2-ene molecule positioned in the channel; C) position-optimized *trans*-3,4-dimethylhex-2-ene molecule retaining the perpendicular orientation of the original C_4 molecules.

tal evaluation of the but-2-ene oligomerization activity of these catalysts (Table 1), the reaction temperature was increased stepwise starting at 353 K until the but-2-ene conversion exceeded 60%. With all catalysts, double-bond shift was rapid and thus in the expression of conversion, all C_4 double-bond isomers were lumped and considered as unconverted feed.

The H-ZSM-57 catalyst gave a high butene conversion of 89% at 353 K. With the other zeolites, higher temperatures of 383–483 K were necessary to achieve similar conversions (Table 1). H-ZSM-57 combined high activity with high selectivity for C_8 alkenes (86%). C_{12} alkenes were formed in minor amounts. Other catalysts were substantially less selective for C_8 .

H-ZSM-57 was active even at 298 K. The but-2-ene conversion of 33% after 1 h dropped below 1% in the second hour on stream at this temperature. At 353 K, the but-2-ene conversion decreased more slowly with time on-stream. After

conversion of about 20 g butene per gram of zeolite, the butene conversion was still 60% with a C_8 selectivity of 91%. An increase in temperature of 20 K restored the initial activity and led to still slower decay with time on-stream. The temperature was increased stepwise to 473 K to balance deactivation with preservation of the high C_8 selectivity. Cracking and other side reactions started occurring only at temperatures exceeding 473 K. H-ZSM-57 zeolite can be periodically regenerated by calcination in air.

The expected primary products from the acid-catalyzed condensation of two but-2-ene molecules and of but-1-ene with but-2-ene are 3,4-dimethylhexenes and 3-methylheptenes, respectively. At 298 K, these products represented 76 and 9% of the C_8 product fraction, respectively. The other products were isomers in which methyl groups had shifted. At higher reaction temperatures, methyl shifts were more important. Branching and de-branching of C_8 products were limited. At 423 K, the C_8 fraction contained only 2.8% linear octenes and 1.4% tribranched octenes.

Molecular modeling and repulsion energy calculations^[17] (Table 2) were useful for rationalizing the oligomerization behavior of H-ZSM-57. Propene, *n*-butene, and *n*-pentene molecules fit in the lobe and leave enough free space in the channel for reaction with any linear or methyl-branched alkene. Isobutene can dimerize and codimerize with isopentenes, but reaction with heavier methyl-branched alkenes is sterically hindered. *n*-Hexenes, methylpentenes, and methylhexenes are repulsed from the lobe. When adsorbed in the channel, they can be combined with C_3 , *n*- C_4 , or *n*- C_5 alkenes in the lobe. Among the dimethyl-branched alkenes, the

Table 2. Excess repulsion potential [kJ mol⁻¹] of alkenes in ZSM-57.^[a]

Alkene in channel	Alkene in lobe							
	<i>n</i> - C_3	<i>n</i> - C_4	<i>i</i> - C_4	<i>n</i> - C_5	<i>i</i> - C_5	<i>n</i> - C_6	MB- C_6	MB- C_7
C_3	0	0	0	0	1	13	11	25
<i>n</i> - C_4	0	2	0	0	2	11	6	24
<i>i</i> - C_4	0	1	0	0	0	12	23	51
<i>n</i> - C_5	0	1	7	0	3	38	12	51
<i>i</i> - C_5	0	2	8	1	27	16	23	27
<i>n</i> - C_6	0	2	0	2	2	35	12	100
MB- C_6	0	3	11	2	28	16	44	n.d.
<i>n</i> - C_7	0	2	7	2	2	9	29	n.d.
MB- C_7	5	5	85	14	28	41	n.d.	n.d.
DB- C_8 ^[a]	31	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] MB and DB: monomethyl-branched and dimethyl-branched isomers, respectively; n.d. = not determined.

Table 1. Screening of the zeolite catalyst for the oligomerization of 2-butene.^[a]

Zeolite	Topology code	Si/Al	<i>T</i> [K]	Conversion [%]	Product distribution [wt %]				
					C_6 – C_7	C_8	C_9 – C_{11}	C_{12}	C_{13} ^[b]
H-ZSM-57	MFS	27	353	89	0.3	85.7	1.1	12.8	0.0
H-ZSM-48	–	50	383	82	0.9	60.5	9.9	24.9	3.8
H-Beta	BEA*	12.5	393	79	1.2	50.7	14.1	28.9	5.0
H-Ferrierite	FER	6.2	413	66	1.5	69.5	7.6	19.8	1.6
H-SAPO-11	AEL	–	413	73	1.4	60.7	7.7	24.8	5.5
H-ZSM-11	MEL	45	413	90	2.3	56.6	10.8	26.8	3.6
H-ZSM-22	TON	30	423	82	0.9	57.6	4.0	30.4	7.2
H-MCM-22	MWW	45	433	95	6.1	52.2	22.2	19.5	0.0
H-ZSM-5	MFI	26	435	72	5.9	56.8	13.9	23.4	0.0

[a] Feed composition: 2-butene (16 wt %); propane (78 wt %); pentane (6 wt %), *W/Fo*: 115 kg mol⁻¹. [b] C_{13} and higher oligomers.

dimethylpentenes undergo least repulsion when combined with propene in the lobe. Severe repulsion is encountered with dimethylhexenes in the channel in combination with any alkene in the lobe.

The experimentally observed low formation of C_{12} alkenes from C_4 alkenes on H-ZSM-57 (Table 1) is explained by the low reactivity of the C_8 fraction which mainly comprises dimethylhexenes. The C_{12} products were dibranched or tribranched, confirming their formation out of linear or monobranched C_8 isomers, respectively.

In an experiment with pent-1-ene at 373 K and at a pentene conversion of 60%, dimethyl-branched C_{10} alkenes were selectively formed. The subsequent formation of C_{15} alkenes by reaction of pentenes with dimethyl-branched isodecenes was suppressed, as expected.

Other experiments were concerned with a $C_3 + C_4$ mixture (Figure 4). Propene and butene were equally reactive. The primary products were C_6 – C_9 alkenes originating from dimerization and codimerization of C_3 and C_4 alkenes and

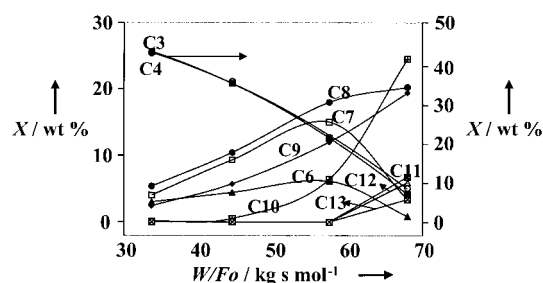


Figure 4. Distribution X [wt %] of the reaction products of a propene–butene mixture converted in the supercritical phase on H-ZSM-57 at different contact times W/Fo [kg s mol^{-1}] at 423 K (W = catalyst weight; Fo = molar flow rate of alkenes at reactor inlet. The feed composition is given in Table 1).

codimerization of methylpentenes and C_3 alkenes, all favored in the lobe and channel system (Table 2). At long contact times, the monomethyl-branched C_6 isomer and the monomethyl- and dimethyl-branched C_7 alkenes reacted further to give C_8 – C_{13} alkenes. These molecules with two or more methyl branchings cannot be properly coadsorbed with any of the shorter molecules and are inert to this catalyst.

In binary $C_3 + C_5$ and $C_4 + C_5$ mixtures, the two alkenes were also equally reactive (Table 3). The products had carbon numbers from C_6 up to C_{13} , peaking at C_8 – C_{11} . The main products from the $C_3 + C_5$ mixture can be explained by the favored dimerization and trimerization of C_3 alkenes, codimerization of C_3 and C_5 alkenes, and dimerization of C_5 alkenes. Alternative condensations occurred less, since they involve less appropriate molecule combinations. The mixture of $C_4 + C_5$ leads to C_8 , C_9 , and C_{10} alkenes through favored dimerizations and codimerizations. The less significant formation of C_{12} and C_{13} products is explained by condensations of methyl-branched C_8 isomers with C_4 and C_5 alkenes, respectively.

In all these experiments, the saturation selectivity of the feed alkenes into the corresponding alkanes was below 0.8%. With other zeolites, the selectivity for double-bond saturation

Table 3. Conversion and distribution of products in the oligomerization of binary alkene mixtures over H-ZSM-57 at 423 K.

	$C_3 + C_4$ ^[a]	$C_3 + C_5$ ^[b]	$C_4 + C_5$ ^[c]
	Conversion [%]		
C_3	86	93	–
C_4	83	–	90
C_5	–	85	87
	Product distribution [wt %]		
C_6	1	5	0
C_7	4	0	0
C_8	24	41	9
C_9	23	22	36
C_{10}	29	10	37
C_{11}	8	18	0
C_{12}	7	6	12
C_{13}	4	0	5

[a] Feed composition: propene (7 wt %), 1-butene (7 wt %), isobutane (76 wt %), pentane (5 wt %); W/Fo : 68 kg s mol^{-1} . [b] Feed composition: propene (9.8 wt %), 1-pentene (8.9 wt %), butane (74.5 wt %), isobutane (6.8 wt %); W/Fo : 72 kg s mol^{-1} . [c] Feed composition: 1-butene (7 wt %), 1-pentene (10 wt %), propane (10 wt %), isobutane (76 wt %); W/Fo : 158 kg s mol^{-1} .

was substantially higher, for example, around 10% with H-ZSM-22 (Table 1).

The exceptional activity, stability, and selectivity of H-ZSM-57 in alkene oligomerizations is the result of the lobate–pore structure, which favors a crosswise arrangement of alkenes and thus suppresses hydride transfer. With H-ZSM-57, the molecular weight of the oligomers can be tailored by mixing the appropriate short alkenes.

Experimental Section

Catalytic experiments were performed in a fixed-bed down-flow reactor filled with zeolite powder compressed into particles with diameters of 250–500 μm . The dead volume in the reactor tube was filled with glass beads of similar diameters. The feedstocks were prepared by mixing pure compounds in a storage vessel equipped with magnetic stirrer and pressurized at 7 MPa. The liquid feedstock was delivered to the reactor by means of an electronic mass flow controller for liquids. The pressure in the reactor was kept at 6 MPa. Product samples taken at the reactor outlet with a sampling valve with internal volume of 0.1 μL were analyzed on-line by gas chromatography. The skeletal branching and carbon number of the oligomers were analyzed off-line by gas chromatography with a catalytic hydrogenation module.

For the evaluation of the Lennard-Jones (12-6) interaction potential of an alkene and H-ZSM-57 zeolite, a rigid and purely siliceous MFS framework was considered. Dispersion and repulsion constants were taken from reference [17]. All calculations were done on a framework fragment of 1280 oxygen atoms. The geometry of the organic molecule was optimized by using Hyperchem software from Hypercube Inc. This geometry-optimized molecule was introduced into the lattice fragment, and its position optimized following a translation–rotation steepest descent procedure in three dimensions. The excess repulsion potential (Table 2) is the difference between the potential energy of the considered alkene and that of a position-optimized monomethyl-branched alkene of the same molecular weight. These potentials are –79, –91, –107, –120, –133, –144, –154, and –164 kJ mol^{-1} for C_6 to C_{13} isomers, respectively.

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Tribochemical Activation of Iron Oxide for the Reduction of NO with CO: How Lattice Defects Can Influence the Catalytic Activity

Thomas Rühle, Olaf Timpe, Norbert Pfänder und Robert Schlögl*

The cleavage of NO_x molecule into its constituent elements is an important goal for environmental catalysis.^[1] Massive metal oxides are, in principle, a suitable class of compounds^[2] and have been discussed for a long time as potential catalysts,^[3] however had neither the required activity nor stability against reduction.

Iron oxide is non-toxic and chemisorbs NO very well^[4] and is thus a promising material, provided that the active centers can be regenerated by a reducing agent such as CO.^[5] Iron oxide nano particles are currently the subject of extensive investigations in NO reduction processes.^[6] nonstationary kinetic measurements^[7] show that in hematite (α -Fe₂O₃) NO is absorbed and activated at defects in the oxygen sublattice.^[8] Conversion into N₂ proceeds via the intermediate N₂O. The active sites are regenerated by the reaction of CO with oxygen. In comparison to other catalysts iron oxide exhibits a high selectivity for N₂, but not for N₂O,^[6,9] an important feature for application in environmental catalysis.

When the reaction with CO is not limited to the surface active sites then the catalysts is rapidly following the

chemistry of the blast-furnace reduction of iron oxide. This reaction pathway requires the diffusion of iron out of the bulk material and onto the surface.^[10] Thus it is necessary to maximize the number on active sites but at the same time the bulk diffusion of the iron atoms has to be controlled.

The tribochemical (that is, induced through high-energy ball milling) activation of commercial hematite leads to a clear improvement in the catalytic properties of the material. The conversion–temperature profile (Figure 1) shows a

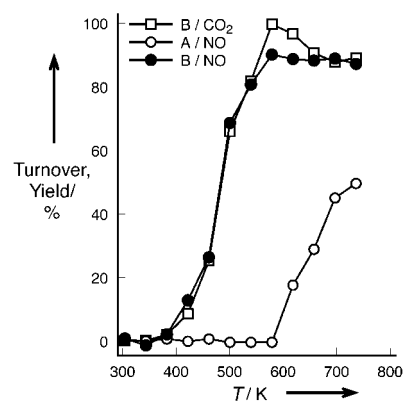


Figure 1. Conversion–temperature profile of the NO–CO reaction on pure unactivated (A) and activated iron oxide (B) (For B/CO₂ it is not the conversion but the yield curve). After activation the Brunauer–Emmett–Teller (BET) surface is not measurably different from the starting value. The modified residence time^[7] was in each case 100 kg mol^{−1}.

considerable increase in catalytic activity after the grinding process. At the temperature at which the unactivated oxide begins to react the activated sample shows its greatest activity. Compared to supported oxide particles^[7] that give active catalysts with an internal surface of 302 m² g^{−1} the catalyst presented herein, which has an internal surface of only 8.2 m² g^{−1}, is significantly more active, as can be seen by comparing the measured values at 50% conversion (here 482 K, supported sample 578 K) and at 80% conversion (here 532 K, supported sample 615 K). The comparison of the almost identical NO conversion and CO₂ yield curves in Figure 1 demonstrates clearly the excellent selectivity^[11] of the catalyst to N₂, that is achieved here even without a high surface-area activated charcoal support and the addition of promoter oxides.^[12] This result also demonstrates the high stability of the ground iron oxide against irreversible reduction.

The effect of the tribochemical treatment on the activity was emphasized by comparing the activation energies of the NO reduction by iron oxide and by a highly dispersed platinum catalyst^[13]. Figure 2 shows the Arrhenius plot of the corresponding data from stationary measurements. The activation improves the specific activity of the iron oxide compared to that of the supported platinum metal. Moreover, the apparent activation energy of the reaction on the iron oxide reduces from 82 to 45 kJ mol^{−1} and is thus comparable to that of the platinum catalyst (50 kJ mol^{−1}; a value that compares well with literature data^[14]). This observation is noteworthy because the mechanism of the reactions on platinum and iron ought to be different.^[15]

[*] Prof. Dr. R. Schlögl, Dipl.-Chem. T. Rühle, Dr. O. Timpe, N. Pfänder
Fritz-Haber-Institut der Max-Planck Gesellschaft
Faradayweg 4–6, 14195 Berlin (Germany)
Fax: (+49) 30-8413-4401
E-mail: andrea@fhi-berlin.mpg.de

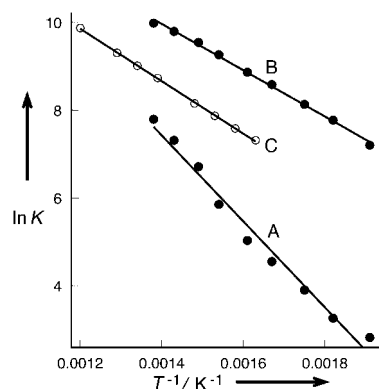


Figure 2. Determination of the apparent activation energy from the NO conversion; A) unactivated iron oxide, B) platinum on aluminum oxide, C) activated iron oxide. The errors are about twice as large as the data symbols for sample A.

The catalytic activity remained constant for 30 hours, during which the concentrations of the reaction gases and the temperature were changed many times in a steps-wise manner. This confirms the excellent stability of the active sites up to a maximum temperature of 773 K. Thermal pretreatment up to 873 K did not have a negative effect on the catalytic behavior.

The chemical stability after activation was tested through pulse treatment under reducing conditions. Figure 3 shows pulse responses to CO (5% in He carrier gas) at 673 K. After

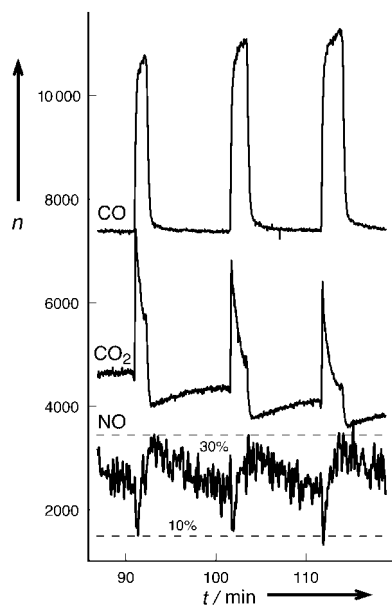


Figure 3. Pulse responses of an activated catalyst to the addition of CO (5% in He carrier gas) to the reaction gas mixture; n = IMR mass spectrometer count rate [events per second].

a very short period of improved NO conversion the SCR activity (SCR = selective catalytic reduction) reduces by 70% conversion, then after the end of the reductive pulse, slowly recovers to the initial value. The regeneration process, which has a time span of around 5 min, is assigned to the reoxidation of the iron (2+) centers in the bulk material; these result

from a fast reaction under the influence of CO, as the CO₂ pulse responses in Figure 2 suggest. The unactivated sample is completely and irreversibly deactivated by a single one of these CO pulses

The stability of the activated catalysis to gaseous oxygen is an important point as the defects near to the surface of the oxygen lattice in hematite seem to be responsible for the catalytic properties. Figure 4 reveals that the selectivity of the

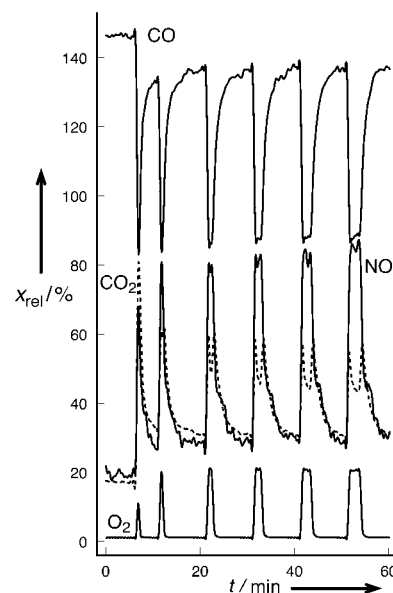


Figure 4. Pulse response of an activated catalyst to the addition of O₂ (2% in He carrier gas) to the reaction gas mixture. The pulse response in the CO₂ channel is shown as a broken line; x_{rel} = relative amount [%].

SCR reaction in the presence of oxygen (2% in He) is significantly diminished, however, rapidly recovers. The pulse profiles for CO and CO₂ are clearly not mirror images of one another which indicates that, in addition to the expected competition between the SCR reaction and the total oxidation of CO, other processes must also be occurring. After longer exposure to oxygen the conversion of CO₂ shows a transient effect that is distinguished by the combustion of carbon which does not come directly from the gas phase. Apparently the catalyst is able to store CO when the catalyst surface is transformed into a particular oxidation state that is higher than that under stationary operation.^[16] In response to the composition of the gas phase the surface of the iron oxide changes its composition^[17] and thus, its coverage of CO and NO. As a result of this behavior we observe a change in the selectivity of the SCR reaction and a CO storage effect.

The catalytic behavior demonstrates that the tribochemical activation leads to considerable disorder in the oxygen lattice at the surface, but without the occurrence of irreversible deactivation by reduction. The limitation of the catalytic activity by diffusion processes in the oxygen lattice was confirmed by modeling the nonstationary kinetic measurements for N₂O reduction on a supported iron oxide sample.^[18] The resulting activation energy for oxygen diffusion (47 kJ mol⁻¹) corresponds very well to the value determined here for the activated sample.^[19]

A significant change in the internal structure of the hematite was demonstrated by means of transmission electron microscopy (TEM). Figure 5A shows at the edge of an oxide particle a nano particle (green) that has been formed by the

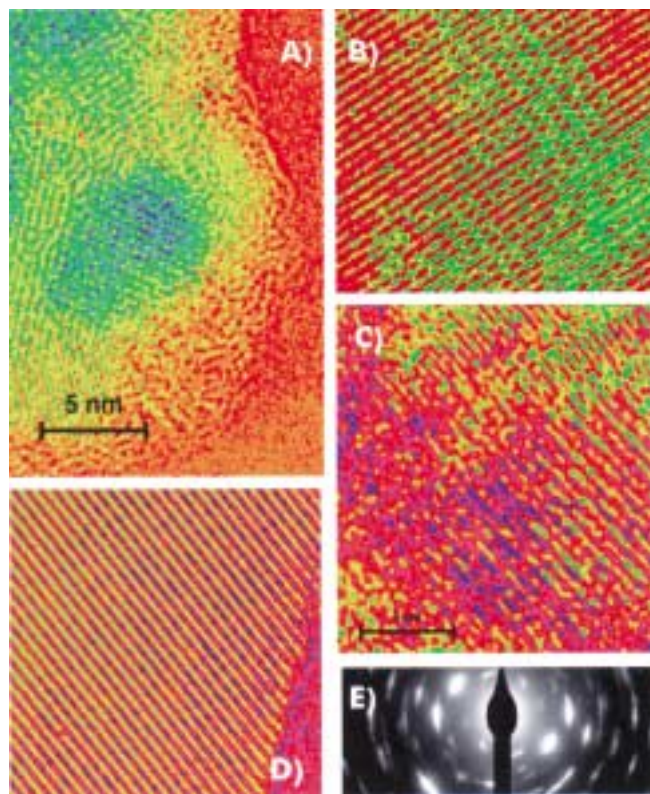


Figure 5. TEM image (Philips CM200FEG, 200 kV, samples dispersed by ultrasound in CCl_4 , measured on charcoal) of an activated catalyst (images A–C, E) and the untreated material (image D). In images A–C the (104) crystal planes with an average separation of 0.27 nm and in image D the (012) crystal planes with a separation of 0.37 nm can be seen. The crossed lattice planes in the oxide cluster (green) in image A correspond to the (024) crystal plane with a separation of 0.18 nm.

grinding process and that is surrounded by an amorphous layer which also forms the outer surface of the particle. The internal structure of the polycrystalline particle^[20] is highly disrupted (Figure 5B and C). It seems plausible that the amorphous regions (green in Figure 5B, blue in Figure 5C) and breaks in the (104) crystal plane (green in Figure 5C) hinder the migration of the iron ions in the bulk material. Under the present reaction conditions this migration controls the reduction to iron.^[21] Figure 5D displays the intact structure of starting material prior to grinding, which is free from an amorphous covering layer (red in Figure 5D). The diffraction pattern of the activated hematite (Figure 5E) does not show any foreign-phase reflections and indicates that disrupted crystalline regions (large dotlike reflections) and highly amorphous substance (ring structure) lie next to each other. The X-ray powder diffraction diagram^[22] also does not show a foreign phase but indicates the loss of around 90 % of the scattering power of the sample after activation.

The inhibition of the migration of iron ions by tribochemical activation up to “the grinding equilibrium”^[23] was

confirmed by temperature programmed reduction (TPR) in a hydrogen atmosphere. From the integrated mass loss^[24] the contribution of vacant oxygen sites^[25] was determined to be 1.8 mass %. The profile of the two-step reaction^[26] from hematite to magnetite (Fe_3O_4) and then to iron has a relative hydrogen consumption of 1:9 which indicates the phase purity of the activated sample. The profile of the reduction of the ground material indicates two double structures which intergrade in the ratio of 10:90, the first of which (at 584 and 724 K) can be assigned to the reduction of the crystalline hematite and the second (at 640 and 803 K) corresponds to a kinetically delayed reduction reaction. The quantitative agreement of this analysis with the loss of scattering power in the X-ray powder diffraction shows that around 90 % of the mass of the hematite becomes amorphous and thus kinetically hinders the reduction.

Through variation of the heating rate in the TPR^[27] and a deconvolution corresponding to the two differently damaged lattice sections of hematite it was determined that only the formation of magnetite from hematite^[28] is kinetically hindered while the subsequent reaction to metallic iron is in fact significantly easier after activation.^[29]

The results show that it is possible to increase the number of defects in the oxygen lattice that are necessary for catalysis and at the same time to increase the kinetic stability of the activated iron oxide in the form of hematite.^[30] This apparent contradiction to chemical intuition^[31] can be explained in that the metal-oxide reduction is at least a two-step process and the rate-limiting steps (the reorganization of the iron lattice for the formation of the magnetite and the oxygen migration for the formation of iron) are dependent in different ways upon the induced lattice defects. This example shows further that the controlled change of the bulk structure of a material really can cause significant improvements in catalytic activity and stability without the need to alter the usual “design variables” of heterogeneous catalysis, the chemical composition and the particle size.

Experimental Section

Hematite (Fluka, 99.5 % pure) was activated in a Retsch-Kugelmühle S2 in a steel grinding vessel. Dry grinding gave the grinding equilibrium after 45 h (first 24 h with 50 kg ms^{-2} and then 21 h with 100 kg ms^{-2}). The determination of the metallic impurities by X-ray fluorescence analysis before/after the activation gave the following values: Cu: 1130/1190; Cr: 1640/2000; Ni: 140/400 ppm. Photoelectron spectroscopy (XPS) could not detect the presence of any of the impurities in the surface of the sample.

The activity measurements were carried out in quartz-glass flow reactor (blind activity with SiC substitute filling: 4 % conversion of NO at 723 K) using 20 mg of activated sample. A flow of dry helium (100 mL min^{-1}), that contained NO (1700 ppm) and CO (10000 ppm), was employed as the reaction gas mixture. Detection was with an ATOMIKA 1500 IMR mass spectrometer (IMR = ion molecule reaction), which with the use of the primary gasses Kr, Xe, and CF_3I could analyze quantitatively the gas components NO, CO, NO_2 , CO_2 without cross sensitivity. The amount of N_2 was determined partially quantitatively through electron impact ionization mass spectrometry. For the IMR mass spectroscopic quantification relative sensitivity factors of 1:100:600 for NO:CO:CO₂ were used. For calibration benzene was used as an internal standard.

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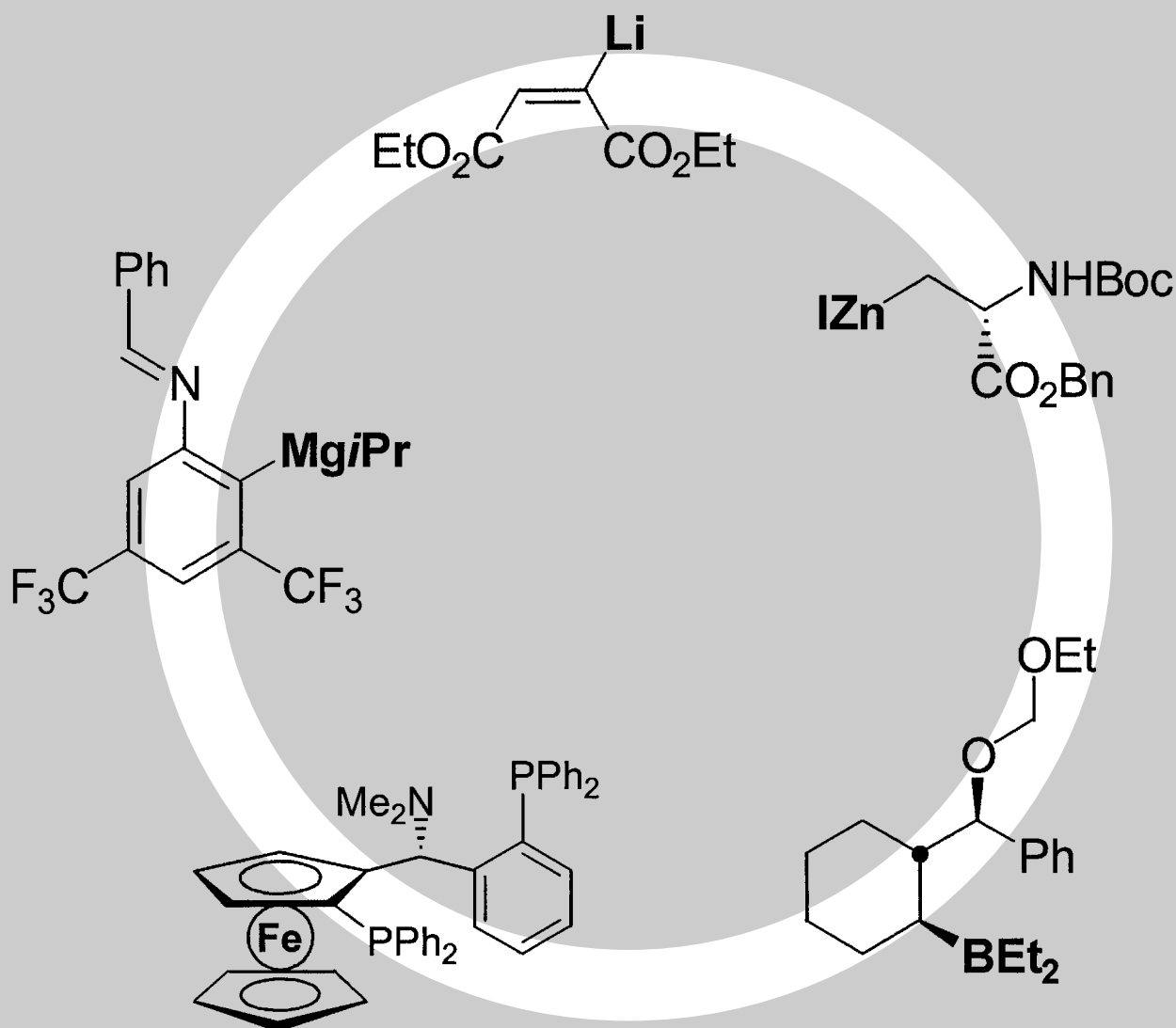
Cover Picture

**Burckhard Seelig, Sonja Keiper, Friedrich Stuhlmann,
and Andres Jäschke**

The cover picture shows the catalysis of a Diels–Alder reaction by an artificial ribozyme. A series of anthracene and maleimide derivatives are converted with high efficiency, and with suitable substituents enantioselectivities greater than 95 % *ee* can be achieved. Shown in the mirror is a picture of a synthetic ribozyme built from unnatural L-ribonucleotides, which, as expected, catalyzes the formation of the other enantiomer of the product. The threadlike molecules in the background symbolize the combinatorial RNA pool from which the ribozymes were originally isolated. More about the fascinating properties of these ribozymes is reported by Jäschke and co-workers on page 4576 ff. (The 3D model of a folded RNA molecule was generated with the program MOLMOL from the data set 1EHT.pdb.)



Polyfunctionalized organometallic complexes have great tolerance towards many functional groups making them particularly suitable for multistep syntheses without complicated protecting group strategies



New Applications of Polyfunctional Organometallic Compounds in Organic Synthesis**

Andreas Boudier, Lars O. Bromm, Matthias Lotz, and Paul Knochel*

Dedicated to Professor Rolf Huisgen on the occasion of his 80th birthday

In the second half of the twentieth century much effort was invested in the preparation of highly reactive polar organometallic reagents. The high reactivity of these reagents precluded the presence of many functional groups and often good chemoselectivities and stereoselectivities could only be achieved by transmetalation reactions. The synthesis of increasingly complex

target molecules and the desire to avoid tedious protection–deprotection steps has led inevitably to the use of functionalized organometallic reagents in retrosynthesis. In the last fifteen years, the generation of organic derivatives of numerous metals and metalloids (Li, Mg, B, Zn, Sn) was investigated. In this review the most important preparations and applica-

tions of organometallic reagents in organic synthesis will be covered, with particular emphasis on organozinc reagents.

Keywords: asymmetric catalysis • asymmetric synthesis • C–C coupling • organometallic compounds • synthetic methods

1. Introduction

The complexity of organic target molecules is constantly increasing and novel strategies allowing the efficient formation of new carbon–carbon bonds between functionalized moieties are needed. Conventional approaches using extensive protecting-group strategies are not satisfactory^[1] because of a mediocre atom economy.^[2] Radical reactions are more attractive but are more difficult to tune than polar reactions.^[3] On the other hand, polyfunctional organometallic complexes provide a general entry into complex molecules and extensive applications in total synthesis have been described.^[4] There are two difficulties associated with organometallic reagents: 1) to tolerate the functional groups present their preparation requires mild reaction conditions; 2) their reactivity is in many cases too low so that a transition metal catalyst is required. The reactivity of an organometallic species generally increases with the increasing ionic nature of the carbon–metal bond and thus with the difference of electronegativity between the metal center and the carbon atom (Figure 1).^[5]

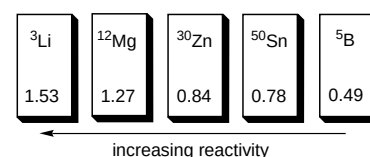


Figure 1. Metals used for the formation of polyfunctional organometallic complexes and electronegativity differences between the metal and carbon (Allred–Rochow electronegativity scale).

The polar organolithium compounds display a high reactivity toward most functional groups. The generation of polyfunctional organolithium species is only possible at low temperature or in the presence of an electrophile (Barbier reaction^[6]). At the other end of the reactivity spectrum are zinc, tin, and boron which form covalent bonds with carbon. These elements show little reactivity towards many electrophilic reagents but thus, tolerate many functional groups. Decisive for the synthetic applications of these non-polar organometallic compounds is the presence of low-lying orbitals which facilitate transmetalations. This review article emphasizes the synthetic applications of the most useful classes of polyfunctional organometallic complexes, and especially the recent results with polyfunctional organozinc compounds.

2. Polyfunctional organolithium reagents

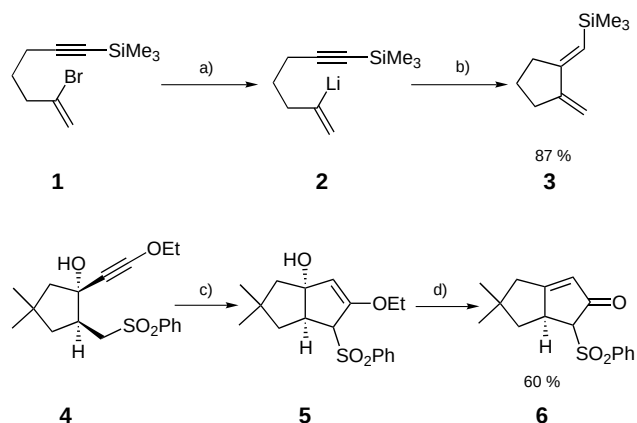
Organolithium compounds have played a central role in the development of organometallic chemistry for organic syn-

[*] Prof. Dr. P. Knochel, Dipl.-Chem. A. Boudier, Dipl.-Chem. L. O. Bromm, Dipl.-Chem. M. Lotz
Department of Chemistry
Ludwig-Maximilians-Universität
Butenandtstrasse 5–13, 81377 München (Germany)
Fax: (+49)89-2180-7680
E-mail: Paul.Knochel@cup.uni-muenchen.de

[**] Frequently used abbreviations are defined at the end of the article.

thesis.^[7] These reagents have to be prepared at low temperature because they attack even moderately reactive functionalized compounds such as silylacetylene. This property has been exploited in a very useful diene synthesis: Thus, the reaction of the enyne **1** with *t*BuLi at -100°C affords cleanly the difunctional lithium compound **2** which undergoes a smooth cyclization at 0°C providing the diene **3** (Scheme 1).^[8] A similarly straightforward carbolithiation^[9] is observed with alkoxyacetylenes. Thus, the lithiated sulfone obtained from **4** affords, after cyclization and hydrolysis via **5**, the enone **6**.^[10]

The remarkable pioneer work of Parham et al.^[11] has shown that various functionalized aryl- or heteroaryl lithium derivatives can be prepared by a bromine–lithium exchange. The aryllithium **7** is only stable at low temperature and has to be trapped with a highly reactive electrophile. With ketones under these conditions good yields of products such as **8** are obtained (Scheme 2). Alternatively, the unstable aryllithium can be transmetalated to the corresponding zinc reagent which is stable at 25°C and can react with a broad variety of electrophiles in the presence of a transition metal catalyst.^[12] Thus, the functionalized alkenyl iodide **9** is converted into the



Scheme 1. Intramolecular carbolithiations. a) *t*BuLi (2 equiv), pentane/diethyl ether, -100°C ; b) 0°C , 1 h; c) *n*BuLi (2 equiv), $-78^{\circ}\text{C} \rightarrow 0^{\circ}\text{C}$, 0.5 h; d) H_3O^+ .

corresponding zinc compound **10** which, in the presence of $\text{CuCN} \cdot 2\text{LiCl}$,^[13] smoothly adds to ethyl propiolate furnishing the unsaturated diester **11**.^[14]

Paul Knochel was born in 1955 in Strasbourg (France). He did his undergraduate studies at the University of Strasbourg and completed his Ph. D. at the ETH Zürich under the supervision of Prof. D. Seebach (1982). He spent 4 years at the CNRS at the University Pierre and Marie Curie (Paris) with Prof. J.-F. Normant and one year of post-doctoral studies at the Princeton University in the laboratory of Prof. M. F. Semmelhack. In 1987 he became assistant professor at the University of Michigan in Ann Arbor and full professor in 1991. He then accepted a C4-professor position at the Philipps-University in Marburg (Germany). In 1999, he moved to the Chemistry Department of the Ludwig-Maximilians-University (LMU) in Munich. His research interests are the development of new synthetic methods for organic chemistry using organometallic reagents, new asymmetric catalysts, and natural product synthesis.



A. Boudier

L. O. Bromm

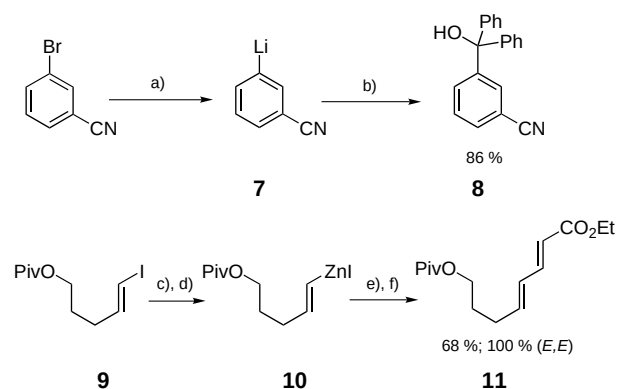
M. Lotz

P. Knochel

Andreas Boudier, born 1973 in Saarlouis (Germany), passed his undergraduate studies at the University of Saarbrücken (Germany, 1992–94), Strasbourg (France, 1994–97), and Houston (USA, 1997). He started his Ph. D. in Prof. Knochel's group in 1997 at the Philipps-University in Marburg and moved with him in 1999 to the LMU Munich. His work concentrates on the preparation and reactions of chiral dialkylzinc compounds.

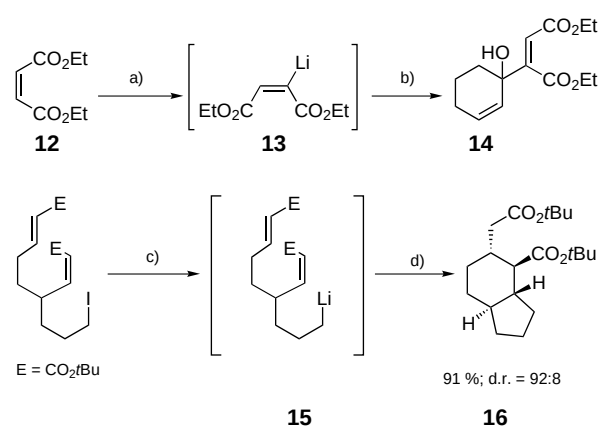
Lars Olav Bromm, born 1973 in Lich (Germany), did his undergraduate studies at the Philipps-University in Marburg (Germany) and at the University of Manchester Institute of Science and Technology (UMIST). After completing his undergraduate studies with Prof. P. Knochel on diastereoselective rearrangements of organoboranes, he moved with Prof. P. Knochel to the LMU Munich in 1999 to start a Ph. D. on the rearrangements of organoboranes and selective activation of C–H-bonds.

Matthias Lotz was born in Marburg (Germany) in 1972. He did his undergraduate studies at the Philipps-University in Marburg (1993–99). He joined the Knochel group in 1998 for his diploma thesis and started his Ph. D. at the LMU Munich in the same group in 1999. His work concentrates on the development of new ferrocenyl-type catalysts and their application in asymmetric catalysis.



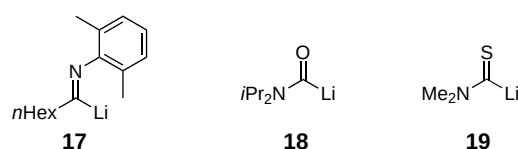
Scheme 2. Reactions of aryl and alkenyl zinc reagents. a) *n*BuLi, THF/hexane, -100°C ; b) benzophenone; c) *n*BuLi, THF/diethyl ether/hexanes, -100°C , 4 min; d) ZnI_2 , THF; e) $\text{CuCN} \cdot 2\text{LiCl}$, THF/ Et_2S , -20°C ; f) ethyl propiolate, -20°C , 2 h.

Under appropriate reaction conditions, the presence of an ester group is tolerated in the generation of an organolithium species. Thus, satisfactory yields of **14** are obtained by adding diethyl maleate (**12**) to a cooled solution of *N*-lithio-2,2,6,6-tetramethylpiperidine in THF and quenching immediately the short-lived intermediate **13** with an excess of an electrophile (Scheme 3).^[15] A rapid iodine–lithium exchange allows the generation of a lithium compound **15** bearing two ester functions. This reagent undergoes a Michael addition furnishing with an excellent stereoselectivity the *trans*-bicyclo[4.3.0]nonane **16** in 91 % yield.^[16]

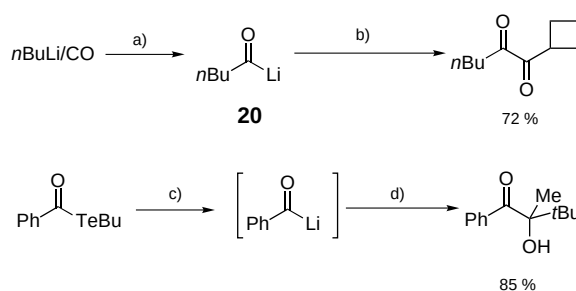


Scheme 3. Synthesis and reactions of functionalized alkenyllithium compounds generated for ester groups. a) *N*-lithio-2,2,6,6-tetramethylpiperidine, THF, -78°C ; b) 2-cyclohexenone; c) *n*BuLi, THF, -100°C ; d) $-100^{\circ}\text{C} \rightarrow -30^{\circ}\text{C}$.

The presence of heteroatoms in close proximity to the carbon–lithium bond facilitates the formation of an organolithium species as long as the various functional groups are tolerated.^[17] The direct lithiation with lithium powder in the presence of a catalytic amount of 4,4'-di-*tert*-butylbiphenyl (DBB), as popularized by Yus et al.^[18], proves to be a very convenient method for preparing a broad range of polyfunctional organolithium reagents.^[17–19] Thus, the difficult to prepare imido (17), carbamoyl (18), or thiocarbamoyl (19) lithium compounds could be generated.^[19,18c] The direct



preparation of acyllithium compounds such as **20**, either by a direct low-temperature route from RLi/CO ^[20,21] or a lithium–tellurium exchange reaction,^[22] has been successfully performed. In these direct preparation methods, the acyllithium species is generated in the presence of an electrophile (Scheme 4).

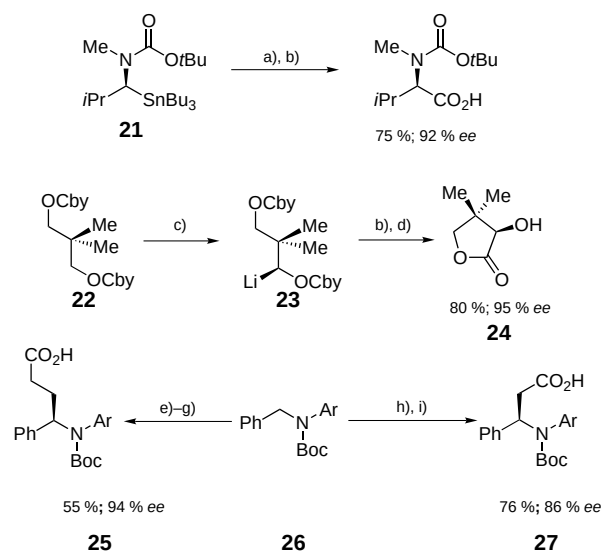


Scheme 4. Generation of acyllithium compounds. a) THF, -100°C ; b) ethyl cyclobutanecarboxylate, -110°C ; c) *n*BuLi, THF/diethyl ether, -105°C ; d) methyl-*tert*-butylketone.

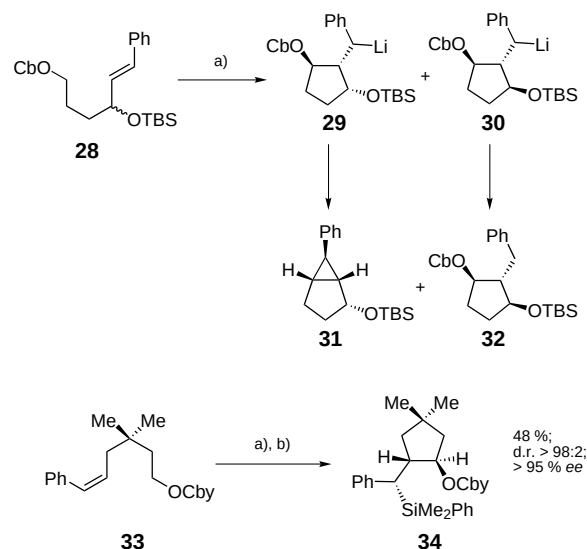
A remarkable development in the preparation of chiral functionalized organolithium reagents has led to numerous synthetic applications.^[23] The chiral compounds first prepared were functionalized in the position α to oxygen.^[24] Chiral organolithium reagents have also been obtained functionalized at the position α to nitrogen,^[25] sulfur,^[26] and, selenium.^[27] Optically pure or enriched lithium compounds can be prepared from the corresponding α -substituted organostannane compounds such as **21**^[28] or with the aid of the enantioselective deprotonation developed by Hoppe's group^[23a] using (–)-sparteine as a chiral inductor (Scheme 5).^[29]

This method has been successfully extended by Beak et al.,^[30] and others,^[31] to organolithium compounds functionalized at the position α to nitrogen. Thus, the enantioselective deprotonation of the 1,3-dicarbamate **22** with *s*BuLi/ (–)-sparteine provides the functionalized lithium reagent **23** which is readily carbonylated affording, after acidic deprotection, (*R*)-pantolactone (**24**) in 95 % *ee*;^[29] β - and γ -amino acid derivatives such as **25** and **27** are obtained from *N*-Boc-*N*-(*p*-methoxyphenyl)benzylamine (**26**) in high enantiomeric purity.^[32]

Enantioselective carbometallation reactions have also been performed by using the asymmetric deprotonation assisted by (–)-sparteine. The cyclization of the racemic carbamate **28** gives a 1:1 mixture of the two compounds **31** and **32**. The intermediate **29** undergoes a fast intramolecular cyclopropane formation while the diastereomer **30** is stable under the reaction conditions and is hydrolyzed to the diastereomerically pure cyclopentane **32** (Scheme 6).^[33]



Scheme 5. Preparation and reactions of chiral organolithium compounds. a) *n*BuLi, THF, -95°C , 10 min; b) CO_2 ; c) *s*BuLi, (–)-sparteine; d) HCl, Δ ; e) *n*BuLi, (–)-sparteine; f) acroleine; g) $\text{CrO}_3/\text{H}_2\text{SO}_4$; h) *n*BuLi, (–)-sparteine, 1-bromo-3-methylbut-2-ene; i) O_3 , then CrO_3 . (Cby = carbamoyl group.)



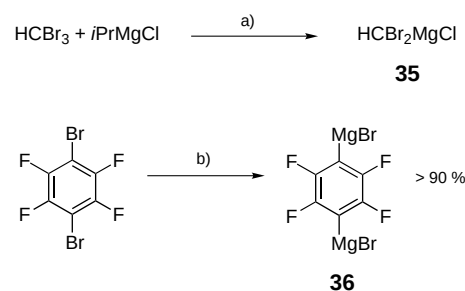
Scheme 6. Intramolecular enantioselective carbolithiation. a) *s*BuLi, (–)-sparteine, diethyl ether, $-78^{\circ}\text{C} \rightarrow -40^{\circ}\text{C}$; b) PhMe_2SiCl . (Cb, Cby various carbamoyl groups.)

The stereochemistry of up to three chiral centers can be controlled in these cyclizations and the carbamate **33** undergoes a smooth cyclization providing, after silylation, the benzyl silane **34** with excellent stereoselectivity (Scheme 6).^[34] The intermolecular version of this reaction is also possible and the addition of alkyl lithium compounds to cinnamaldehyde and cinnamylamine also proceeds with excellent stereoselectivity.^[35]

3. Polyfunctional organomagnesium reagents

Although organomagnesium reagents bear a more covalent carbon–metal bond than organolithium reagents and thus

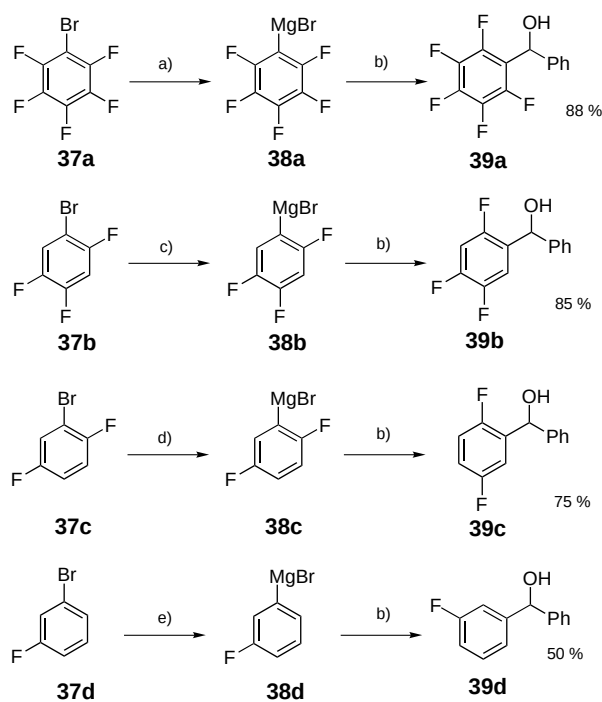
would appear suitable for use in organic synthesis, only a few methods for generating functionalized organomagnesium species have been described.^[36] The use of highly active magnesium, such as “Rieke-magnesium”,^[37] is not possible since the presence of electron-poor functional groups such as a carboxy or cyano group inhibits the formation of the Grignard reagent.^[38] Recently, the halide–magnesium exchange reaction was employed for preparing polyfunctional aryl-, heteroaryl-, or alkenylmagnesium compounds.^[39] The mild conditions required for the bromine–magnesium exchange were first discovered by Villiéras who demonstrated that highly reactive magnesium carbenoids such as **35** can be prepared at -78°C .^[40] Even dimagnesium derivatives like **36** have been prepared using the Br/Mg exchange reaction (Scheme 7).^[41]



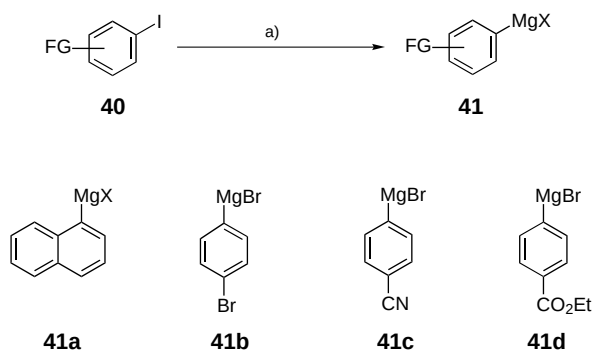
Scheme 7. Applications of the bromine–magnesium exchange. a) THF, -78°C ; b) EtMgBr (2 equiv), THF.

Both the iodine–magnesium and the bromine–magnesium exchange are accelerated by the presence of electron-withdrawing groups in the organic halide. Thus, in the fluorinated bromobenzene derivatives **37a–d** a significant difference is observed in the rates of the exchange reactions.^[42,43] Whereas bromopentafluorobenzene (**37a**) reacts with *i*PrMgBr at -78°C within 30 min giving the corresponding Grignard reagent **38a**, 1-bromo-2,4,5-trifluorobenzene (**37b**) requires a reaction temperature of -10°C for the Br/Mg exchange reaction to **38b**. Both organomagnesium compounds react cleanly with PhCHO giving the benzyl alcohols **39a** and **39b** in 85 and 88 % yield, respectively. For the 1-bromo-2,5-difluorobenzene (**37c**), the use of the more reactive *i*Pr₂Mg and a reaction temperature of 20°C are required to drive the reaction to completion. The 1-bromo-3-fluorobenzene (**37d**) is converted into the corresponding magnesium reagent at 20°C only by using an excess of *i*Pr₂Mg (3 h). Treatment with PhCHO leads to the alcohol **39d** in only 50 % yield because of the competitive reaction of excess *i*PrMgR with the electrophile PhCHO (Scheme 8).

In contrast, the iodine–magnesium exchange is possible at lower temperatures. Thus, an unactivated aryl iodide such as 1-naphthyl iodide (**40a**) undergoes the I/Mg exchange at 25°C within 0.5 h using 0.5 equivalents *i*Pr₂Mg. In the presence of electron-withdrawing groups such as a bromine, cyano, or ethylcarboxylate, a fast exchange reaction occurs between -25 and -40°C leading to polyfunctional arylmagnesium compounds such as **41b–d** which are perfectly stable at these low temperatures allowing for the first time a general approach to functionalized arylmagnesium compounds (Scheme 9).^[44]



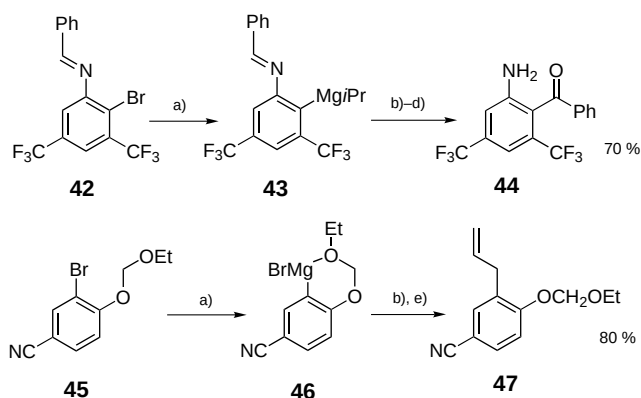
Scheme 8. Rate of the bromine–magnesium exchange depends upon the degree of fluorination of the bromoarene. a) $i\text{PrMgBr}$, THF, -78°C , 0.5 h; b) PhCHO ; c) $i\text{PrMgBr}$, THF, -10°C , 1 h; d) $i\text{PrMgBr}$, THF, 20°C , 2 h; e) $i\text{PrMgBr}$, THF, 20°C , 3 h.



Scheme 9. Entry to functionalized organomagnesium compounds by an I/Mg exchange. a) $i\text{PrMgBr}$ (1.0 equiv) or $i\text{Pr}_2\text{Mg}$ (0.5 equiv), THF, $-25^\circ\text{C} \rightarrow -40^\circ\text{C}$. (FG = functional group.)

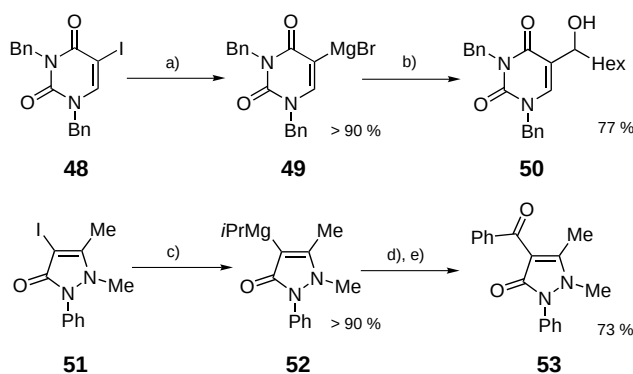
If there are sufficient electron-withdrawing groups in the molecule the bromine–magnesium exchange can also be useful in preparative chemistry. Thus, the bromobenzene derivative **42** reacts with $i\text{Pr}_2\text{Mg}$ at 0°C and provides the imine-functionalized magnesium compound **43**. After a copper-catalyzed benzylation and acidic workup, the aniline **44** is obtained in 70% yield.^[42] The presence of an *ortho*-directing group, as in **45**, considerably facilitates the exchange reaction. For example the chelate stabilized organomagnesium compound **46**, is converted by allylation into the benzonitrile **47** in 80% yield (Scheme 10).^[42]

The exchange reaction can also be applied to a variety of heterocyclic iodides or bromides. Thus, the 5-iodouracil derivative **48** reacts smoothly with $i\text{PrMgBr}$ in THF (-40°C , 0.5 h) and affords the corresponding magnesium derivative **49** which displays a much higher reactivity than the



Scheme 10. Synthesis and reactions of functionalized organomagnesium derivatives. a) $i\text{Pr}_2\text{Mg}$, THF, 0°C , 45 min; b) CuCN (10 mol %); c) PhCOCl $-30^\circ\text{C} \rightarrow 0^\circ\text{C}$; d) 2 M HCl; e) allyl bromide.

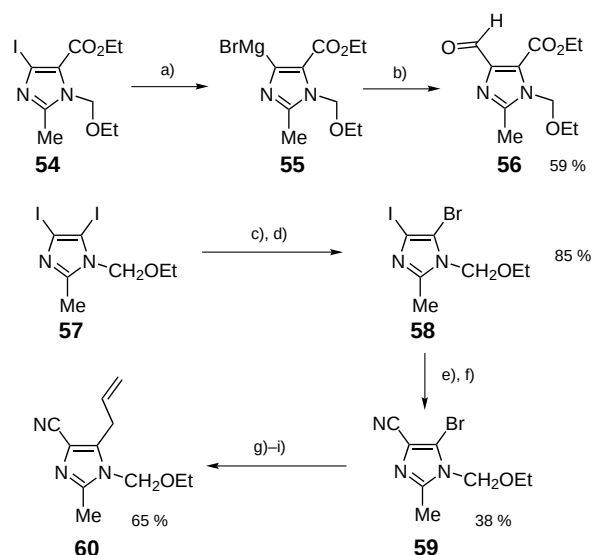
corresponding zinc derivative^[45] reacting readily with, for example, aldehydes to give alcohols such as **50**. Similarly, the iodoantipyrine **51** can be converted into the corresponding heteroaryl magnesium compound **52**, which reaction with PhCOCl (after transmetalation with $\text{CuCN} \cdot 2\text{LiCl}$) to the ketone **53** in 73% yield (Scheme 11).^[46]



Scheme 11. Synthesis of heterocyclic Grignard reagents by halide–magnesium exchange and subsequent reactions. a) $i\text{PrMgBr}$, THF, -40°C , 30 min; b) $n\text{HexCHO}$, $-40^\circ\text{C} \rightarrow \text{rt}$; c) $i\text{Pr}_2\text{Mg}$, CH_2Cl_2 , -25°C , 2 h; d) $\text{CuCN} \cdot 2\text{LiCl}$; e) PhCOCl , 0°C .

Functionalized iodoimidazoles like **54** undergo an exchange reaction at -40°C . The resulting Grignard reagent **55** reacts with DMF furnishing the aldehyde **56** in 59% yield.^[46] The 4,5-diiodoimidazole (**57**) undergoes the I/Mg exchange preferentially at the 5-position. It is possible to perform a contra-thermodynamic exchange at the 4-position, by converting the iodine substituent into a bromine (Scheme 12).

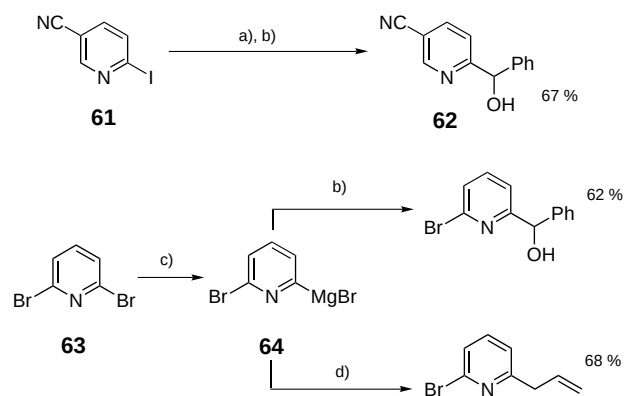
Thus, the treatment of **57** with $i\text{Pr}_2\text{Mg}$ in a mixture of THF and *N*-butylpyrrolidinone (NBP) at -35°C leads to the incorporation of the magnesium reagent at the 5-position. Reaction with $(\text{BrCl}_2)_2$ affords the 5-bromo-4-iodoimidazole derivative **58**. A second exchange with $i\text{PrMgBr}$ gives a magnesium compound which can be cyanated by *N*-cyano-benzotriazole giving the 5-bromo-4-cyanoimidazole (**59**). This heterocyclic bromide undergoes a fast Br/Mg exchange (THF,



Scheme 12. Synthesis and reactions of functionalized magnesium imidazole derivatives. a) *i*PrMgBr, THF, -40°C , 1 h; b) DMF; c) *i*Pr₂Mg, THF/NBP, -35°C , 1 h; d) (BrCl₂C)₂, -35°C , 1.5 h; e) *i*PrMgBr, -50°C , 0.5 h; f) *N*-cyanobenzotriazole, THF, $-78^{\circ}\text{C} \rightarrow \text{rt}$, 2 h, inverse addition; g) *i*PrMgBr, THF, -50°C , 10 min; h) CuCN·2LiCl; i) allyl bromide.

-50°C , 10 min) leading, after copper(I)-catalyzed allylation, to the imidazole **60** (Scheme 12).^[46]

The presence of the CH₂OEt group greatly accelerates this exchange reaction. Functionalized pyridines such as **61** undergo the I/Mg exchange within a few minutes, showing again the importance of electron-withdrawing groups. After reaction with PhCHO, the expected alcohol (**62**) is obtained in 67% yield (Scheme 13).^[47] Various dibromoheterocycles^[43]

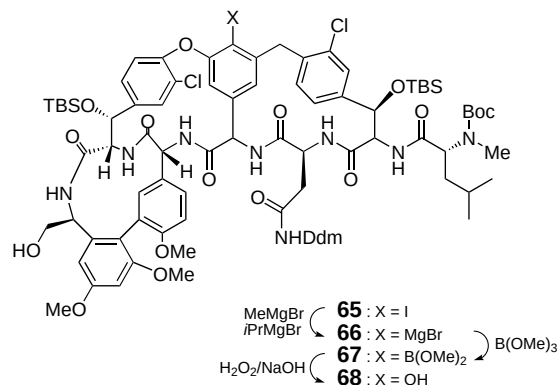


Scheme 13. Selective generation of pyridylmagnesium derivatives and subsequent reactions. a) *i*PrMgBr (1.1 equiv), THF, -78°C , 2 min; b) PhCHO; c) *i*Pr₂Mg, 20°C , 4 h; d) allyl bromide.

were found to undergo a chemoselective mono Mg/Br-exchange reactions. Thus, 2,6-dibromopyridine^[48] (**63**) undergoes a selective exchange with *i*Pr₂Mg (THF, 20°C , 4 h) furnishing the magnesium derivative **64** which can be trapped by various electrophiles (Scheme 13).^[42]

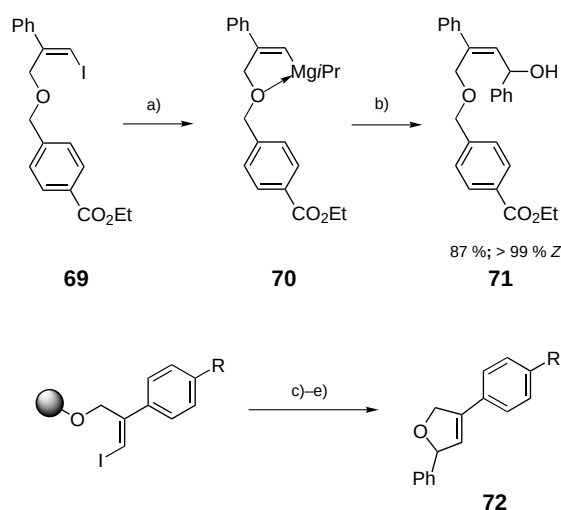
A Michael addition can be performed by treating the polyfunctional Grignard reagents with an enone in the presence of TMSCl (1 equiv) and CuCN·2LiCl (10 mol %).^[49] In the course of the synthesis of the antibiotic

Vancomycin, the iodine–magnesium exchange is used to perform a difficult oxidation reaction. The aryl iodide **65** undergoes a selective I/Mg exchange by treatment with MeMgBr and *i*PrMgBr (excess) leading to the organomagnesium derivative **66**. Reaction with B(OMe)₃ affords the boronic ester **67** which is oxidized to the corresponding phenol **68** with an alkaline solution of H₂O₂ (Scheme 14).^[50]



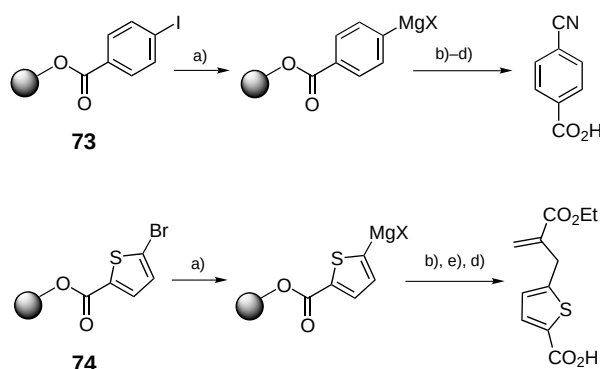
Scheme 14. Iodine–magnesium exchange in the synthesis of Vancomycin.

Besides aryl and heteroaryl iodides or bromides, alkenyl iodides also undergo an I/Mg exchange, however it is considerably slower. Thus, the reaction of (*E*)-iodooctene with *i*Pr₂Mg requires 18 h at 25°C for complete conversion. Again, the presence of a chelating group in the proximity of the halide helps the I/Mg exchange. Thus the allyl ether **69** undergoes an I/Mg exchange at -70°C within 12 h giving the chelate stabilized organomagnesium derivative **70** which inturn reacts with PhCHO furnishing the (*Z*)-allyl alcohol **71** ($> 99\%$ *Z*). This reaction sequence can be efficiently performed on the solid phase and leads to dihydrofurans of type **72**. The cleavage from the solid phase is performed under strong acidic conditions (CF₃CO₂H) which induce the cyclization reaction (Scheme 15).^[51]



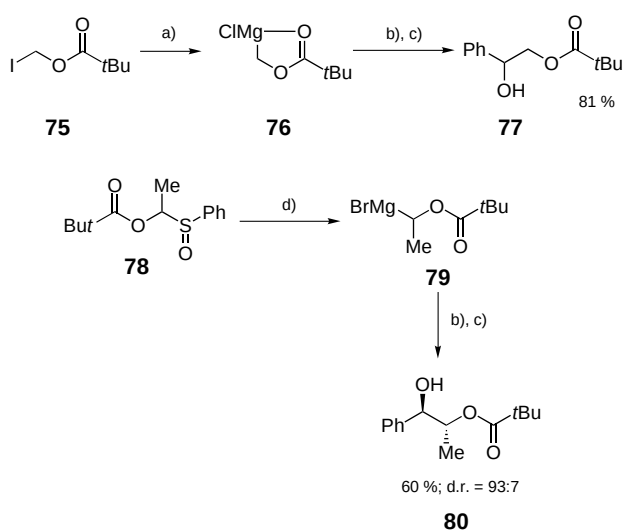
Scheme 15. Preparation and reactions of functionalized alkenylmagnesium compounds in solution and in the solid phase. (97%–98% HPLC purity). a) *i*Pr₂Mg, THF, -70°C , 12 h; b) PhCHO, $-70^{\circ}\text{C} \rightarrow \text{rt}$; c) *i*PrMgBr, THF:NMP (40:1), -40°C , 1.5 h; d) PhCHO; e) TFA:CH₂Cl₂ (1:9).

Similarly, a range of aryl iodides or heteroaryl iodides or bromides such as **73** and **74** attached to a Wang-resin are readily converted to the Grignard reagent using an excess of *i*PrMgBr (7–10 equiv). Remarkably, the ester linker is not attacked under these reaction conditions (-35°C , 0.5–1.0 h).^[44,51] This reaction constitutes one of the most general and convenient preparation methods for an organometallic species^[52] on the solid phase (Scheme 16) and is ideally suited for applications in combinatorial chemistry.^[53]



Scheme 16. Preparation and reactions of functionalized aryl- and heteroarylmagnesium compounds on a solid phase. (>94% HPLC purity). a) *i*PrMgBr (7 equiv), THF, -35°C , 0.5–1 h; b) CuCN·2LiCl; c) TosCN; d) $\text{CF}_3\text{CO}_2\text{H}$; e) ethyl(α -bromomethyl)acrylate.

Sensitive intermediates like the functionalized carbenoid **76** could be prepared from the readily available iodomethyl pivalate (**75**). Using *i*PrMgCl, the I/Mg exchange was complete within 15 min at -78°C . Treatment of **76** with PhCHO in the presence of TMSCl (excess) affords the 1,2-diol derivative **77** in 81%. As an extension of this reaction, the sulfoxide **78** was found to undergo a sulfoxide–magnesium exchange^[54] leading to the substituted magnesium carbenoid **79** which, after reaction with PhCHO, furnishes the alcohol **80** (Scheme 17).^[55]



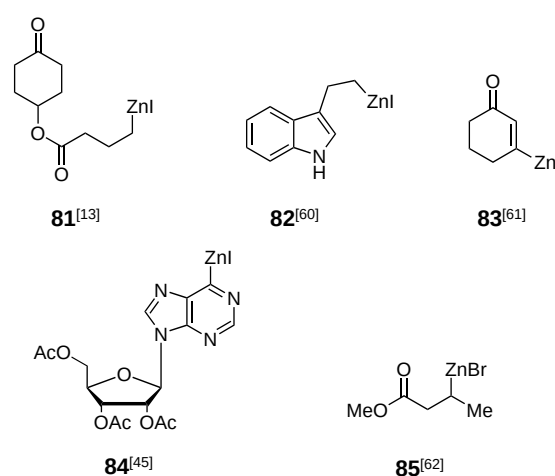
Scheme 17. Synthesis and reactions of functionalized organomagnesium carbenoids. a) *i*PrMgCl, THF/NBP, -78°C , 15 min; b) PhCHO; c) TMSCl; d) *i*PrMgBr, THF, -78°C , 15 min.

4. Polyfunctional organozinc reagents

Organozinc derivatives have an almost covalent carbon–metal bond and are therefore less reactive than organolithium reagents or organomagnesium compounds. This low reactivity allows for the presence of many functional groups in organozinc compounds.^[12,56–58]

4.1 Preparation of organozinc reagents by halogen–zinc exchange

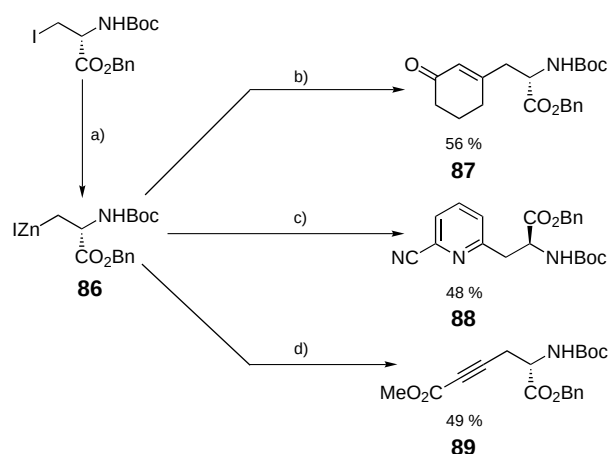
A broad range of zinc organometallics such as **81**–**84** have been prepared by the direct insertion of zinc (added in the form of zinc dust) to a polyfunctional alkyl iodide, or as for **85**, by using the reaction of highly activated “Rieke-zinc”^[37,59] with an alkyl bromide. Interestingly, activated organic halides



like benzylic, allylic, or propargylic halides undergo the insertion reaction far more readily, allowing the use of the corresponding bromide, or in several cases, the corresponding chloride or phosphate.^[12,63,64]

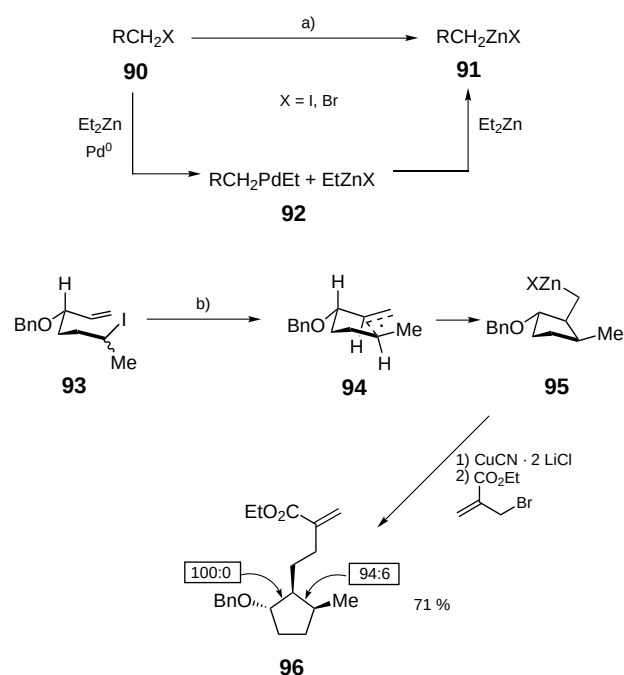
Ultrasound^[65] has in some cases led to more efficient and faster zinc insertions than by conventional methods. This approach proves to be especially useful for the preparation of the zinc–serine derivative **86**. Although all these organozinc reagents are relatively unreactive they undergo fast transmetalations with most transition metal salts (except those of manganese; transmetalation is also not observed with lanthanides).

Thus, various transition metal catalysts (Pd,^[66] Cu,^[13] Fe,^[67] Co,^[67,68] Ti,^[69] Ni^[70]) catalyze the reaction of polyfunctional zinc compounds with functionalized electrophiles leading to a broad range of multifunctional organic molecules. Thus, the reaction of **86** with 3-iodo-2-cyclohexenone in the presence of catalytic amounts of Pd⁰ provides the protected α -amino-acid **87**. Similarly the Pd⁰-catalyzed reaction of **86** with 6-bromo-2-cyanopyridine furnishes the protected α -aminoacid **88**. With a copper-catalyst (CuCN·2LiCl), the addition of methyl 2-bromopropionate^[71] leads to the protected acetylene- α -aminoacid **89** (Scheme 18).



Scheme 18. Amino acid synthesis using a chiral functionalized organozinc intermediate. a) Zn, THF, 35 °C, ultrasound; b) 3-iodo-cyclohex-2-en-1-one, Pd⁰; c) 2-bromo-5-cyanopyridine, Pd⁰; d) CuCN · 2 LiCl, methyl bromopropionate.

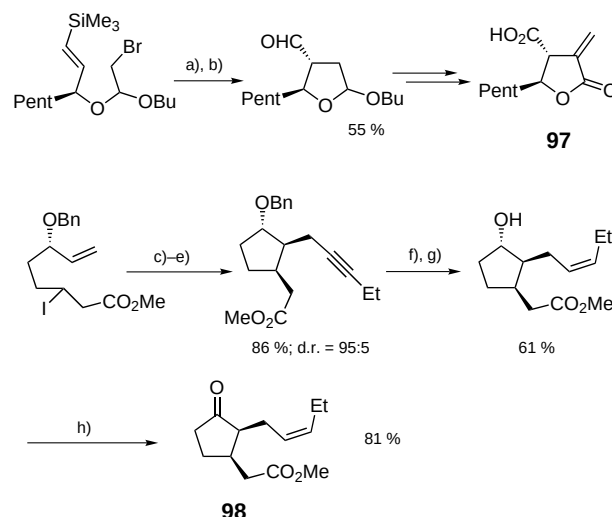
Organozinc halides of type **91** are also obtained using a palladium- or nickel-catalyzed halide–zinc exchange reaction.^[70,72] The initial oxidative addition of Pd⁰ to the alkyl iodide or alkyl bromide **90** proceeds by a radical mechanism (Scheme 19).^[73] The palladium intermediate **92** undergoes a



Scheme 19. Radical pathway for the palladium catalyzed iodine–zinc exchange reaction. a) Et₂Zn (2 equiv), THF, Pd⁰, 25 °C, 2 h; b) Et₂Zn (2 equiv), [PdCl₂(dppf)] (1.5 mol %), 25 °C, 5 h.

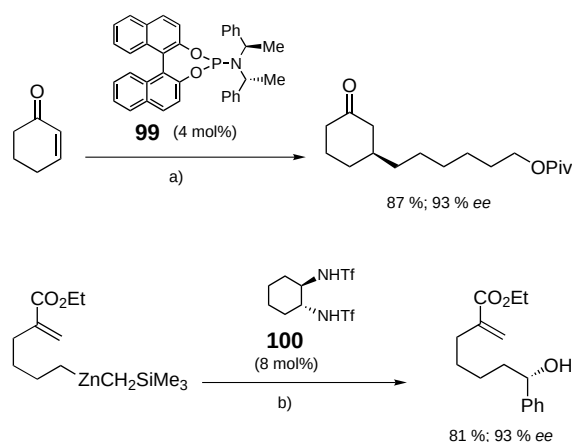
transmetalation with Et₂Zn leading to the organozinc reagent **91**. The radical nature of the halide–zinc exchange is best demonstrated by the stereoconvergent cyclization of the unsaturated alkyl iodide **93** (1:1 mixture of diastereomers) which leads to the almost diastereomerically pure compound **95** with stereocontrol of three adjacent centers.

The radical transition state **94** explains the stereoconvergence of the reaction as well as the stereochemistry of the product. After allylation with ethyl (2-bromomethyl)acrylate, the cyclopentane derivative **96** is obtained in 71 % yield.^[74] This type of radical cyclization which provides an organo-metallic product is well suited for the preparation of polyfunctional natural products such as (–)-methylenolactocin (**97**)^[75] and *cis*-methyl jasmonate (**98**)^[76] (Scheme 20).



Scheme 20. Synthesis of (–)-methylenolactocin and *cis*-methyl jasmonate. a) Et₂Zn, LiI, [Ni(acac)₂] cat., THF, 40 °C; b) O₂, TMSCl, THF, –5 °C; c) Et₂Zn, [Ni(acac)₂] cat., THF, 25 °C; d) CuCN · 2 LiCl; e) 1-bromo-1-butyne, –55 °C, 48 h; f) H₂, Pd/BaSO₄ cat., pyridine, 92 %; g) BCl₃, CH₂Cl₂, –78 to –10 °C; h) Dess–Martin oxidation.

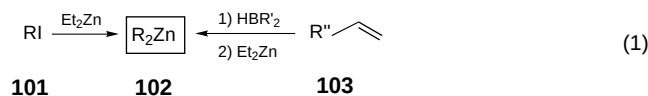
Like organozinc halides, diorganozinc compounds are an important class of organozinc reagents. The absence of halides makes these reagents especially useful in asymmetric additions (1,2- and 1,4-additions).^[77,78] Thus, the use of the chiral ligand **99** (4 mol %) and Cu(OTf)₂ (2 mol %) allows the highly enantioselective addition of polyfunctional dialkylzinc compounds to cyclic enones (Scheme 21).^[78] Also, the chiral 1,2-triflamide **100**^[79] in combination with the mixed dialkylzinc compounds^[80] of type RZnCH₂SiMe₃ allows the addition of



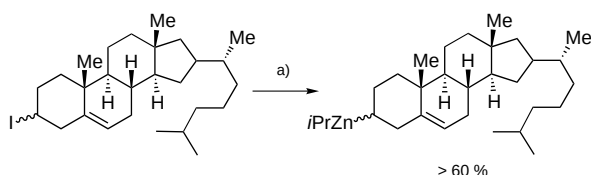
Scheme 21. Asymmetric catalysis with functionalized zinc complexes. a) Zn((CH₂)₆OPiv)₂, Cu(OTf)₂ (2 mol %); b) PhCHO, Ti(OiPr)₄, diethyl ether, –20 °C, 26 h.

functionalized R groups to aldehydes in high enantiomeric excess (Scheme 21).^[81] Furthermore, with mixed dialkylzinc compounds only the synthetically useful R group is transferred.

Several general methods are available to prepare the dialkylzinc compounds **102**, either starting from alkyl iodides **101** or more attractively from olefins **103** [Eq. (1)]. In the first



case, the functionalized alkyl iodide is treated with Et_2Zn or $i\text{Pr}_2\text{Zn}$ in THF. The presence of small amounts of Cu(I) salts like CuCN catalyzes the exchange reaction.^[82,83] A mixed metal catalysis (using MnBr_2 and CuCl) allows the exchange reaction to be performed with alkyl bromides, however the products are alkylzinc bromides and not dialkylzinc compounds.^[84] Interestingly, the iodine–zinc exchange can be strongly accelerated by irradiation, thus avoiding the use of an excess of Et_2Zn .^[85] In the case of secondary alkyl iodides, which require $i\text{Pr}_2\text{Zn}$ for the exchange reaction, the use of $i\text{Pr}_2\text{Zn}$ generated in situ from $i\text{PrMgBr}$ and ZnBr_2 (0.5 equiv) is possible. This method is very useful for the preparation of complex dialkylzinc compounds (Scheme 22).^[86]



Scheme 22. An example of the iodine–magnesium exchange using $i\text{Pr}_2\text{Zn}$ prepared in situ. a) $i\text{PrMgBr}$ (3.0 equiv), ZnBr_2 (1.5 equiv), diethyl ether, rt, 1 h.

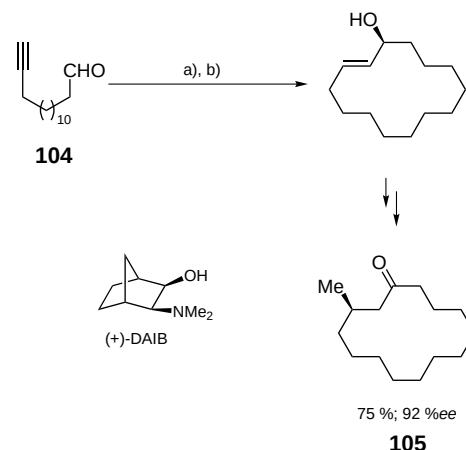
4.2. Preparation of organozinc reagents by the boron–zinc exchange reaction

This section describes the second method of preparation for dialkylzinc compounds, the boron–zinc exchange sequence as well as two of its most important applications: the preparation of chiral dialkylzinc reagents and the diastereoselective allylic C–H activation.

4.2.1. Scope and limitations

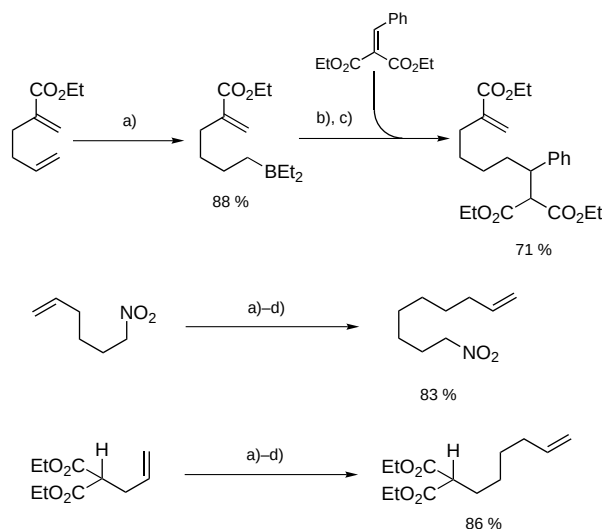
Organoboranes react with dialkylzinc compounds under mild conditions. The driving force of the reaction is the formation of stable dialkylzinc compounds, and in some cases, the formation of volatile organoboranes such as BMe_3 (bp = -22°C).^[87] The reaction can be used to prepare primary dialkylzinc reagents, dibenzylic organozinc compounds by using Me_2Zn or Et_2Zn , or secondary dialkylzinc reagents by using $i\text{Pr}_2\text{Zn}$. Alkenylzinc derivatives can also be prepared, in almost quantitative yield, using Et_2Zn .^[88–90] This reaction has been elegantly applied by Oppolzer et al. for an enantiose-

lective synthesis of (*R*)-(–)-muscone (**105**) from the alkyne **104** (Scheme 23). Remarkably, the aldehyde function is not reduced by $c\text{Hex}_2\text{BH}$ during the hydroboration reaction.^[90,91]



Scheme 23. Enantioselective muscone synthesis. a) $c\text{Hex}_2\text{BH}$, hexane, 0°C ; b) (+)-DAIB, (1 mol %), Et_2Zn .

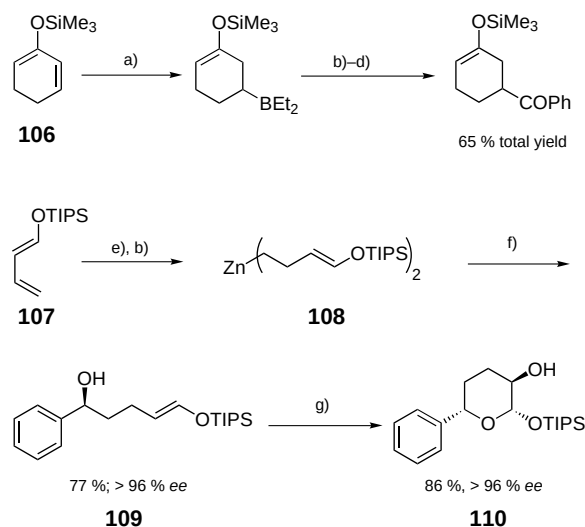
In general, the reaction conditions for the boron–zinc exchange are so mild that a number of sensitive functional groups such as acrylic ester, nitro, diethyl malonate, and enol ether are tolerated (Scheme 24).^[92] The functionalized organozinc intermediates react smoothly with various electrophiles in the presence of a copper(I) catalyst, thus extending the scope of the organoborane chemistry; organoboranes themselves react only with selected electrophiles (Scheme 24).^[92]



Scheme 24. Synthesis of functionalized dialkylzinc compounds by the boron–zinc exchange. a) Et_2BH , diethyl ether, 25°C ; b) Et_2Zn (neat), 0°C ; c) $\text{CuCN} \cdot 2\text{LiCl}$; d) allyl bromide (excess).

The hydroboration of dienic silyl enol ethers such as **106** or **107** followed by a boron–zinc exchange affords zinc complexes such as **108** which are difficult to prepare from the corresponding organic halide because of its instability and high tendency to undergo elimination reactions. The func-

tionalized organozinc reagent **108** undergoes a highly enantioselective addition to PhCHO in the presence of catalytic amounts of **100**. The resulting chiral alcohol **109** can be selectively oxidized giving the enantiomerically pure tetrahydropyran **110** (Scheme 25).^[93]



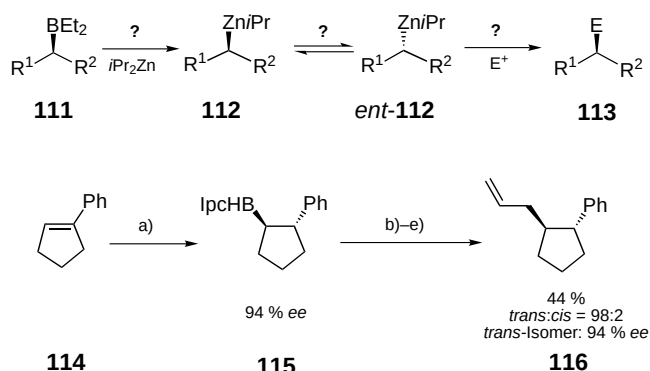
Scheme 25. Synthesis and reactions of functionalized dialkylzinc reagents by the hydroboration of 1,3-dienes. a) Et_2BH , diethyl ether, 25°C , 12 h; b) Et_2Zn , 0°C ; c) $\text{CuCN}\cdot 2\text{LiCl}$; d) PhCOCl ; e) Et_2BH , 50°C , 4 h; f) PhCHO , **100** (8 mol %), $\text{Ti}(\text{O}i\text{Pr})_4$, toluene, -20°C , 12 h; g) $t\text{BuOOH}$, $[\text{VO}(\text{acac})_2]$ cat., hexane, rt, 8 h.

The boron–zinc exchange has also been applied to the preparation of 1,3-dizincapropene derivatives which are difficult to prepare by other methods.^[94] Because of the high tolerance of the hydroboration reaction and the boron–zinc exchange towards functional groups, this reaction sequence represents the most versatile method for preparing polyfunctional dialkylzincs.

4.2.2. The preparation of chiral zinc complexes using the boron–zinc exchange reaction

As a result of the highly covalent character of the carbon–zinc bond, secondary organozinc derivatives should be configurationally stable. This is supported by early ^1H NMR studies which show that the activation energy required for inversion is higher than 26 kcal mol^{-1} ,^[95] which has been confirmed by more recent measurements.^[96] However, the preparation of chiral organozinc compounds such as **112** (Scheme 26) and their use for synthetic applications requires the fulfillment of several conditions: First, the boron–zinc exchange with secondary organoboranes such as **111** has to proceed stereoselectively. Second, the chiral zinc reagent formed (**112**) has to be configurationally stable under the reaction conditions used for its generation (solvent, temperature) and under the conditions used for its reactions with electrophiles. Finally, the reaction with electrophiles leading to products of type **113** has to proceed stereoselectively. Failing to fulfill any of these conditions will make the whole reaction sequence synthetically useless.

Fortunately, reaction conditions could be found to make the reaction sequence highly stereoselective.^[97–100] Thus, the hydroboration of 1-phenylcyclopentene (**114**) with (–)-mono-isopinocampheylborane (–)-IpcBH₂ (99 % ee)^[101] provides, after recrystallization, the chiral organoborane **115**. This organoborane is first treated with Et_2BH at 50°C for 16 h leading to a sterically less-crowded diethylorganoborane which undergoes smoothly the boron–zinc exchange within 5 h at room temperature. Stereoselective allylation of the resulting organozinc compound in the presence of $\text{CuCN}\cdot 2\text{LiCl}$ affords the expected product **116** in 44 % yield with an excellent diastereoselectivity (*cis:trans* = 2:98) and enantiomeric purity of 94 % ee (Scheme 26).^[98]

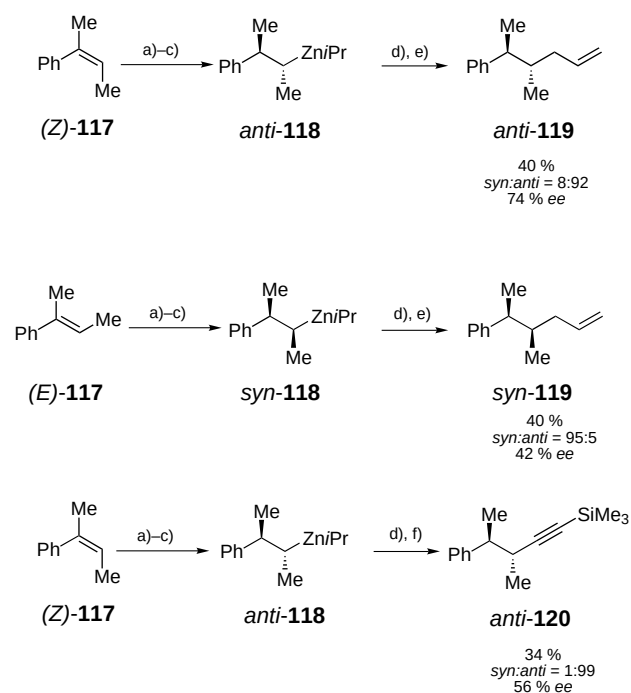


Scheme 26. Synthesis and reactions of chiral dialkylzinc compounds. a) (–)-IpcBH₂, diethyl ether, -35°C ; b) Et_2BH , 60°C , 13 h; c) $i\text{Pr}_2\text{Zn}$, rt, 4 h; d) $\text{CuCN}\cdot 2\text{LiCl}$; e) allyl bromide.

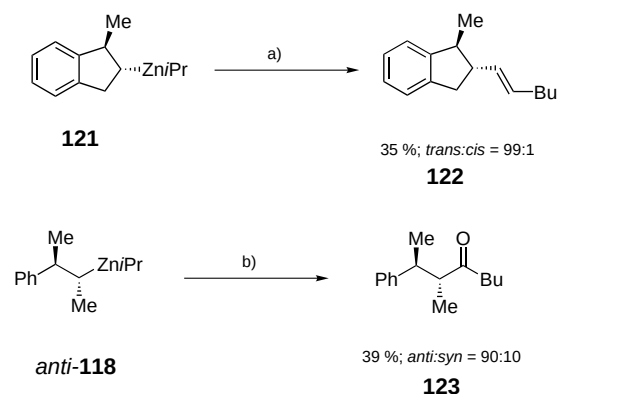
The exchange reaction can be applied to open-chain systems and allows the preparation of chiral acyclic zinc complexes with excellent diastereoselectivities. The enantioselectivity in these reactions is determined by the first asymmetric hydroboration step. Thus, starting from the styrene derivative (*Z*)-**117**, the dialkylzinc compound *anti*-**118** is obtained preferentially. Allylation leads to *anti*-**119** (*syn:anti* = 8:92; Scheme 27). Using the (*E*)-styrene, the same reaction sequence furnishes *syn*-**118**, and after allylation *syn*-**119** (*syn:anti* = 95:5).^[99] The copper(II)-catalyzed cross-coupling with bromoalkynes gives especially good diastereoselectivities and provides diastereomerically pure alkynes such as **120** (Scheme 27).^[99]

Palladium(0)-catalyzed reactions with electrophiles can be performed stereoselectively. The Pd⁰-catalyzed cross-coupling of the organozinc complex **121** and *anti*-**118** with an alkyl iodide or an acid chloride provides the desired products **122** and **123**, respectively, with excellent diastereoselectivity (Scheme 28).^[100]

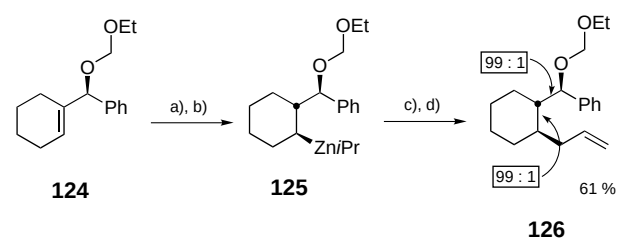
Preliminary experiments have shown that with this method the stereocontrol of three neighboring centers can be achieved. The chiral allyl ether derivative **124** undergoes a fully diastereoselective hydroboration with Et_2BH . After boron–zinc exchange (25°C , 4 h) the zinc complex **125** is obtained. The subsequent allylation affords the expected product **126** with high diastereoselectivity (Scheme 29).^[102]



Scheme 27. Synthesis and reactions of chiral open-chain dialkylzinc compounds. a) (–)-IpcBH₂, –35 °C, 48 h; b) Et₂BH, 60 °C, 16 h; c) iPr₂Zn, 25 °C, 5 h; d) CuCN · 2 LiCl; e) allyl bromide, –78 °C; 1–2 h; f) 1-bromo-2-trimethylsilylacetylene, –78 °C; 1–2 h.



Scheme 28. Asymmetric generation of chiral dialkylzinc compounds and Pd⁰-catalyzed reactions. a) [Pd(dba)₂] (2 mol %), P(oTol)₃ (4 mol %), 0 °C → rt, 12 h, 1-iodohex-1-ene; b) [Pd(dba)₂] (2 mol %), P(oTol)₃ (4 mol %), 0 °C → rt, 12 h, BuCOCl.

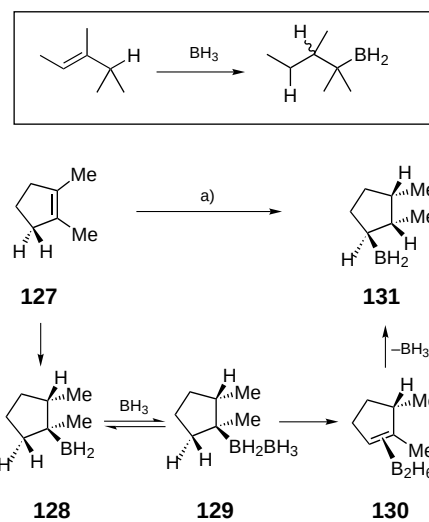


Scheme 29. Diastereoselective hydroboration and boron–zinc exchange followed by allylation. a) Et₂BH, 60 °C, 13 h; b) iPr₂Zn, 25 °C, 4 h; c) CuCN · 2 LiCl cat.; d) allyl bromide.

4.2.3. Diastereoselective C–H activation

The activation of inactive C–H bonds is an important research area.^[103] The hydroboration of tetrasubstituted

olefins followed by a thermal rearrangement allows a formal C–H activation reaction at the allylic position. Mechanistic studies of Rickborn and Field^[104] have shown that thermal rearrangement of hindered organoboranes may proceed stereoselectively. The mechanism of the thermal rearrangement is complex, but it has been reported that the presence of excess borane catalyzes the rearrangement, so that, in the case of 1,2-dimethylcyclopentene (**127**) the intermediates are the initial hydroboration product **128**, which in the presence of excess borane forms the organoborane **129** (Scheme 30). This intermediate rapidly rearranges at 50 °C via a transition state,^[105] tentatively represented by **130**, giving **131** as the only diastereomer (Scheme 30).

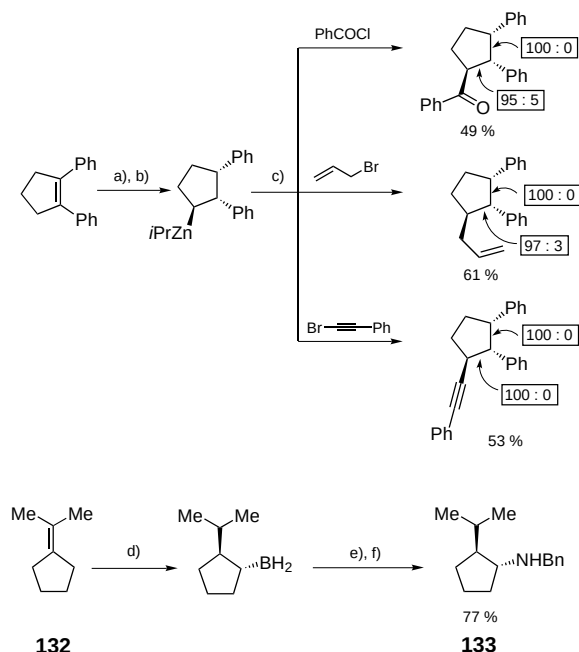


Scheme 30. The thermal rearrangement of organoboranes. a) BH₃ · THF, 50 °C, 3 h.

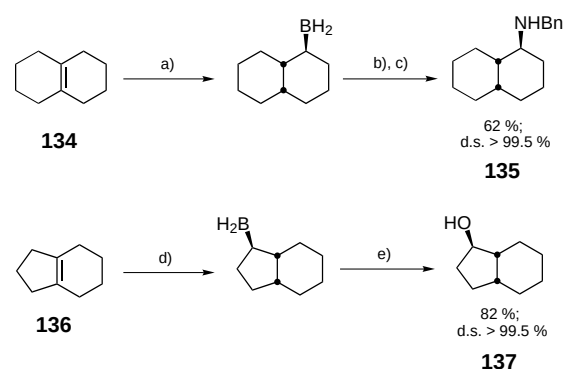
This rearrangement allows the functionalization of a range of tetrasubstituted cyclic olefins. The resulting organoborane can be transmetalated to the corresponding zinc derivative by treatment with iPr₂Zn and reacts with several electrophiles with retention of the configuration of the stereocenter next to zinc (Scheme 31).^[106] In the case of exocyclic tetrasubstituted alkenes such as **132**, the migration occurs in the ring affording, after an electrophilic amination, the *trans*-substituted benzylic amine **133** as one diastereomer (Scheme 31).^[107]

For bicyclic systems, very selective migrations take place. In the case of the bicyclo[4.4.0]decene (**134**), only one diastereomer of **135** is obtained after migration and electrophilic amination.^[106] Remarkably the migration in the unsymmetrical bicyclo[4.3.0]nonene (**136**), gives only **137**, derived from a migration in the five-membered ring. This may be because of the greater configurational flexibility of the five-membered ring which can therefore better accommodate the transition state required for the rearrangement (Scheme 32).

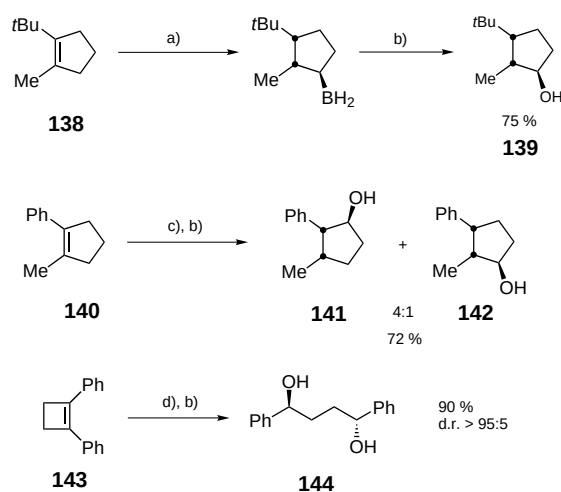
In the case of unsymmetrically substituted cyclopentenes such as **138**, only the migration to the site adjacent to the methyl substituent is observed, affording, after oxidation, the cyclopentanol **139** in 75 % as only one stereoisomer (Scheme 33). In the case of 1-phenyl-2-methylcyclopentene (**140**) the two possible tertiary organoboranes migrate with



Scheme 31. Stereoselective generation and reactions of secondary organoborane compounds. a) $\text{BH}_3 \cdot \text{THF}$, 50°C , 3 h; b) $i\text{Pr}_2\text{Zn}$, 25°C , 3 h; c) $\text{CuCN} \cdot 2\text{LiCl}$; d) $\text{BH}_3 \cdot \text{THF}$, 50°C , 8 h; e) BCl_3 ; f) BnN_3 .



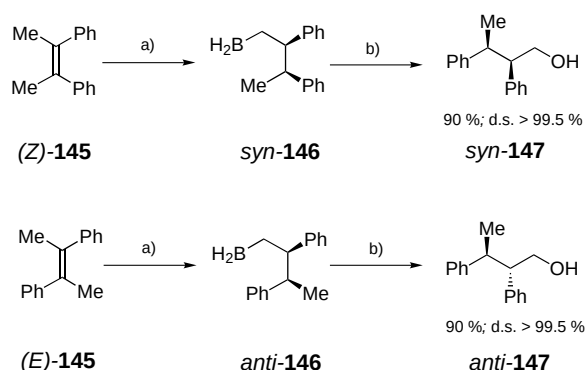
Scheme 32. Stereoselective thermal rearrangement and reactions of bicyclic organoboranes. a) $\text{BH}_3 \cdot \text{THF}$, 70°C , 6 h; b) BCl_3 ; c) BnN_3 ; d) $\text{BH}_3 \cdot \text{THF}$, 50°C , 3 h; e) NaOH , H_2O_2 .



Scheme 33. Stereoselective rearrangements of monocyclic tertiary organoboranes. a) $\text{BH}_3 \cdot \text{THF}$, 50°C , 3.5 h; b) NaOH , H_2O_2 ; c) $\text{BH}_3 \cdot \text{THF}$, 50°C , 18 h; d) $\text{BH}_3 \cdot \text{THF}$, 50°C , 5 h.

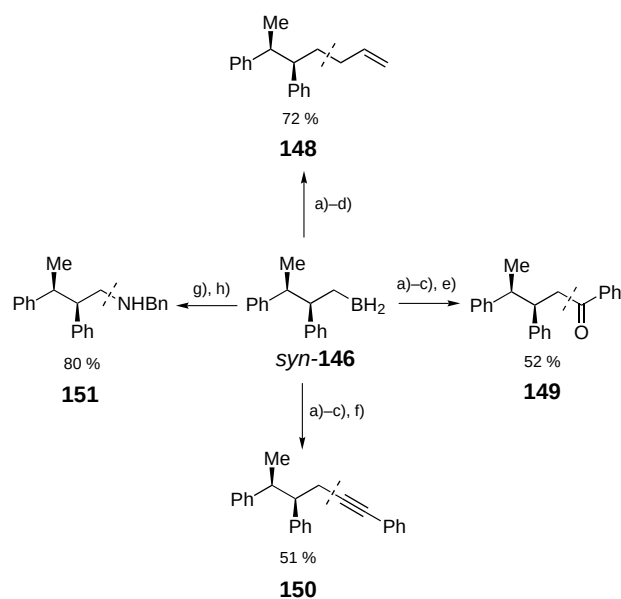
different rates. After heating to 50°C for 18 h, the two regioisomeric alcohols **141** and **142** are obtained as a 4:1 mixture after oxidative workup.^[108] Interestingly, for cyclobutene derivatives such as **143**, a ring-opening reaction is observed leading to the *meso*-diol **144** with good stereoselectivity (Scheme 33).^[106]

Remarkably, highly diastereoselective rearrangements are also observed with open-chain tetrasubstituted olefins. Thus, after oxidation, the olefin (*Z*)-**145** produces only the alcohol (*syn*)-**147**, whereas the (*E*)-**145** furnishes the corresponding *anti*-alcohol (*anti*)-**147** (Scheme 34).^[107]



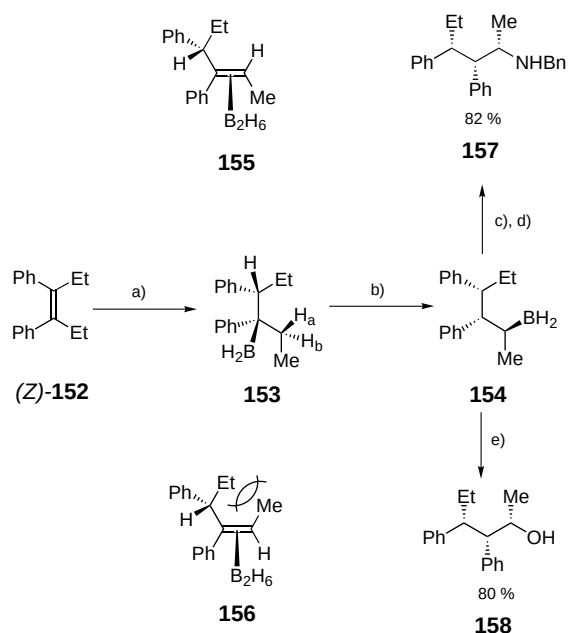
Scheme 34. Rearrangement of acyclic tertiary organoboranes. a) $\text{BH}_3 \cdot \text{THF}$, 70°C , 12 h; b) NaOH , H_2O_2 .

Bubbling ethylene through a solution of *syn*-**146** leads to the formation of the corresponding diethylborane derivative. Subsequent transmetalation of the organoborane by reaction with Et_2Zn produces the corresponding organozinc derivative which reacts with a range of carbon electrophiles leading to various products such as **148–150**. The direct electrophilic amination of *syn*-**146** furnishes the diastereomerically pure amine **151** (Scheme 35).^[107]



Scheme 35. Stereoselective reactions of *syn*-**146**. a) ethylene; b) Et_2Zn ; c) $\text{CuCN} \cdot 2\text{LiCl}$; d) allyl bromide; e) PhCOCl ; f) 1-bromo-2-phenylethyne; g) BCl_3 ; h) BnN_3 .

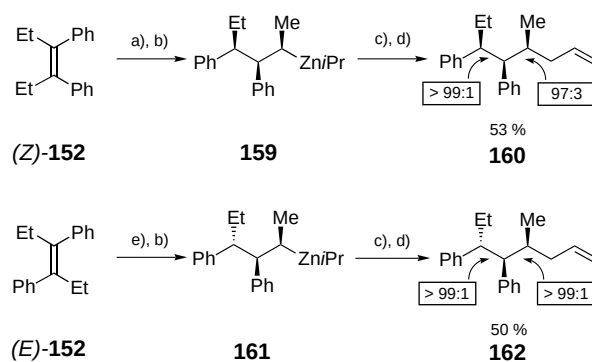
An extension of the reaction is possible by using substrates with two diastereotopic hydrogen atoms at the α position. Thus, (*Z*)-3,4-diphenylhexene (*Z*)-**152**, after hydroboration gives the tertiary organoborane **153** bearing two diastereotopic hydrogen atoms (H_a and H_b) at the α -position. Remarkably, after heating the reaction mixture to 70 °C for 12 h only one diastereomeric organoborane **154** is obtained. This is best explained by assuming that the resulting diborane–olefin complexes are intermediates of the rearrangement (see Scheme 30). By abstraction of H_a , the diborane–olefin complex **155** is formed, whereas by abstraction of H_b , the diborane–olefin complex **156** is formed. The high steric hindrance between the methyl substituent and the *cis*-1-phenylpropyl substituent precludes the formation of **156** and only an intermediate, such as **155**, may form in the course of the reaction. After oxidation or amination of **154** the two expected products **157** and **158** are isolated. The stereochemistry of the products has been confirmed by X-ray analysis (Scheme 36).^[109]



Scheme 36. Diastereoselective rearrangement allowing the stereocontrol of three adjacent carbon centers. a) $\text{BH}_3 \cdot \text{THF}$; b) 70 °C, 6 h; c) BCl_3 ; d) BnN_3 ; e) NaOH , H_2O_2 .

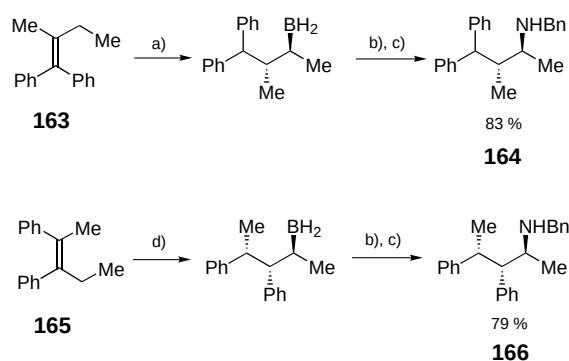
In combination with the boron–zinc exchange, the thermal rearrangement of organoboranes allows the control of the stereochemistry of three adjacent carbon centers.^[109] Thus, the hydroboration and boron–zinc exchange of (*E*)- and (*Z*)-**152** produces two epimeric organozinc reagents **159** and **161**, which after a copper(i)-catalyzed allylation, lead to the diastereomerically almost pure products **160** and **162** (Scheme 37).^[109]

Interestingly, by using unsymmetrically substituted acyclic olefins only one migration direction is observed. Thus, 1,1-diphenyl-2-methyl-1-butene (**163**) furnishes only the product from rearrangement in the direction of the ethyl group, no migration in the direction of the methyl group is observed. After electrophilic amination the benzylic amine **164** is



Scheme 37. Diastereoselective allylation of diastereomerically pure secondary dialkylzinc compounds. a) $\text{BH}_3 \cdot \text{THF}$, 65 °C, 12 h; b) $i\text{Pr}_2\text{Zn}$, rt, 2 h; c) $\text{CuCN} \cdot 2\text{LiCl}$ (20 mol %); d) allyl bromide, –78 °C, 1–2 h; e) $\text{BH}_3 \cdot \text{THF}$, 50 °C, 6 h.

formed as one diastereomer. Similarly, (*Z*)-2,3-diphenyl-2-pentene (**165**) affords, after thermal rearrangement and amination, the benzylic amine **166** as one diastereomer (Scheme 38).^[109]

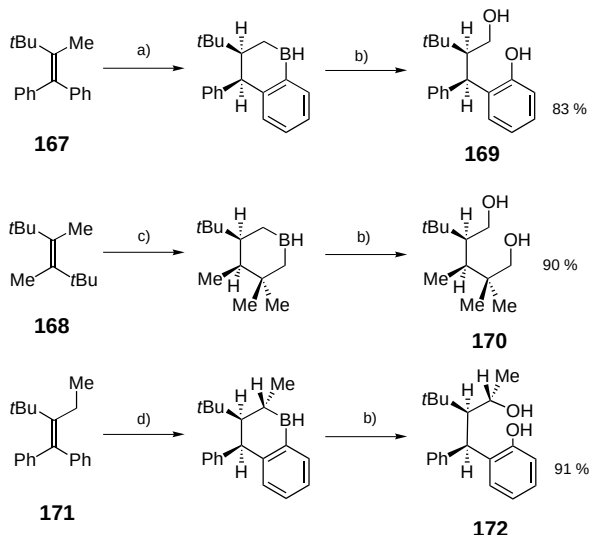


Scheme 38. Stereoselective and regioselective thermal rearrangement: a) $\text{BH}_3 \cdot \text{THF}$, 50 °C, 4 h; b) BCl_3 ; c) BnN_3 ; d) $\text{BH}_3 \cdot \text{THF}$, 65 °C, 12 h.

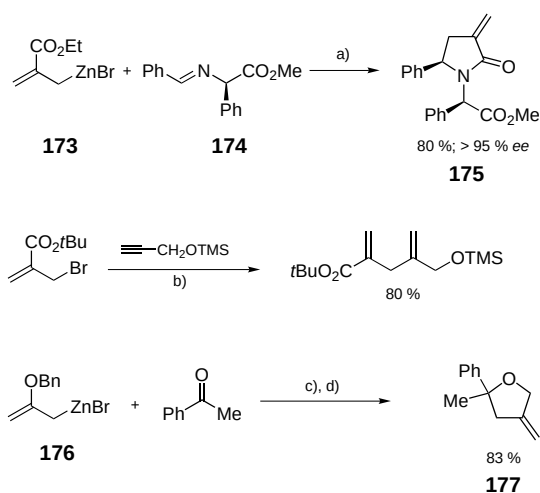
Remote C–H activations are possible and after hydroboration and rearrangement the olefins **167** and **168** undergo a remote C–H activation, subsequent oxidation produces in both cases only one diastereoisomer (**169** and **170**, respectively). By using the ethyl substituted precursor **171** control of the stereochemistry of three adjacent centers is possible leading to the hydroxyphenol **172** in 91% yield (Scheme 39).^[107,110]

4.3. Functionalized allylzinc reagents

Allylzinc compounds are highly reactive and react with most functionalized organic electrophiles.^[111] Some functionalized allylzinc compounds can be prepared by the direct insertion of zinc added in powder form. Thus, Villieras et al. showed that the reaction of ethyl (2-bromomethyl)acrylate with zinc powder in THF produces the functionalized zinc reagent **173** in excellent yield.^[112] Its reaction with chiral imines such as **174** furnishes α,β -unsaturated lactams^[113] such as **175** (Scheme 40) with excellent diastereoselectivity. The allylzinc reagent **173** can also be prepared *in situ*^[114] and added to alkynes. The functionalized allylzinc compound **176**



Scheme 39. Diastereoselective remote C–H activation. a) $\text{BH}_3 \cdot \text{THF}$; 50°C , 12 h; b) NaOH , H_2O_2 ; c) $\text{BH}_3 \cdot \text{THF}$; 40°C , 72 h; d) $\text{BH}_3 \cdot \text{THF}$; 50°C , 4 h.

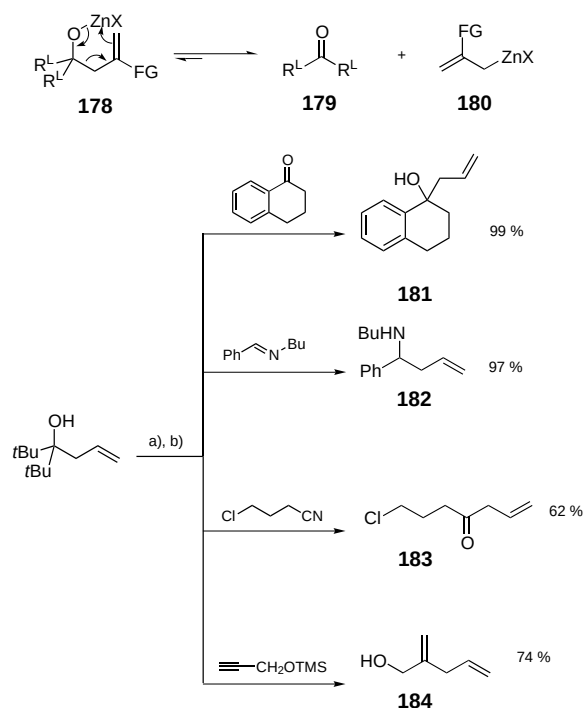


Scheme 40. Reaction of functionalized allylic organozinc reagents. a) THF , 25°C ; b) Zn , THF , 45°C , 0.5 h; c) THF , rt; d) $[\text{Pd}(\text{PPh}_3)_4]$ cat., 65°C , 16 h.

has been used to perform several new types of cyclization leading to, for example, 3-exo-methylene tetrahydrofurans like **177** (Scheme 40).^[115]

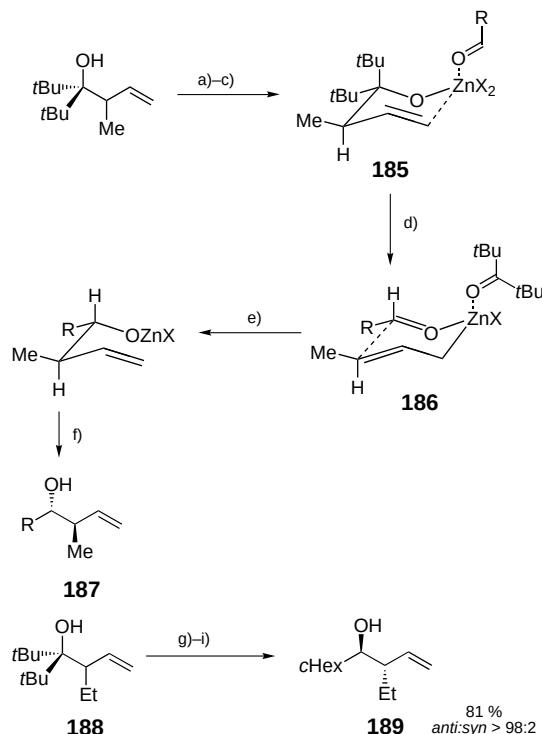
A new method for preparing the functionalized allylzinc reagents **180** uses the corresponding homoallylzinc alcoholates **178** as masked allylzinc reagents.^[116, 117] The driving force for the fragmentation is the reduction of steric hindrance in **178** and the formation of the ketone **179** (R^L = large substituent such as *t*Bu).^[118] If this fragmentation is performed in the presence of an organic electrophile, an allylation reaction takes place leading to products such as **181–184** (Scheme 41).

Interestingly, substituted homoallyl alcoholates undergo stereoselective additions to aldehydes whereas the corresponding substituted allylic organozinc compounds generated by conventional methods (insertion of zinc metal) display no stereoselectivity on addition to aldehydes. This behavior can be best explained by assuming that the homoallylzinc alcoholate **185** coordinates the aldehyde before fragmentation. The



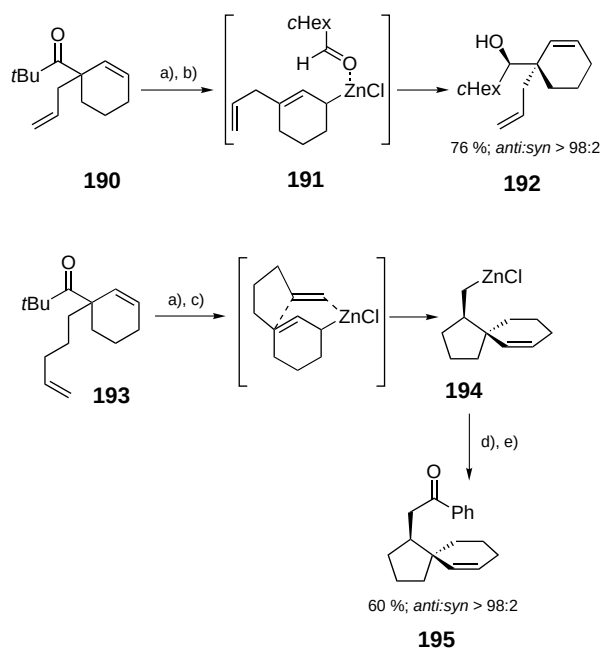
Scheme 41. Generation of allylzinc, by the fragmentation of sterically hindered homoallylzinc alcoholates, and subsequent reaction. a) *n*BuLi; b) ZnCl_2 . (FG = functional group.)

crotylzinc reagent formed (**186**) immediately reacts with the aldehyde providing the homoallylic alcohol **187**. Especially high stereoselectivities are obtained with secondary aldehydes (Scheme 42).^[116] The fragmentation of **188** provides after reaction with cyclohexanecarbaldehyde the homoallylic alcohol **189**.



Scheme 42. Diastereoselective addition reactions of substituted allylzinc compounds generated by fragmentation. a) *t*BuLi; b) RCHO ; c) ZnX_2 ; d) fragmentation; e) allylic rearrangement; f) H_2O ; g) *n*BuLi, -78°C ; h) *c*HexCHO; i) ZnCl_2 , -78°C .

The method can be applied to generate highly substituted allylzinc reagents such as **191**. Its reaction with *c*HexCHO furnishes the secondary alcohol **192** bearing a quaternary carbon center at the α -position. The zinc alcoholate is best generated by the direct addition of *n*BuLi to the ketone **190** at -78°C .^[119] The method can also be used for metallo-ene reactions.^[120] Thus, the addition of *n*BuLi to the ketone **193** initiates the fragmentation reaction and addition to the double bond of the zinc reagent **194** which is benzoylated in the presence of CuCN·2LiCl giving the ketone as only one diastereomer (Scheme 43).

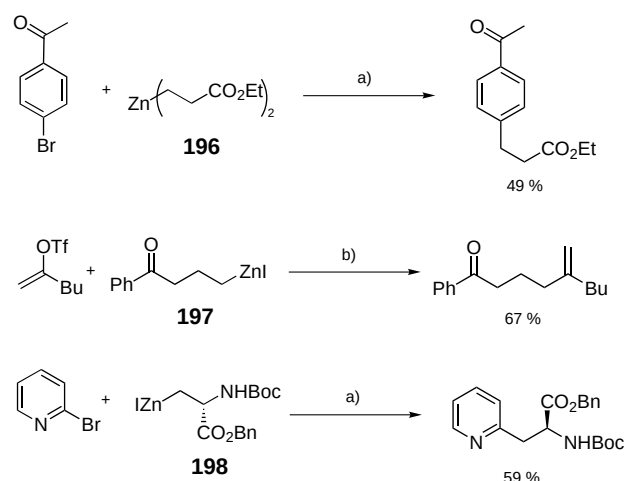


Scheme 43. Zinc-ene reaction and diastereoselective reaction of highly substituted organozinc reagents. a) *n*BuLi, -78°C ; b) *c*HexCHO, ZnCl₂, $-78^\circ\text{C} \rightarrow \text{rt}$, 4 h; c) ZnCl₂; d) CuCN·2LiCl; e) PhCOCl, rt.

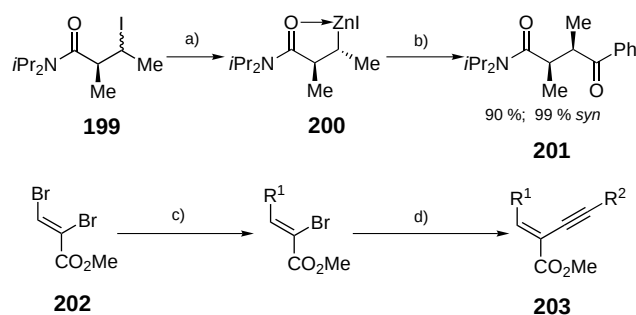
4.4. Cross-coupling reactions with polyfunctionalized organozinc compounds

Polyfunctional organozinc compounds have been extensively used in cross-coupling reactions.^[121, 122] Only examples of the method pioneered by Negishi,^[66, 121] the Pd⁰-catalyzed cross-coupling of functionalized alkyl or arylzinc reagents with aryl or alkyl iodides, bromides or triflates are given. Various palladium complexes have been used as catalysts and P(*o*Tol)₃ has been an especially useful ligand. Thus, ester- and keto-functionalized zinc reagents such as **196**,^[123] and **197**,^[124] respectively, or the zinc-serine derivative **198**^[125] undergo selective coupling with aryl bromides or triflates in satisfactory yields (Scheme 44).

Interestingly, a stereoconvergent insertion of zinc into *syn*- or *anti*-iodoamide **199** furnishes the same organozinc reagent **200** which undergoes a Pd⁰-catalyzed cross-coupling with benzyl chloride leading only to the *syn*-product **201** (Scheme 45).^[126] A chemoselective cross-coupling can be performed with the 2,3-dibromoacrylate (**202**). Two successive

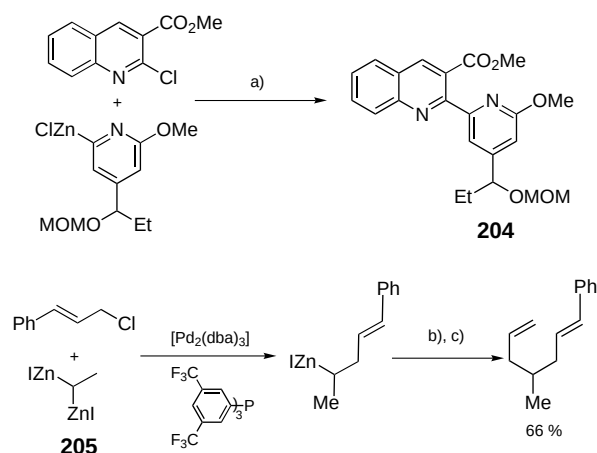


Scheme 44. Negishi cross-coupling reactions of functionalized organozinc reagents. a) [PdCl₂{P(*o*Tol)₃}₂] cat., THF, rt; b) [Pd(PPh₃)₄] cat., THF, rt.



Scheme 45. Pd⁰-catalyzed cross-coupling reaction of a chiral secondary zinc reagent and regioselective alkylation of 2,3-dibromoacrylates. a) Zn; b) PhCOCl, Pd⁰; c) R¹ZnCl, [Pd(PPh₃)₄] THF, rt, d) R²C≡CZnCl, [Pd(PPh₃)₄].

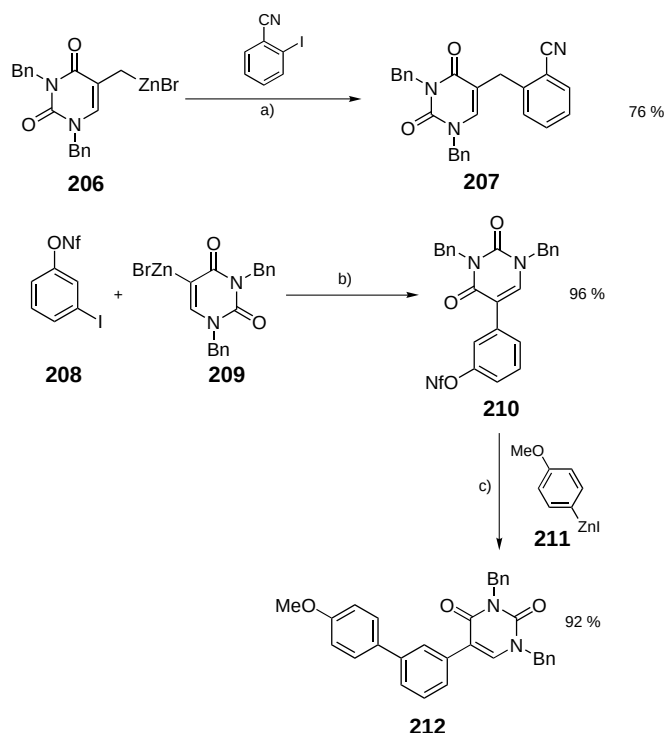
cross-coupling reactions with organozinc reagents furnished highly functionalized enynes of type **203** (Scheme 45).^[127] This cross-coupling can be readily performed with heterocyclic building blocks^[128] leading to precursors such as **204** which can be converted to camphothecin (Scheme 46). A novel application of dimetallic reagents has been described by Utimoto



Scheme 46. Pd⁰-catalyzed cross-couplings. a) Pd⁰ cat., THF, reflux; b) CuCN; c) allyl bromide.

and Matsubara and co-workers using 1,1-bis(iodozinc)ethane (**205**) for the sequential coupling with two different electrophiles.^[129]

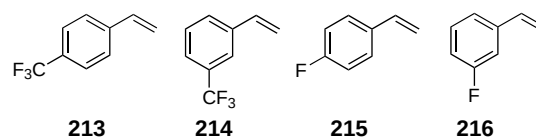
An alternative catalytic system is [Pd(dba)₂] and P(2-furyl)₃ (tfp) recently introduced by Farina et al.^[130] Under these conditions, heterocyclic benzylzinc complexes such as **206** undergo smooth cross-coupling with various aryl iodides leading to 5-substituted uracil compounds of type **207**.^[131] Especially suited for cross-coupling reactions with organic zinc derivatives are iodo-aryl nonaflates such as **208** (nonaflate (ONf) = nonafluorobutanesulfonate). The reaction of the zinc-uracil derivative **209** with **208** affords **210**. This nonaflate undergoes a smooth cross-coupling with the arylzinc derivative **211** providing the functionalized diphenyl **212** in high yield (Scheme 47).^[132]



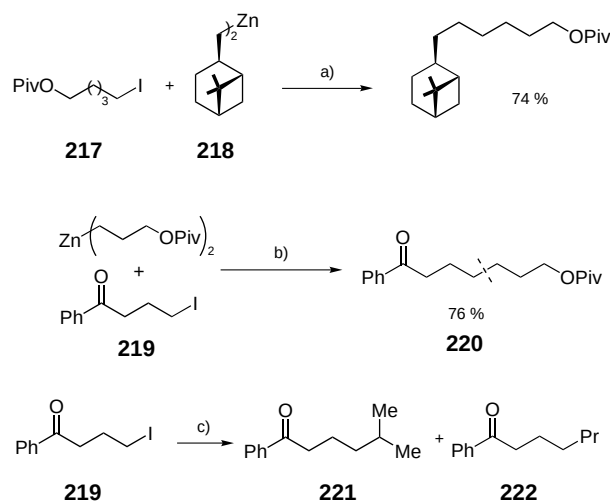
Scheme 47. Synthesis of uracil- and thymine-derivatives by a Pd⁰-catalyzed cross-coupling. a) [Pd(dba)₂] (2.5 mol %), tfp (5 mol %), 25 °C, 12 h; b) [Pd(dba)₂] (0.5 mol %), tfp (1 mol %), 25 °C, 1 h; c) [Pd(dba)₂] (2 mol %), dppf (2 mol %), 55 °C, 36 h.

Whereas Pd-catalysis is well suited for C_{sp}²–C_{sp}² cross-couplings, Ni-catalysis is advantageous for C_{sp}³–C_{sp}³ cross-couplings. Very few transition metal catalyzed reactions allow the selective cross-couplings between C_{sp}³-centers. Copper(I) has been successfully used, however often stoichiometric amounts of copper salts are necessary.^[133, 134] The Pd⁰-catalyzed cross-coupling between C_{sp}³-centers is not efficient because of the slow reductive–elimination step of the intermediate [(L_n)(R¹)Pd(R²)], leading to the desired product R¹–R². Since this reductive elimination is too slow, exchange of the ligands can compete leading to the formation of [(L_n)(R¹)Pd(R¹)] and [(L_n)(R²)Pd(R²)], which after reductive elimination, provide homocoupling products R¹–R¹ and R²–R² as side products. The use of a nickel(0) catalyst in

conjunction with a promoter ligand such as *p*- or *m*-tri-fluoromethylstyrene (**213** and **214**) or *p*- or *m*-fluorostyrene (**215** and **216**) allows efficient cross-coupling reactions between functionalized alkyl iodides (such as **217**) and dialkylzinc compounds such as **218**.^[135, 136] In the case of activated alkyl iodides like **219**, acetophenone can also be used as a



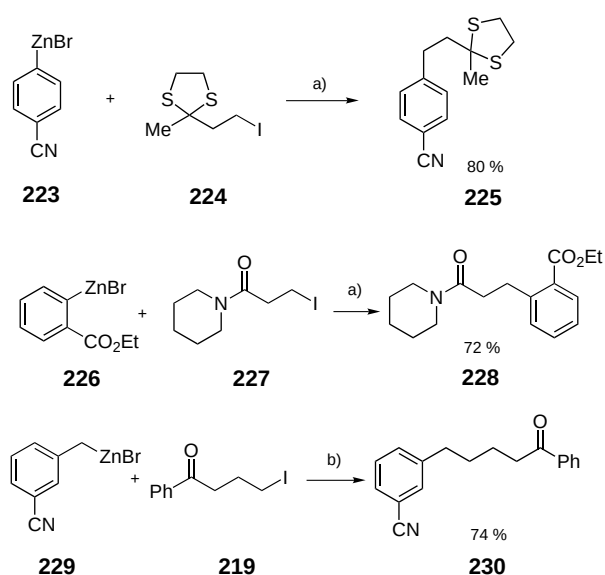
promoter affording the polyfunctional product **220**. Interestingly, in the case of the cross-coupling with secondary dialkylzinc compounds such as *i*Pr₂Zn, the presence of a promoter such as acetophenone improves the yield of the desired cross-coupling product **221** and reduces the amount of the coupling product **222** that results from a β-hydrogen migration and readdition (Scheme 48).^[136]



Scheme 48. Ni⁰-catalyzed cross-coupling reactions between primary and secondary dialkylzinc compounds and primary alkyl iodides. a) [Ni(acac)₂] (10 mol %), THF:NMP (2:1), **214** (1.0 equiv); b) [Ni(acac)₂] (10 mol %), THF:NMP (2:1), –35 °C, 10 h; c) *i*Pr₂Zn, [Ni(acac)₂] (10 mol %), PhC(O)Me (0.5 equiv), THF:NMP (2:1). Without PhC(O)Me: 51 % **221** and 17 % **222**. With PhC(O)Me: 62 % **221** and 3 % **222**.

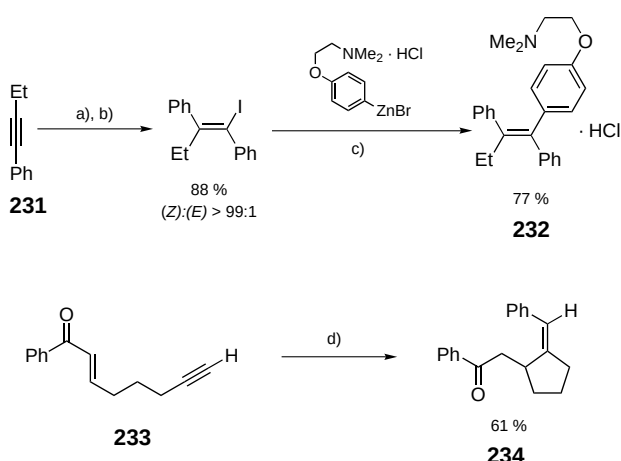
This cross-coupling can be extended to the reaction between arylzinc halides and alkyl iodides, a reaction rarely described in the literature. Thus, the arylzinc bromide **223** reacts with the functionalized alkyl iodide **224** in the presence of the promoter **213** affording the product **225** in 80 % yield. The organozinc reagent **223** was obtained from the corresponding organolithium reagent and the organozinc compound **226** from the corresponding organomagnesium compound. The cross-coupling of **226** with the alkyl iodide **227** gives the benzoic acid derivative **228** in 72 % yield (Scheme 49).^[137] Benzylzinc bromides such as **229** were found to be unreactive towards cross-coupling with various alkyl iodides, however the addition of tetrabutylammonium iodide (3 equiv)^[138] considerably facilitates this reaction leading, in the case of coupling with **219** in presence of *p*-fluorostyrene

(**215**) as promoter, to the desired product **230** in 74 % yield (Scheme 49).^[139]



Scheme 49. Ni^0 -catalyzed cross-coupling between arylzinc reagents and functionalized primary alkyl iodides. a) $[\text{Ni}(\text{acac})_2]$ (10 mol %), THF:NMP (2:1), **213** (1.0 equiv), -20°C , 4 h, b) $[\text{Ni}(\text{acac})_2]$ (10 mol %), Bu_4NI (3 equiv), 0°C to rt, 4–16 h, **215** (20 mol %), THF/NMP.

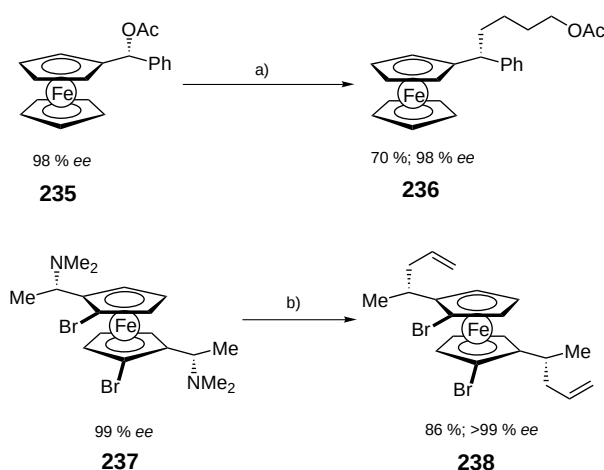
The complex $[\text{Ni}(\text{acac})_2]$ catalyzes the carbozincation of alkynes. The reaction proceeds with excellent regio- and stereoselectivity in many cases and allows, among other things, the stereoselective preparation of tetrasubstituted olefins. The procedure has been used to prepare the anti-cancer drug (*Z*)-Tamoxifen (**232**) from 1-phenyl-1-butyne (**231**).^[140] A range of analogues of **232** can be prepared by this method.^[141] In the presence of a catalytic amount of $[\text{Ni}(\text{cod})_2]$ organozinc halides add to alkynes such as **233** and subsequently undergo a ring closure by the intramolecular addition to an enone affording the *exo*-alkylidene cyclopentane **234** (Scheme 50).^[142]



Scheme 50. Ni^0 -catalyzed carbozincations of alkynes; synthesis of (*Z*)-Tamoxifen (**232**) a) Ph_2Zn , $[\text{Ni}(\text{acac})_2]$ (25 mol %), THF/NMP, -35°C , 3 h; b) I_2 ; c) $[\text{Pd}(\text{dba})_2]$ (4 mol %), tfp (16 mol %), THF, 50°C , 3 h; d) Ph_2Zn / PhZnCl , $[\text{Ni}(\text{cod})_2]$ (5 mol %).

4.5. Use of organozincs for the preparation of chiral ferrocenyl ligands

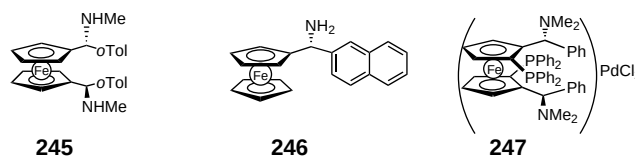
The preparation of new chiral ligands has been one of the most researched fields in the nineties and considerable progress has been made.^[143] The use of zinc complexes has allowed the development of new ferrocenyl ligands.^[144] It was found that chiral ferrocenyl acetates^[145] of type **235** react, with almost complete retention of configuration, in the presence of $\text{BF}_3 \cdot \text{OEt}_2$ with various organozinc derivatives leading to interesting products of type **236**.^[146] This reaction has been extended to the more stable (α -aminoalkyl)ferrocene derivatives such as **237**. Performing the reaction in the presence of acetyl chloride leads to the desired ferrocenes of type **238** with retention of configuration (Scheme 51).^[147]



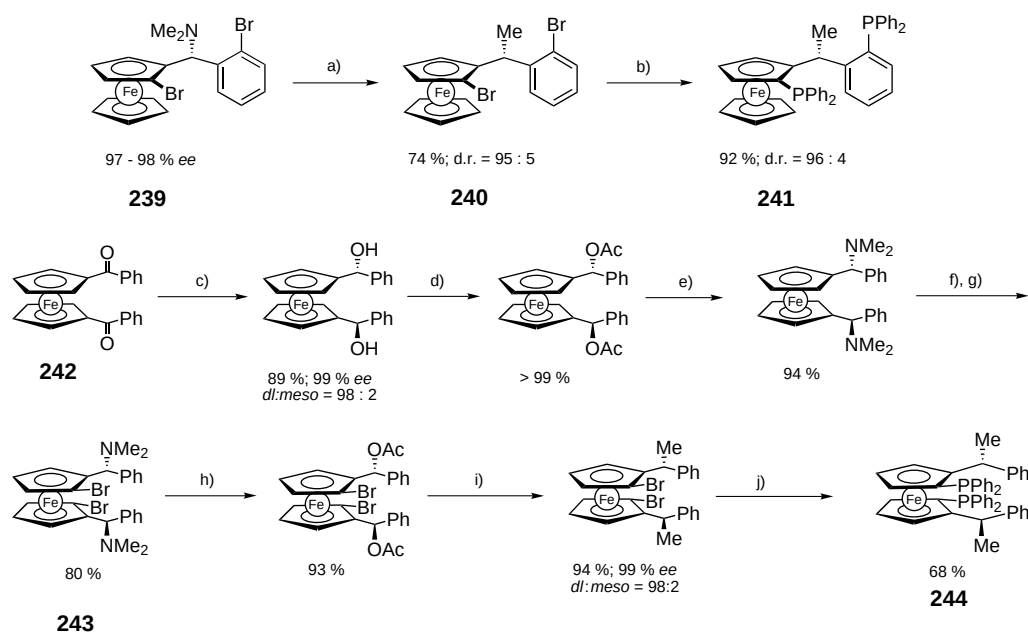
Scheme 51. Synthesis of chiral ferrocene derivatives. a) bis(4-acetoxybutyl)zinc, $\text{BF}_3 \cdot \text{OEt}_2$. b) allylzinc bromide (3 equiv), AcCl (2.4 equiv), THF, -30°C .

More recently, a novel class of 1,5-diphosphanes such as **241** has been prepared by a substitution reaction. Monoaminoferrocenyl derivatives **239** are converted in one step into the chiral ferrocene **240**.^[148] Using the methods developed in our laboratory^[144] the synthesis of the C_2 -symmetrical ligands of type **244** (Ferriphos) were obtained from the ferrocenyl diketone **242** via the key intermediate **243** (Scheme 52).^[147, 149]

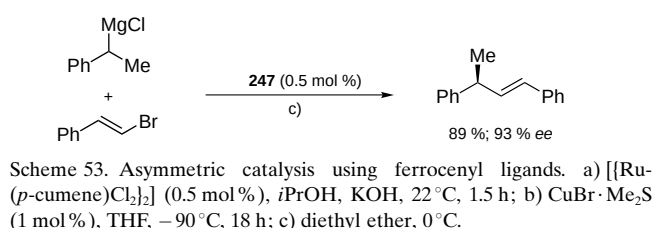
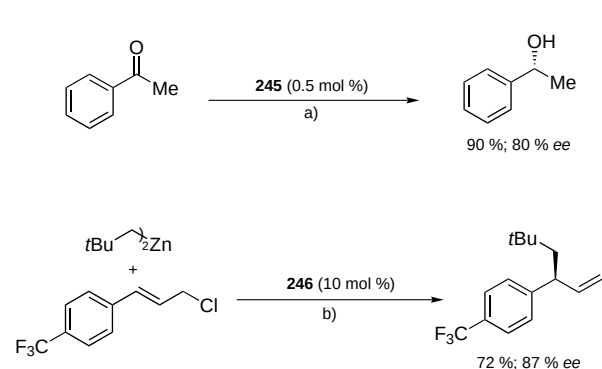
All these ferrocenyl ligands proved to be excellent ligands for various metal-catalyzed asymmetric reactions. Thus, the C_2 -symmetrical aminoferrocenyl derivative **245** catalyze the transfer hydrogenation of aromatic ketones.^[150] The mono-



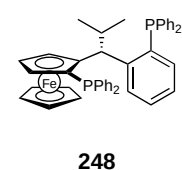
ferrocenyl compound **246** proves to be excellent for the asymmetric alkylation of allyl chlorides.^[151] The palladium complex **247** gives excellent results for the cross-coupling between chiral benzylmagnesium reagents and alkenyl bromides (Scheme 53).^[144]



Scheme 52. Preparation of new chiral ferrocenyl ligands. a) Me_2Zn (4 equiv), AcCl (2 equiv); b) BuLi (2.2 equiv), ClPPh_2 (2.3 equiv); c) $\text{BH}_3 \cdot \text{SMe}_2$ cat.; d) Ac_2O , pyridine, rt, 15 h; e) Me_2NH , $\text{THF}/\text{H}_2\text{O}$, rt, 15 h; f) $t\text{BuLi}$, diethyl ether, 0°C , 0.5 h; g) $(\text{CCl}_2\text{Br})_2$, THF , 0°C ; h) Ac_2O , 100°C , 2.5 h; i) Me_2Zn , $\text{BF}_3 \cdot \text{OEt}_2$, THF , $-78^\circ\text{C} \rightarrow \text{rt}$, 1.5 h; j) BuLi , THF , -70°C , 0.25 h, ClPPh_2 .



Remarkable enantioselectivities were obtained in the reduction of α -acetamidoacrylates and 1,3-dicarbonyl compounds by using the Ferriphos ligands such as **244**^[147, 149] and the ligands **241** and **248**. Interestingly these two last ligands these two last ligands provide reduction products with the opposite configuration (Scheme 54).^[148]

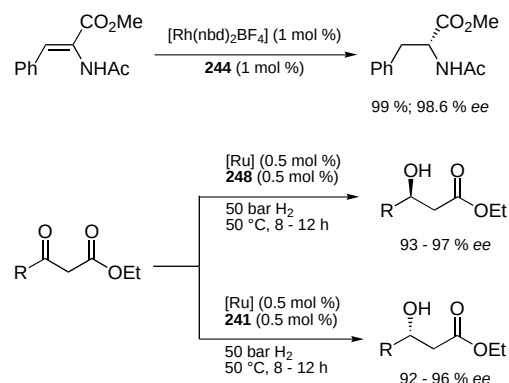


The discovery of these new ligands was a consequence of the development of new synthetic methods for the stereoselective manipulation of ferrocene derivatives.^[144–148] The umpolung of the ferrocene

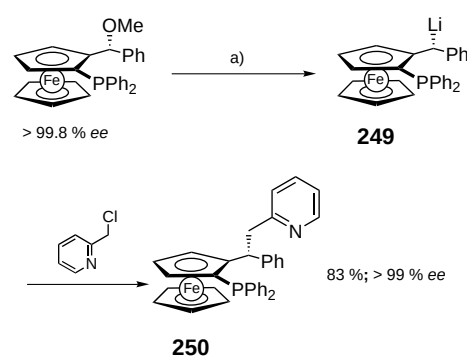
reactivity, through chiral α ferrocenylalkyllithium derivatives such as **249**, opens up new synthetic possibilities for the development of new chiral ferrocenyl ligands such as **250** (Scheme 55).^[152]

5. Conclusion and perspectives

Functionalized organometallic complexes have become key intermediates for retrosynthetic analysis and numerous applications have been described in the literature. Organozinc compounds have played a central role in the development of these reagents but other classes of compounds such as organoboranes may also play a large



Scheme 54. Asymmetric reactions using ferrocenyl ligands.



Scheme 55. Preparation of a new P,N-ligand via a chiral α -lithiated ferrocene. a) Lithium naphthalene, -78°C .

role in the future. Transition metal catalysis was essential for the development of many new reactions of organozinc compounds and further studies in this direction should expand further the broad field of application of functionalized organometallics.

Abbreviations

Ac	acetyl
acac	acetylacetonato
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
cod	cycloocta-1,5-diene
dba	dibenzylidenacetone
Ddm	4,4'-dimethoxydiphenylmethyl
dppf	1,1'-bis(diphenylphosphanyl)ferrocene
MOM	methoxymethyl
nbd	norbornadiene
NBP	<i>N</i> -butylpyrrolidinone
NMP	<i>N</i> -methylpyrrolidinone
<i>o</i> Tol	2-tolyl
Piv	pivaloyl
TBS	<i>tert</i> -butyldimethylsilyl
Tf	trifluoromethylsulfonyl
TFA	trifluoroacetic acid
tfp	tri(2-furyl)phosphane
TIPS	triisopropylsilyl
TMS	trimethylsilyl
Tos	toluene-4-sulfonyl

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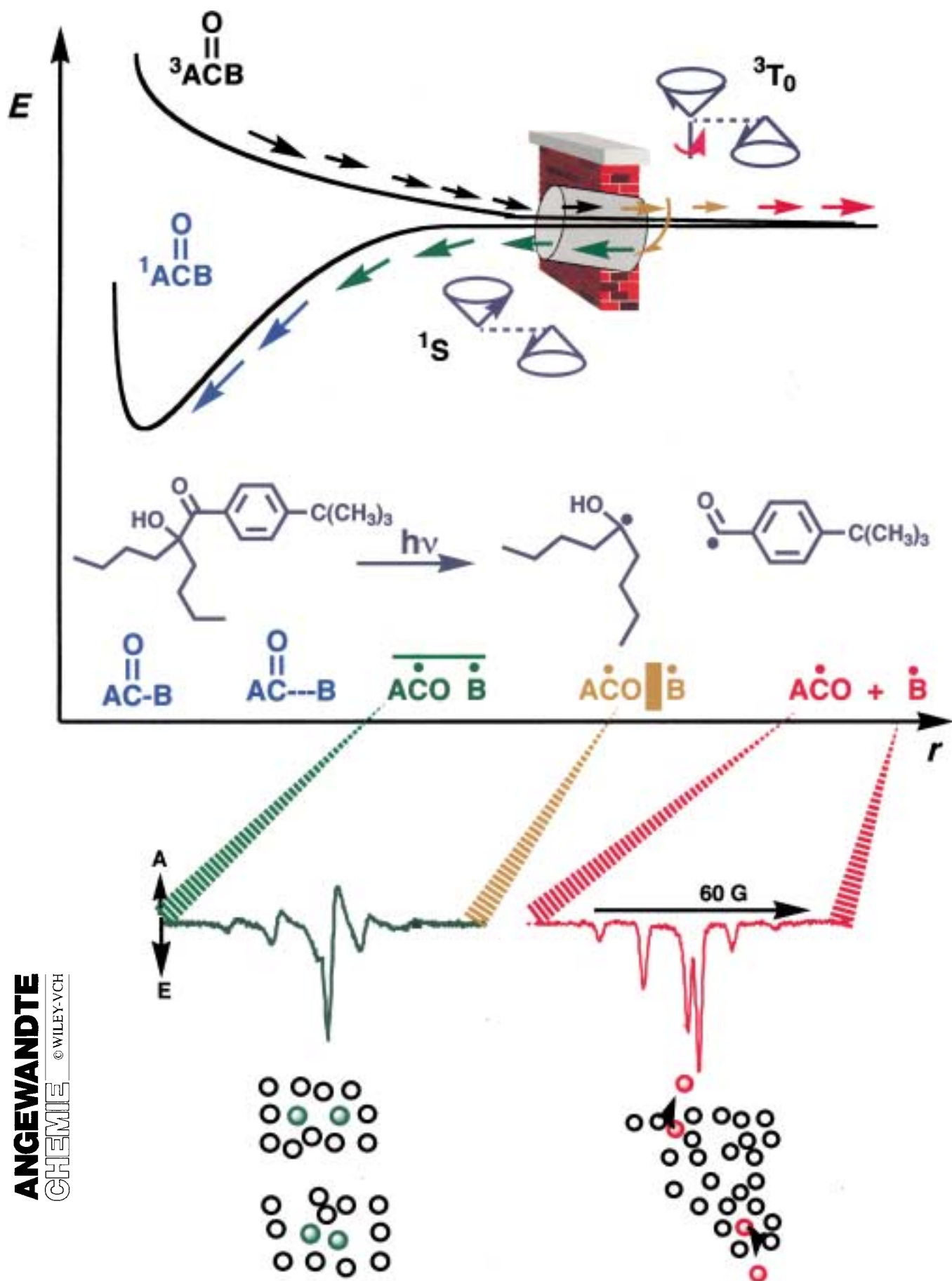
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Detailed monitoring of the photochemical α -cleavage of ketones is possible with the TREPR technique.

Electron Spin Polarization and Time-Resolved Electron Paramagnetic Resonance: Applications to the Paradigms of Molecular and Supramolecular Photochemistry

Nicholas J. Turro,* Mark H. Kleinman, and Erdem Karatekin

Most molecular and supramolecular organic photochemical reactions involve paramagnetic reactive intermediates (such as molecular triplet states, triplet radical pairs, and free radicals). In a number of cases these species are created with “anomalous” spin populations which are far from thermal equilibrium. Such paramagnetic species are said to be “spin polarized” and may be observed di-

rectly by time-resolved electron paramagnetic resonance (TREPR). The TREPR technique can be applied to exploit spin polarization, which, in addition to providing an enormous signal to noise enhancement, also reveals the mechanisms involved in photochemical reactions. TREPR spectroscopy provides a means of tracking the reaction of radicals with molecules and the nonreactive interactions of radicals

with other radicals in real time. The latter interactions provide a systematic investigation of supramolecular interactions of geminate radicals in micelles.

Keywords: EPR spectroscopy • paradigms • photochemistry • radicals • supramolecular chemistry

1. Paradigms, Electron Polarization, Time-Resolved Electron Paramagnetic Resonance, and Photochemistry

This review attempts to tie together the paradigms of several fields: organic photochemistry, time-resolved electron paramagnetic resonance (TREPR), electron spin polarization (ESP), and supramolecular chemistry. A major goal of the authors is to provide organic chemists and physical organic chemists with a nonmathematical overview and appreciation of how TREPR and ESP can contribute to an understanding of the paramagnetic species (triplet states, radical pairs, and radicals) which are commonly produced in organic photochemical reactions. Although the approach is qualitative and exemplary, the interested reader is referred to any one of a number of excellent quantitative reviews on TREPR and ESP which have appeared.^[1–7]

This review presents a general working paradigm for organic photochemistry which can be examined by the TREPR technique and for which ESP plays a major role in providing both mechanistic, structural, and dynamic information on the paramagnetic intermediates involved in photo-

chemical reactions. After a brief qualitative and conceptual review of EPR emphasizing the importance of the simpler aspects of transitions between magnetic energy levels, the phenomenon of ESP and its impact on TREPR spectroscopy is described. With this background the reader is provided with an exemplar photochemical reaction, the α -cleavage of carbonyl compounds, which is examined in detail by first using magnetic energy level diagrams to predict the attributes expected from TREPR spectra. These expectations are then compared to experimental examples of TREPR spectroscopy of the paramagnetic intermediates involved in the photochemical α -cleavage of carbonyl compounds. Examples are then given of 1) the creation of ESP by intersystem crossing from excited singlet states of carbonyl compounds; 2) the creation of ESP by the primary photochemical reactions of spin-polarized molecular triplets; 3) the transfer of ESP by reaction of polarized radicals with molecular scavengers; 4) the transfer of ESP by nonreactive collisions between radicals; and 5) the sorting of ESP by nonreactive collisions between radicals. The account concludes with a description of how TREPR can be employed to examine supramolecular structure and dynamics of geminate radical pairs adsorbed in micelles.

[*] Prof. Dr. N. J. Turro, M. H. Kleinman, E. Karatekin
Department of Chemistry
University of Columbia
Havemeyer Hall, New York, NY 10027 (USA)
Fax: (+1) 212-932-1289
E-mail: turro@chem.columbia.edu

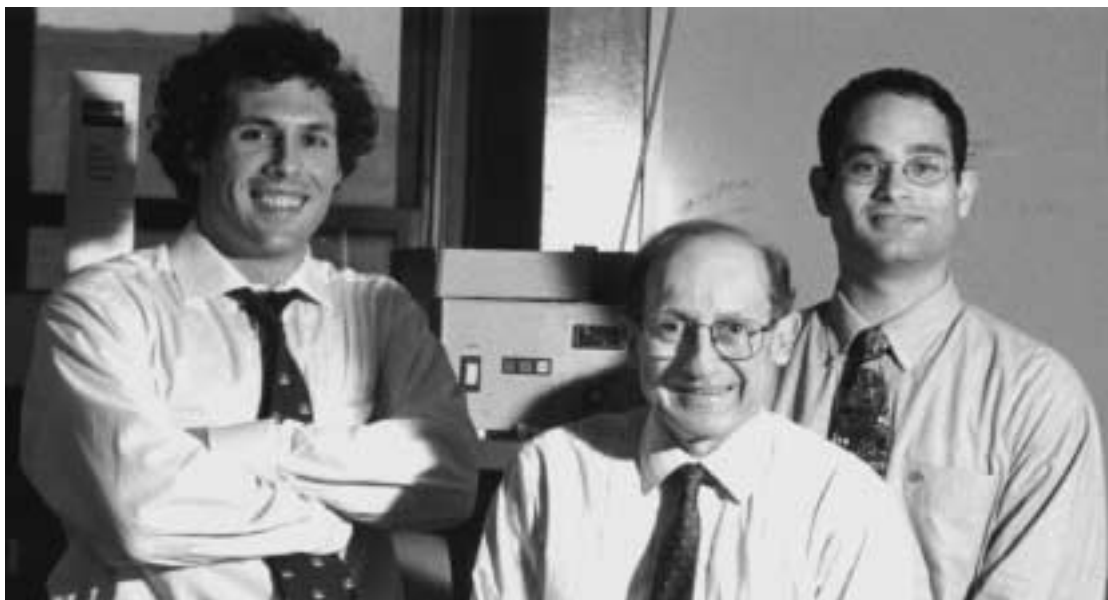
1.1. Paradigmatic Science and Paradigmatic Chemistry

According to Kuhn,^[8] ordinary scientific research is executed under the implicit control of *paradigms*. The term

“paradigm” is often used loosely in several different contexts: 1) There are *global paradigms* referring to an entire constellation of beliefs, values, techniques, methods, etc. that are shared by the practitioners of a given scientific discipline. To a large extent the nature of a scientific discipline is determined by the shared global paradigms, which achieve their authority over the practitioners from the sociological and community-based structure of a science. 2) There are also everyday *working paradigms* which are based on experience with specific areas of normal, everyday research and which tend to be generally and broadly applicable when investigating systems having some precedent; 3) Finally there are *exemplar paradigms* or specific concrete examples, widely considered by the community to be outstanding research accomplishments, which serve as models for previous, current, and future research by practitioners.

1.2. The Paradigm of Molecular Organic Photochemistry

The global, working, and exemplar paradigms of molecular organic photochemistry determine the everyday “normal science” activities of organic photochemists by defining entities and transitions between entities that are generally involved in typical organic photochemical reactions.^[9] Let us consider the development of these three paradigms defining the entities and transitions that are essential for organic photochemical reactions involving a transformation from a reactant R to a product P. A very condensed representation of the global paradigm is found in Scheme 1. In words, the global paradigm states that an organic photochemical reaction generally proceeds as follows: the absorption of a photon by R produces an electronically excited state *R that undergoes primary photochemical reactive transitions to form a reactive intermediate I. At this point the photochemistry has ended.



E. Karatekin

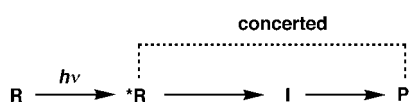
N. J. Turro

M. H. Kleinman

Nicholas J. Turro was born in Middletown (Connecticut, USA), in 1938. He received his Ph.D. degree from Caltech in 1963 and joined the faculty at Columbia University in 1964, where he is currently the William Schweitzer Professor of Chemistry. His research interests include the use of photochemistry and spectroscopy to investigate the structure and dynamics of molecular and supramolecular systems.

Mark H. Kleinman was born in Montreal (Québec, Canada) in 1969. Heading westward, he worked with C. Bohne at the University of Victoria (British Columbia, Canada) where he studied the dynamics of radicals in supramolecular systems and received his Ph.D. in 1998. He is currently working with N. J. Turro, G. W. Flynn, and P. Schlosser as a Dreyfus postdoctoral fellow at Columbia University. His main research interests include triplet state mediated photochemistry and the application of spectroscopic techniques to investigate dynamic processes in biological and environmental systems.

Erdem Karatekin was born in Ankara (Turkey) in 1972. He received his B.S. degree in chemical engineering from the University of Louisville, Kentucky. He has recently completed his Ph.D. degree under the mentorship of N. J. Turro and B. O'Shaughnessy at Columbia University, where his research focused on the use of photochemical techniques in the study of free radical polymerization and polymer reaction kinetics. After leaving Columbia he joined the Institut Curie in Paris for a post-doctoral position with F. Brochard and J. L. Viovy.

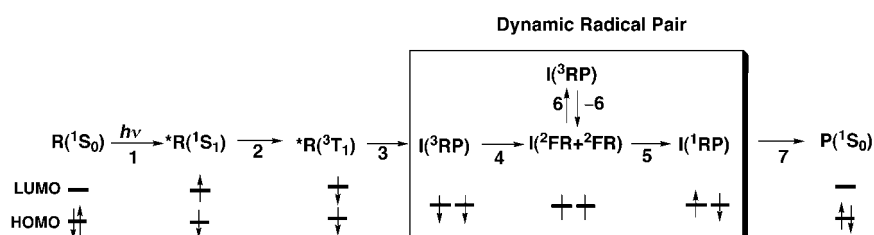


Scheme 1. Schematic representation of a global paradigm for molecular organic photochemistry. Concerted reactions (dashed line) are rare in photochemical reactions.

The reactive intermediate I then proceeds through secondary ordinary thermal processes to the isolated product P. Simply put, the photochemist will start an analysis of a photoreaction $R+h\nu \rightarrow P$ by considering the entities and transitions involved in the pathways shown in Scheme 1 and then proceed with the analysis by including all of the explicit and implicit knowledge that is “globally” accepted by the photochemical community.

1.2.1. A Working Paradigm for Organic Photochemistry

The global paradigm of Scheme 1 may be expanded and elaborated into a general working paradigm for the photo-reactions of families of organic molecules. For any family of molecules the working paradigm usually considers orbital and spin descriptions (configurations) of the entities (R, *R, I, and P) shown in Scheme 1. It is usually sufficient to start by classifying *R and I according to the orbital nature and spin orientation of the electrons in the singly occupied (SOMO), highest occupied ground state orbital (HOMO), and lowest unoccupied ground state orbital (LUMO) as shown in Scheme 2, which represents a working paradigm for the



Scheme 2. A working paradigm for the photochemistry of organic molecules, in particular, for the photochemistry of ketones. The arrows are schematic representations of electronic spins. In the case of the electronic spins indicated for $I(^2FR)$, the lines represent spins that have orientation but do not interact with each other. See text for a description of mechanistic steps.

molecular photochemistry of ketones (AC(O)B), the family of molecules that are of greatest interest in this account. In the working paradigm the structures of the generalized concept of excited states and reactive intermediates are amplified to include the orbital and spin structures of *R and I. In addition, certain details of the dynamic behavior of intermediates that have been shown to be commonly important, such as the dynamic behavior of radical pairs and free radicals (box in Scheme 2), are included. Throughout this account much of what is written about radical pairs will be analogous to the chemistry of biradicals,^[2, 10, 11] which are beyond the scope of this review.

In Scheme 2, the superscripts 1, 2, and 3 are employed to denote the spin multiplicities of the entities. These terms and the spin features will be explained in more detail in Section 1.3.3. Both starting material and products are ground-state singlet states, $R(^1S_0)$ and $P(^1S_0)$, and possess an

electronic configuration with two electrons in the HOMO (and all lower energy orbitals) and no electrons in the LUMO. *R may be either a singlet $*R(^1S_1)$ or a triplet $*R(^3T_1)$ species, where both possess one electron in the HOMO and one electron in the LUMO. Similarly, I may be either a singlet $I(^1RP)$ or a triplet $I(^3RP)$ species, but in this case each possesses one electron in separate SOMOs. Depending on the ketone structure, each of these entities may be assigned an electronic orbital configuration (for example, n,π^* , π,π^*). In the photochemistry of ketones, the intermediate I actually represents interrelated groups of entities that can be termed “dynamic radical pairs” (RP) and “free radicals” (FR, box in Scheme 2). The concept of the dynamic radical pair is essential for an understanding of organic molecular and supramolecular photochemistry and will be discussed at several junctures in this account.

Based on a wide range of experience, a photochemist is likely to accept Scheme 2 as an initial working paradigm for investigating and analyzing the photochemistry of ketones. Thus, a photoreaction of a ketone is generally assumed to involve the following connected transformations: the absorption of a photon by $R(^1S_0)$ produces an electronically excited singlet state $*R(^1S_1)$ (step 1) which undergoes an efficient conversion into an electronically excited triplet state $*R(^3T_1)$ through a transition termed intersystem crossing (ISC, step 2). In this step, spin is apparently created as $*R(^1S_1)$ and has a net spin of zero, but $*R(^3T_1)$ has a net spin of one. $*R(^3T_1)$ is usually the electronically excited entity responsible for the photochemistry of ketones, that is, $*R(^3T_1)$ undergoes the primary photochemical reaction (step 3).

Implicit in the global paradigm of photochemistry is the notion that all primary photochemical processes are elementary steps and that all elementary steps obey spin conservation laws (total spin is conserved in an elementary reaction). Thus the primary photochemical reaction (step 3) from $*R(^1T_1)$ produces a geminate triplet radical pair $I(^3RP)$. The dynamic radical pair must operate under spin selection rules, that is, $I(^3RP)$ cannot undergo elementary radical–radical reactions to form singlet molecules such as $P(^1S_0)$, since such an elementary step would violate spin selection rules. As a result, the geminate triplet pair separates (step 4) to produce two free radicals $I(^2FR+^2FR)$. In spin terminology, each free radical is termed a doublet and labeled with a superscript 2. The ^2FR s undergo diffusion excursions (see Section 3.1.1) and are considered to be part of the dynamic radical pair system indicated in Scheme 2. In the absence of scavengers (which are left out at this point for the sake of simplicity) two ^2FR 's, after certain diffusional excursions in solution, will eventually encounter to produce random singlet radical pairs $I(^1RP)$ (step 5) or triplet radical pairs $I(^3RP)$ (step 6) in the ratio of 1:3 (based on spin statistics). According to the working paradigm the isolated products (P) may only form from the radical–radical reactions of the singlet radical pairs $I(^1RP)$ (step 7). The random triplet radical pairs $I(^3RP)$ separate again into $I(^2FR)_2$

(step – 6), and continue to cycle through steps – 6, 6, and 7 until the reaction is complete and all the radicals are consumed. The radical–radical reactions or RP are conventionally termed “combination reactions” which applies to reactions in which the pair actually combine by bond formation to form a molecule, or undergo disproportionation to form two molecules.

In most actual photochemical reactions each entity on the pathway from R to P($^1S_0$) will also have other options that are not shown in Scheme 2. The chemical and quantum yield of formation of P($^1S_0$) depends on how these options compete with those on the pathway from R to P($^1S_0$). Since these options are well known to the photochemist through the global paradigm, they are not explicitly included in the working paradigm. For example, in addition to the radical–radical reactions shown in the example, $^3R(^3T_1)$ may undergo more than one primary photochemical process in step 3 to form other products P($^1S_0$). One of the primary ^2FR formed in step 4 may undergo radical–radical transformations (unimolecular rearrangements or fragmentations) or undergo reactions with scavengers to form secondary free radicals (^2FR)₂, that eventually undergo radical–radical reactions to form other products P($^1S_0$). The working paradigm of Scheme 2 (and the implicit options that are designated by the global paradigm) works extremely well for the molecular photochemistry of ketones in nonviscous homogeneous solutions for which the separation of the triplet radical pair into ^2FR is very efficient. We shall see in Section 3.1 that the working paradigm of Scheme 2 can be readily adapted to accommodate supramolecular systems.

1.2.2. Electron Spin Polarization and TREPR

This review is concerned with the phenomenon of electron spin polarization (ESP) and the TREPR technique, which takes great advantage of ESP. The combination of ESP and TREPR provides the power to directly or indirectly observe *all* of the paramagnetic entities listed in Scheme 2 ($^3T_1$, ^3RP , ^2FR) and to elucidate the connecting transitions leading to or from these species (for example, $^1S_1 \rightarrow ^3T_1$, $^3T_1 \rightarrow ^2FR + ^2FR$, transformations of the dynamic radical pair, $RP \rightarrow ^2FR'$). In addition, we shall see how ESP and TREPR provide a means of elucidating “invisible” intermolecular (supramolecular) interactions, such as nonreactive intermolecular collisions between paramagnetic species, that are implicit in the working paradigm (for example, $^2FR + ^2FR \rightarrow ^3RP$). A further remarkable feature of the TREPR technique is that, in addition to information that will confirm that these elementary steps have occurred, TREPR will reveal some very subtle and somewhat esoteric features of these transitions. For example, the spin sublevel selectivity of each of these reactive and nonreactive transitions can be investigated. Finally, we shall see that ESP is a common feature of the elementary steps involving the paramagnetic entities in Scheme 2 and that ESP serves to enhance the signal-to-noise ratio of the TREPR method and acts as a nonintrusive label of paramagnetic species.

Since TREPR deals with the investigation of photochemical reactions in an applied magnetic field, we will now consider how the molecular photochemistry paradigm must

be modified as the result of the presence of a magnetic field and develop a paradigm as to how electron spin polarization is created, sorted, and transferred.

1.3. Electrons In a Strong Applied Magnetic Field—EPR Spectroscopy

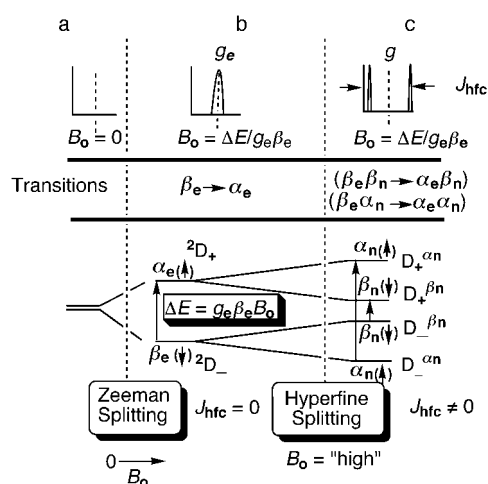
Investigation of photochemical reactions by EPR involves the study of photochemistry in a magnetic field. Thus, a photochemist needs to modify the working paradigm by including the effects of an applied magnetic field on the entities and transitions in Scheme 2. It is expected that all of the paramagnetic entities ($^3T_1$, ^3RP , and ^2FR) and transitions involving these paramagnetic species will be influenced by the presence of a strong applied magnetic field. Since they are diamagnetic, the singlet entities $^1R(S_0)$, $^1R(S_1)$, I(^1RP), and $^1P(S_0)$ are not expected to be influenced by the applied magnetic field.

In this section we provide a qualitative and elementary description of the behavior of the paramagnetic entities and how their transitions are influenced by a strong magnetic field. This description is intended to provide the reader with a feeling of how TREPR may serve as a powerful tool to elucidate the structure and transformations of the entities of the working paradigm for photochemistry. An apology is in order at this point to experts who may be concerned with the level of simplification. The simplification is intended to capture the essential qualitative features of very complex phenomena and follow through with the consequence of a set of rules that can be visualized on a simplified model. The article is intended for the organic chemist who has had little or no detailed experience with EPR. References are given throughout to more detailed and advanced discussions.

1.3.1. The Simplest Case: EPR of a Free Electron

EPR spectroscopy involves the experimental observation of transitions between magnetic sublevels in a magnetic field. Thus, for a qualitative understanding of EPR we need to understand how the sublevels of the paramagnetic entities in Scheme 2 are influenced by the application of a strong applied magnetic field. We shall consider only three simple cases: 1) a single electron; 2) a single electron interacting with a spin $\frac{1}{2}$ nucleus; and 3) two interacting electrons. These cases will serve as a basis for understanding the features of EPR, TREPR, and ESP of all of the paramagnetic entities of Scheme 2.

For the simplest case we consider the effect of an applied external magnetic field (B_0) on a single isolated, “free” electron which does not interact with any other electron spins or nuclear spins.^[12, 13] The spin of an electron S is associated with a magnetic moment μ , and both are vector quantities. When placed in a magnetic field, the electron spin is required by quantum mechanics to assume definite orientations with respect to the direction of the external magnetic field. The energy separating the magnetic energy levels of this electron depends linearly on the strength of the external magnetic field (Scheme 3). The magnetic interaction which causes this



Scheme 3. Energy level diagram describing the origin of EPR spectra of a single electron and an electron coupled to a spin $\frac{1}{2}$ nucleus. Top: Observed EPR spectra derived from electronic transitions; bottom: energy level diagram depicting the transitions in the absence (case a, $B_0 = 0$), “low” levels (case b, Zeeman splitting), and high levels (case c) of an applied magnetic field. For clarity, the energy levels split by hyperfine coupling in case c are pictured with larger energy gaps. See text for a full description.

energy difference is termed the Zeeman interaction, and is given by Equation (1). β_e is the Bohr magneton ($\beta_e = 9.27 \times 10^{-24} \text{ J T}^{-1}$) and g_e is the free electron “ g -factor”, a unitless

$$\Delta E_{\text{Zeeman}} = g_e \beta_e B_0 \quad (1)$$

value which is equal to 2.0023. From Equation (1) it is seen that the energy gap between two magnetic levels is proportional to the magnitude of the applied field B_0 , and the g -factor. The latter is analogous to the chemical shift of NMR spectroscopy. It is important to note that the spin angular momentum difference between the two adjacent magnetic sublevels is always exactly one unit of spin angular momentum, but the energy difference depends on the strength of the applied field and the g -factor of the radical.

When the angular momentum of an electron is aligned parallel to an applied magnetic field (the magnetic moment vector points in the same direction as the field), its magnetic energy is higher than when the angular momentum is aligned antiparallel to it. The higher energy spin orientation is termed an “up” spin ($\uparrow$) or an α spin and will be noted as $\alpha(\uparrow)$. The lower energy spin is termed a “down” spin ($\downarrow$) or a β spin and will be noted as $\beta(\downarrow)$. The effect of an applied magnetic field is to break the degeneracy of the two orientations in energy, with the $\beta(\downarrow)$ level dropping and the $\alpha(\uparrow)$ rising as the field strength increases [Eq. (1)].

In practice, an orbitally unpaired electron is bound to one or more nuclei, such as an unpaired electron in a carbon-centered free radical. This situation causes two effects: 1) the g -factor of the radical, which determines the strength of the Zeeman interaction and the energy gap in Equation (1), will differ from that of the free electron, g_e , and 2) the unpaired electron will interact or “couple” with the magnetic moments of the neighboring nuclei that make up the radical, to produce the so-called “hyperfine coupling” (hfc) of electron spins to nuclear spins (which is analogous to the

spin–spin coupling of nuclear spins). Typical g -factors for organic radicals range between 2.010 and 1.990 (compared to a value of 2.0023 for the free electron). Although these differences in g -factors are small, they can be measured with sufficiently high precision, so that g -factors (like chemical nuclear shifts in NMR) provide valuable information on the structure of a given radical.^[14]

Conventional commercial EPR spectrometers operate at a single frequency, usually between 9 and 10 GHz, so that the EPR transitions for a free electron measured in Equation (1) will occur at magnetic fields of the order of 3500 gauss (3500 G or 0.35 T). The EPR spectrum of a free electron will consist of a single “line” (Scheme 3b), which results from the absorption of a photon whose frequency corresponds to the energy of $g_e \beta_e B_0$ [Eq. (1)]. The photon induces the transitions of electron spins from the more heavily populated $\beta(\downarrow)$ orientation to the $\alpha(\uparrow)$ orientation (and from the lesser populated $\alpha(\uparrow)$ to $\beta(\downarrow)$). This change in orientation, in turn, corresponds to a change in spin of one unit of spin angular momentum ($-\frac{1}{2} \rightarrow +\frac{1}{2}$), which is exactly the spin angular momentum possessed by a photon. The conservation of spin angular momentum in the absorption of electromagnetic radiation serves as the basis for first order selection rules for EPR transitions. Thus, the $\beta \rightarrow \alpha$ transition is said to be EPR “allowed” to a first approximation, as is the reverse transition $\alpha \rightarrow \beta$. Thus, the selection rule to the first approximation is very simple: a radiative EPR transition (absorption or emission) is allowed when the electron flips its orientation from $\beta \rightarrow \alpha$ or $\alpha \rightarrow \beta$. As will be discussed in Section 1.4, the intensity of the absorption will depend on the difference in populations between the spins in the β and α orientations.

1.3.2. EPR Spectrum of an Electron Coupled to a Nucleus of Spin $\frac{1}{2}$

The energy levels for a free electron placed in a magnetic field and the manner in which the levels are modified by the presence of a neighboring nucleus of spin $\frac{1}{2}$ (for example, a proton or a ^{13}C nucleus) are shown in Scheme 3c. The EPR spectrum of a hydrogen atom, the simplest free radical (an electron and a proton) provides a model for the effects of the hyperfine coupling of an unpaired electron and a spin $\frac{1}{2}$ nucleus. The nuclear spin states (α_n or β_n) are shown on the energy levels. (The energy levels are not drawn to scale and the actual ranking of energy levels depends on several factors, such as the sign of the hyperfine coupling constants.) As for the free electron, only the $\beta_e \rightarrow \alpha_e$ EPR absorptive transitions are allowed (arrow in Scheme 3c). In addition, a second selection rule now applies: the nuclear spin cannot change its orientation from α to β or β to α during the electronic spin transition. This rule may be considered as the spin analogue of the Franck–Condon Principle for electronic transitions, and denotes that electron spin reorientation caused by the absorption of a photon occurs much more rapidly than the reorientation of a nuclear spin. Thus, the spin orientation immediately before and after the electron spin flip is the same.

From the spin selection rules, only two EPR transitions are allowed for β_e (arrows in Scheme 3c): $\beta_e \beta_n \rightarrow \alpha_e \beta_n$ and $\beta_e \alpha_n \rightarrow \alpha_e \alpha_n$. From the scheme it is seen that the energies

and therefore the frequencies of these transitions are clearly different so that two lines appear in the EPR spectrum. Working through the paradigm of hyperfine coupling, the two lines are split an equal amount about the position defined by the g -factor of the radical. The separation between the lines is equal to the hyperfine coupling constant J_{hfc} . Note that by convention in EPR spectroscopy, the hfc constant is represented as an “ a ”, however, since more readers are familiar with NMR spectroscopy, here we use “NMR-type” nomenclature J . Coupling with more than one spin causes the number of energy levels to increase, and the mixing of levels requires higher order considerations; but in general, Scheme 3 captures the essence of the qualitative information that is needed to obtain an appreciation of the origin of the EPR effects of interest in this account.

It should be pointed out that the higher the energy of an EPR transition, the lower the field at which the transition occurs at a fixed frequency. Thus the transition for which the α nucleus is involved occurs at lower field than the transition for which the β transition occurs (Scheme 3). From Equation (1) it can be deduced that the larger the g -factor, the lower the center field for a hyperfine pattern.

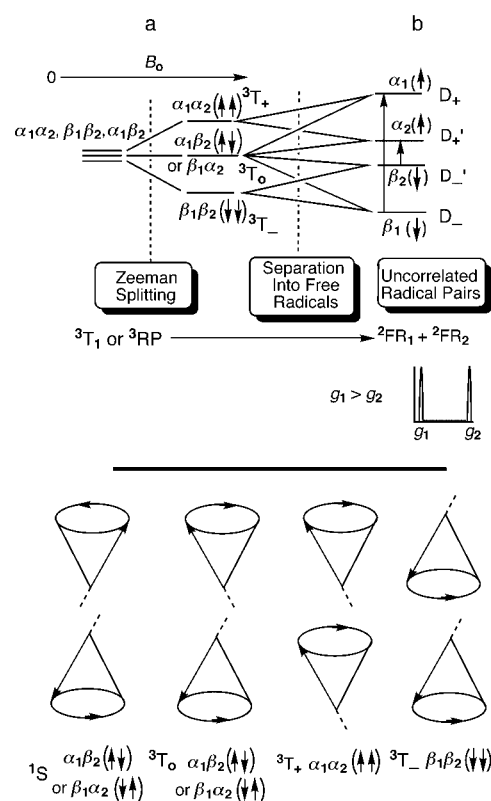
1.3.3. Paramagnetic Species in a Magnetic Field—Singlet (^1S), Doublet (^2D), and Triplet (^3T) States

We are now in a position to discuss the total spin labels of the entities of Scheme 2 and how these labels can be used in magnetic energy level diagrams. The entities with superscript 1 possess a total spin of zero in any orientation in a magnetic field and are termed singlet (^1S) states (for example, $R(^1S_0)$, $R(^1S_1)$, (^1RP) and $P(^1S_0)$). For $^*R$ and I , singlet states result when two orbitally unpaired electrons have an α and β orientation and interact to yield a net spin of 0. Singlets are diamagnetic (nonmagnetic) and their energies are not affected by an applied magnetic field. This means that the energy level of a given singlet state remains a single level in a magnetic field and that there is no Zeeman effect of magnetic state.

The free radical entities of Scheme 2 are described by a superscript 2 and possess a total spin of $\frac{1}{2}$ in the orientation of the spin and are termed doublet (^2D) free radical states (^2FR (^2D)). Doublets are paramagnetic and their energies are influenced, as described in Sections 1.3.1 and 1.3.2, by an applied field which splits doublet species into the two states $^2D_+(\alpha)\uparrow$ and $^2D_-(\beta)\downarrow$. The subscripts + and – are derived from the fact that the $\alpha(\uparrow)$ state has a spin angular momentum of $+\frac{1}{2}$ on the magnetic field axis and the $\beta(\downarrow)$ state has spin angular momentum of $-\frac{1}{2}$ on the magnetic field axis.

Let us now consider the energy diagram that results when two orbitally unpaired electrons (or more generally, two spin $\frac{1}{2}$ particles) interact to create a total spin of 1. In a spin 1 state there are three possible orientations of the spin in a strong magnetic field. These three states are termed $^3T_+$, $^3T_0$, and $^3T_-$. In the absence of the applied field, the three levels have the same energy (except for a small “zero field” energy arising from electron–electron spin interactions). As the field is

applied, the Zeeman interaction splits the energies of the triplet in a manner that depends upon the orientation of the total spin relative to the applied field. The subscripts +, 0, and – correspond to the magnitude of the spin angular momentum on the magnetic field axis of +1, 0, and –1. The $^3T_+$ state, which possesses two $\alpha(\uparrow)$ spins increases in energy and the $^3T_-$ state, which possesses two $\beta(\downarrow)$ spins, decreases in energy as the field strength increases. The energy separation is given by an expression analogous to Equation (1), except that the g -factor refers to the triplet state and the total spin must be taken into account. Scheme 4a shows schematically the magnetic energy sublevel diagram for the interconversion of $^3T_1 \rightarrow ^3RP \rightarrow ^2FR + ^2FR$. The energy diagrams of $^3T_1$ and ^3RP are expected to be similar, since both involve spin-correlated



Scheme 4. Top: Spin-correlation diagram for a triplet state which undergoes a primary photochemical process to produce a pair of free radicals. a) Represents the energy diagram for triplet species (^3T or ^3RP), while case b) is the energy diagram for two free radicals $^2FR_1$ and $^2FR_2$. It is assumed that $^2FR_1$ has a larger g -factor than $^2FR_2$. The energies are not to scale. Bottom: spin vectors for the singlet and three triplet states. See text for more discussion.

pairs. In homogeneous fluid solution ^3RP is generally not detectable as an intermediate because of the very short lifetime of the pair in nonviscous solvents. However, we shall see that in supramolecular systems the lifetime of ^3RP may be extended considerably so that spin-correlated radical pairs can be observed directly by the TREPR technique. We shall make use of the correlation diagram of Scheme 4 when discussing the triplet mechanism for producing ESP in the α -cleavage reactions of ketones in Section 2.3.2.

Both a singlet state ^1S and the $^3T_0$ state possess one $\alpha(\uparrow)$ spin and one $\beta(\downarrow)$ spin. As shown in Scheme 4, the root of the difference between the triplet and singlet states is the relative phase of the spin vectors. The spin vectors for the ^1S state are oriented so as to completely cancel the individual angular momentum and lead to a net spin of zero (antiparallel orientations with respect to the field, 180° out of phase), whereas the spin vectors of $^3T_0$ are oriented so that the total angular momentum adds to one (antiparallel with respect to the field, in phase). $^3T_0$, which possesses one $\alpha(\uparrow)$ spin and one $\beta(\downarrow)$ spin, does not change in energy as the field is applied, because it does not possess a net magnetic moment in the direction of the field (the direction of the total spin is perpendicular to the magnetic field axis). A handy representation of the spin orientation of the three triplet sublevels is $^3T_+[\alpha, \alpha(\uparrow\uparrow)]$, $^3T_0$ [either $\alpha\beta(\uparrow\downarrow)$ or $\beta\alpha(\downarrow\uparrow)$], and $^3T_- [\beta\beta(\downarrow\downarrow)]$. However, although this notation is appropriate for the $^3T_+$ and $^3T_-$ states, the $^3T_0$ state is not properly represented by this notation because it does not properly define the phase of the spins. Indeed, in this representation the singlet state and $^3T_0$ state are not distinguished. This issue will be dealt with in Section 2.2.1 where spin correlation and singlet–triplet interconversion are considered.

The vector representation in Scheme 4) provides a handy shorthand and guide for the spin-correlation diagram to connect the processes $^3T_1 \rightarrow ^3RP \rightarrow ^2FR + ^2FR$, which are of key importance in the photochemical paradigm of Scheme 2. In this notation the singlet state which correlates with the ^2FR is represented as ^1S [either $\alpha\beta(\uparrow\downarrow)$ or $\beta\alpha(\downarrow\uparrow)$]. As discussed above for $^3T_0$, this notation needs to be modified, since at this level, S and T_0 are indistinguishable. Similar to $^3T_0$, the energy of ^1S is not affected by the application of an applied field, because it does not possess a net magnetic moment in any direction and therefore doesn't interact with the field. However, the energy gap between $^3T_0$ and ^1S depends on the distance of separation of the electrons possessing the unpaired spins. In the molecular triplet, the electrons are held close to one another by the molecular framework (Scheme 4a). This proximity leads to a large J value, the electron exchange interaction, which is the basis of the singlet–triplet energy difference. As the system proceeds from $^3T_1 \rightarrow ^3RP \rightarrow ^2FR + ^2FR$, the electrons and their associated spins get further and further apart and the energy gap between $^3T_0$ and ^1S decreases toward a value of zero. When the system forms a pair of free radicals ($^2FR + ^2FR$), the value of J is zero and the system has lost the correlated magnetic characteristics of a spin one system (a triplet, $^3T_1$) and has taken on the magnetic characteristics of two uncorrelated spin $\frac{1}{2}$ systems ($^2D_1$, $^2D_1$, two doublets).

The EPR spectrum of doublets has been discussed above in simple terms (see Scheme 3b). The EPR spectrum of a molecular triplet is very complicated because of the strong spin–spin interactions of the two spins which are tightly coupled together by the molecular framework and the fact that the energy gap between triplet sublevels depends on the orientation of the triplet molecule in the magnetic field. We shall postpone a discussion of the EPR spectrum of $^3T_1$ and ^3RP until actual examples are presented in Section 2.3.

However, formation of ^3RP from $^3T_1$ greatly reduces the spin–spin interactions and in some circumstances, the EPR spectrum may be considered in terms of two interacting doublets (as will be discussed in Section 3.3).

1.4. Electron Spin Polarization (ESP)

An important characteristic of TREPR spectra is the common observation that EPR signals often occur at the same frequencies as in conventional steady-state EPR spectra, but the signals have “anomalous” intensities of “enhanced absorption” (A) or may actually appear in “emission” (E). Since the frequencies of the transitions are not changed, the energy levels involved in the transitions are not changed. How do the anomalous intensities arise? Since they do not arise from anomalies in energy levels, they must arise from anomalies in the populations of the levels, that is, from deviations of the electron spin Zeeman levels from the Boltzmann (thermal or equilibrium) population. We now consider how this can come about during a photochemical reaction.

1.4.1. The Spin Level Population Basis of ESP

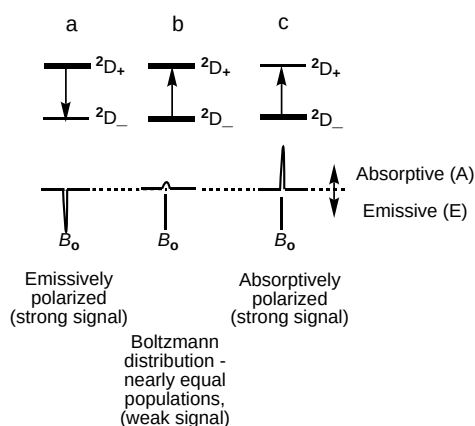
The concepts of ESP and deviations from Boltzmann distributions are not familiar to most organic chemists and, therefore, a simple but hopefully clarifying discussion of ESP is warranted. The EPR signal intensity is proportional to the difference in the populations of the energy levels (Δn) involved in the observed transitions between magnetic sublevels. This energy difference is very small (typically the excess of the lower level is less than 1 part in 10000) in conventional EPR where spin systems are in thermal equilibrium (because $\Delta E_{\text{Zeeman}} \ll k_B T$ at room temperature). However, we shall see that there are several mechanisms commonly occurring in photochemical reactions which produce ESP in an elementary step or sequence of elementary steps.

A convenient and practical experimental definition of ESP is the deviation of an instantaneous population of spins from the equilibrium (Boltzmann) population at a given temperature. At the resonance frequency, the oscillating field of electromagnetic radiation stimulates both upward and downward transitions between energy levels. The probabilities and experimental intensities of upward and downward transitions depend on the difference in the populations of the energy levels. Thus, the phenomena of ESP can be investigated through measurements of the deviations of the intensities of EPR transitions from those expected for Boltzmann populations, and TREPR spectroscopy (which measures instantaneous populations of spins) can be employed to measure ESP. Since ESP does not affect the energy difference between spin levels, the frequency of the EPR transitions is not influenced by the degree of ESP. Thus, the TREPR technique does not influence the structural information (energy differences between levels) that is obtained in systems for which ESP occurs. Experimentally, the TREPR study of radicals produced by nanosecond laser flash photolysis very shortly after

their formation demonstrates that often the radicals possess significant ESP. The extent of spin polarization may be dependent on the hyperfine level, that is to say that, lines corresponding to different nuclear spin states may be polarized to different extents. These unusual patterns created by different polarization mechanisms carry information on both the history of the “parents” of the observed radical pairs and their “children” radicals. These mechanisms will be discussed together with examples in the sections below.

1.4.2. Schematic Description of the Influence of ESP on EPR Spectra

Scheme 5 shows the concept of ESP schematically for a doublet state in a strong magnetic field. Scheme 5b represents the spin system at equilibrium. The population of the two spin sublevels is nearly equal, and this near equality is indicated by



Scheme 5. Schematic representation of Boltzmann (b) and spin-polarized [(a) and (c)] magnetic sublevels and corresponding EPR transitions for a doublet state. The thickness of the lines in the energy diagram refers to the population of the state (a thicker line implies a larger population). The field position of the signal is identical in all cases at B_0 ; the differences are in the intensities of the signals. See text for a more complete description.

a line of the same thickness for both $^2D_+$ and $^2D_-$ levels. In fact the EPR signal at B_0 observed in a conventional steady-state experiment is the result of the small excess of spins in the $^2D_-$ level. If we use the equilibrium population as a reference state, then any deviations from this population in either direction may be defined as a polarized population.

Now consider the situation of the $^2D_+$ level being overpopulated through some process (Scheme 5a, where the overpopulation is indicated by a thicker line for the $^2D_+$ level). If the EPR measurement is made while $^2D_+$ is overpopulated, instead of a weak absorption at B_0 arising from the overpopulation of $^2D_-$, a net emissive phase signal (termed E) is observed at the same frequency (B_0) as for the system at the Boltzmann distribution (Scheme 5b). If the EPR measurement is made while $^2D_-$ is overpopulated (Scheme 5c), instead of a weak absorption at B_0 arising from the overpopulation of $^2D_-$, an enhanced absorption phase (termed A and implying that the measured intensity is stronger than that expected for the Boltzmann population of the energy levels) is observed at the same frequency, B_0 .

1.5. ESP and Chemically Induced Dynamic Electron Polarization

Anomalous EPR signals arising from electron spin polarization (ESP) are lumped under the general term CIDEP (chemically induced dynamic electron polarization) spectra, although, strictly speaking, ESP can be produced by several origins. Indeed, there exist ESP mechanisms which are neither “chemically induced” (that is, by bond breaking or bond forming) nor “dynamic” (diffusion of radicals). There are many examples where nonreactive encounters of triplet excited molecules with doublet free radicals generate ESP.^[15] For example, excitation of a chromophore that is covalently linked to a stable free radical, results in a strong ESP that does not rely on diffusive encounters of the triplets and the doublets. Therefore, we prefer using the simple name electron spin polarization (ESP) and the spectra observed can be termed ESP-TREPR spectra. The observation and interpretation of ESP in TREPR spectra observed during photochemical reactions will be discussed to provide important information concerning the entities involved in photochemical reactions and in elucidating the mechanisms of transformations of these entities.

As mentioned above, ESP-TREPR spectra differ only in the intensities of the “lines” but not in the number of lines or in the frequencies of the lines. Thus, the interpretation of the structure of the species responsible for the spectrum is generally unambiguous. The lifetime of ESP is usually limited by the time needed for the spin population to relax to the Boltzmann distribution, which is, typically, of the order of microseconds in nonviscous liquid solutions. Equipment capable of detecting signals on the submicrosecond time scale is, therefore, essential because of this rapid decay time.

ESP in the measurement of TREPR signals has both technical and scientific advantages. At the technical level, the electron spin polarization creation (ESPC) is a wonderful bonus in that ESP creates a powerful signal-to-noise advantage in measurements of $^3T_1$, ^3RP , and ^2FR (compare with Scheme 2). It is this signal-to-noise advantage resulting from ESP which allows the detection of very low concentrations of paramagnetic entities on the submicrosecond timescale in TREPR experiments. In fact, this signal-to-noise enhancement more than compensates for the loss of signal-to-noise arising from the lack of phase sensitive detection (compare with Section 1.6). At this point, a word of caution is required because of the inherent sensitivity and selective observational powers of this technique. The observation of the TR-ESP spectrum derived from a polarized, paramagnetic product cannot rule out the formation of other “EPR-silent” products. It is always best to use multiple techniques in order to fully characterize the reactive intermediates and products, as well as to draw mechanistic conclusions. Furthermore, at the scientific level, the ESPC and electron spin polarization transfer (ESPT) allow definitive identification of the predecessors of paramagnetic entities and the transitions leading to these entities, thereby providing important mechanistic information on both the entities and transitions involved in photochemical reactions.

1.6. Comments on the Experimental Instrumentation of TREPR

It is relatively straightforward to modify a conventional, commercial EPR spectrometer to operate in the time-resolved mode.^[1, 5, 11] The requirements are the addition of a boxcar integrator, the availability of a pulsed laser, a trigger diode, and a fast preamplifier for the microwave bridge. Such a modified instrument will have a response time in the range of 100 ns. Recently, Elger et al. have introduced an X-band spectrometer with time resolution of about 10 ns.^[16] Radicals possessing ESP polarization are produced in the cavity of the spectrometer by a short (ca. 10 ns) laser pulse, and the EPR transitions are observed with nanosecond time resolution in a fixed external magnetic field B_0 . The transient EPR signals are integrated within a set time window using the boxcar integrator, to yield a single point in the spectrum. The integration window typically starts from 0.1 to a few microseconds following the laser flash, and is open for a few hundred nanoseconds. To obtain an entire spectrum, the external magnetic field is varied incrementally and the measurements are repeated a number of times and averaged to obtain an acceptable level of signal-to-noise. It is an important technical point that TREPR spectra are represented in a direct mode rather than the first derivative form that is typical of steady-state EPR. The spectra thus have an appearance which is closer to that of NMR spectra in that each transition appears as a conventional signal rather than the derivative of the signal.

1.7. Summary

The immersion of the paramagnetic species in the photochemical paradigm of Scheme 2 into a magnetic field causes a splitting of $^3T_1$ and ^3RP into three magnetic sublevels, termed $^3T_+$, $^3T_0$, and $^3T_-$, and causes a splitting of ^2FR into two doublet states, $^2D_+$ and $^2D_-$. The photochemical paradigm of Scheme 2 is now “enriched” in the number of entities and transitions that must be considered along a photochemical pathway which is followed in a strong magnetic field. A set of spin selection rules is also added to the paradigm. It is now natural to ask whether the spin selection rules are followed in photochemical reactions. We shall show how ESP, together with TREPR, provides an outstanding tool for answering this question. Having provided a basis for discussing EPR spectra and the background for the concept of ESP, next we will discuss ESP, and how it may be created, sorted, and transferred.

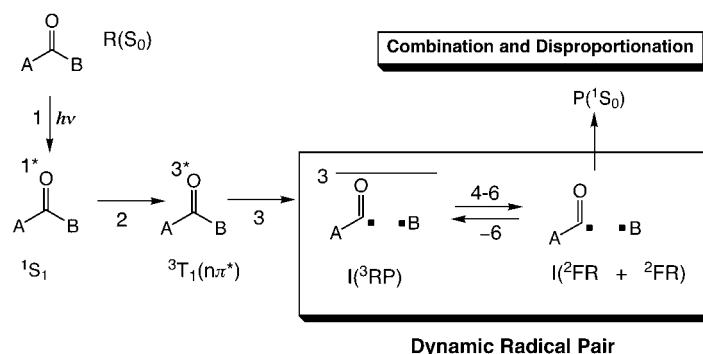
2. Electron Spin Polarization Creation and Transfer

2.1. An Exemplar Paradigm of Organic Photochemistry: The Photochemical α -Cleavage of Carbonyl Compounds

The photochemistry of carbonyl compounds and especially of ketones provides photochemists with a particularly mature and useful set of working and exemplar paradigms.^[5, 17, 18] We

now seek to describe experimental systems involving ketones and carbonyl compounds for which the TREPR technique will provide penetrating tests of the validity of these working and exemplar paradigms. The working paradigm for ketones teaches that the primary photochemical processes of ketones ($^*R(^3T_1) \rightarrow I(^3RP)$, Scheme 2) usually occur from triplet n, π^* states [$^3T_1(n, \pi^*)$] of ketones. The paradigm^[17] further teaches that there are only about four commonly observed primary photochemical processes of $T_1(n, \pi^*)$ states: 1) hydrogen abstraction; 2) electron abstraction; 3) addition to double bonds, and 4) α -cleavage. Of these primary photochemical processes, in this account we shall use α -cleavage (a well-studied unimolecular reaction which produces geminate triplet radical pairs in high yield in both molecular and supramolecular systems) and the subsequent secondary processes involving the dynamic radical pair (Scheme 2) as a working paradigm for the photochemistry of ketones. The α -cleavage reaction, in turn, will serve as an exemplar for the use of TREPR and electron spin polarization to investigate photochemical reactions in molecular and supramolecular systems.

An exemplar paradigm for α -cleavage is readily derived from the working paradigm for the photochemistry (Scheme 2). In the exemplar paradigm (Scheme 6) we replace $^1S_1$, $^3T_1$, ^3RP , ^2FR , and ^1RP with the appropriate structures of a ketone $AC(O)B$, which undergoes a primary photochemical α -cleavage of the $C(O)-B$ bond. For simplicity's sake at this point, we consider that the only products are those that result from combination reactions of radical pairs. We will consider scavenging reactions of free radicals in Section 2.4.



Scheme 6. An exemplar paradigm for the α -cleavage of carbonyl compounds. Excitation of a ketone to an n, π^* triplet excited state leads to α -cleavage and the formation of a dynamic radical pair. See text for discussion.

Let us consider the features of Scheme 6 that are relevant to TREPR, namely the paramagnetic species and the transitions involving paramagnetic species:

- 1) Step 2 is an efficient and rapid intersystem crossing from $^1S_1$ to $^3T_1(n, \pi^*)$ of the ketone $AC(O)B$. This step converts $^1S_1$ (a diamagnetic, EPR “silent” species) into $^3T_1$ (a paramagnetic, EPR “active” species). TREPR spectroscopy allows the direct spectroscopic investigation of the structure of $^3T_1(n, \pi^*)$ and an indirect investigation of the mechanism of spin creation ($^1S_1$ has spin zero; $^3T_1$ has spin one) through examination of the spin sublevel selectively

of the ISC process. The latter produces an electron spin polarized $^3T_1$ state of the ketone. This polarization can be employed to track the subsequent processes involving paramagnetic species indicated in Scheme 6.

- 2) Step 3 is a primary photochemical α -cleavage of $^3T_1$ of the ketone to produce a geminate, triplet radical pair $^3(\text{ACO}\cdot\cdot\text{B})$, which is usually not directly detectable in nonviscous solvents, because the timescale of separation (ca. 100 ps) of the geminate radical pair into free radicals ($^2\text{ACO}\cdot + ^2\text{B}\cdot$, step 4) is much faster than the response time of the TREPR technique (ca. 100 ns).^[*] However, in supramolecular systems, such as micelles, $^3(\text{ACO}\cdot\cdot\text{B})$ may possess a lifetime that is accessible to TREPR spectroscopy, as will be discussed in Section 3.4.2.
- 3) Step 4 involves the separation of the geminate pair to produce spin uncorrelated free radicals $^2\text{ACO}\cdot + ^2\text{B}\cdot$ that are readily investigated directly by TREPR spectroscopy provided that the radicals carry polarization generated from step 2 or 3 (or some other mechanisms, see Section 2.5).
- 4) In subsequent steps (step 5 and 6 in Scheme 2 and Scheme 6), the free radicals formed in step 4 eventually reencounter in a random fashion (compare with the more general situation for the dynamic radical pair of Scheme 2) and form random radical pairs—75 % of the time to form unreactive triplets $^3(\text{ACO}\cdot\cdot\text{B})$ and 25 % of the time to form reactive singlets $^1(\text{ACO}\cdot\cdot\text{B})$. The singlet radical pairs react to yield products by radical–radical recombination reactions and the triplet radical pairs separate to become free radicals again. The latter continue to cycle through reencounters to produce $^3(\text{ACO}\cdot\cdot\text{B})$ and $^1(\text{ACO}\cdot\cdot\text{B})$. We will see that the dynamic radical pair may be investigated by TREPR spectroscopy in both nonviscous solvents and in supramolecular systems such as micelles.

In addition to the information discussed above, TREPR spectroscopy will be shown to be a powerful method for investigating the reactions of the free radicals produced in step 4 and, in micellar systems, to study the electron exchange (J) and the supramolecular interactions between the radicals of the geminate pair.

In the following sections we will first show how the TREPR technique can be employed to investigate the transitions $^1S_1 \rightarrow ^3T_1$ and $^3T_1 \rightarrow ^2\text{FR}$ as well as to demonstrate the validity of the spin-selection rules for step 2–4. After that, we will show how the ESP produced in the $S_1 \rightarrow T_1$ step might be transferred through collisions and reactions and how these processes may be monitored by TREPR spectroscopy. We will also show how to employ supramolecular concepts to slow down the $^3\text{RP} \rightarrow ^2\text{FR}$ step so that the lifetime of the radical pair is long enough to observe ^3RP directly by the TREPR technique. Finally, we will present some selected examples of the use of TREPR spectroscopy to elucidate some important aspects of supramolecular interactions between radicals.

[*] Throughout the text a “+” between two radicals will indicate free radicals. Geminate radicals will be indicated by inclusion in parentheses without the “+” between the structures.

2.2. Application of the TREPR Technique to an Exemplar Paradigm of Photochemical α -Cleavage of Carbonyl Compounds

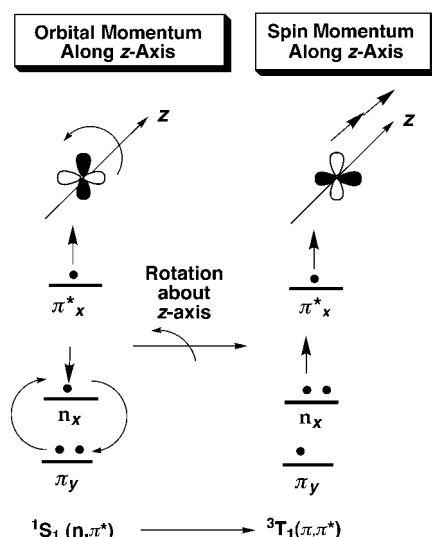
The paradigm of spin mechanics specifies as a fundamental principle that for all elementary processes the total spin must be conserved, and for elementary processes in a magnetic field the projection of spin on the axis of magnetization must be conserved. This principle is responsible for the selectivity, generation, transfer, and sorting of ESP. We now apply this principle to each of the steps in Scheme 6 and then apply the TREPR technique to experimentally test the validity of the principle through the observation of ESP.

2.2.1. The $^1S_1 \rightarrow ^3T_1$ Intersystem Crossing

The $^1S_1 \rightarrow ^3T_1$ transition involves the creation of spin angular momentum (the singlet has no spin; the triplet has a spin of one). Thus, to conserve overall angular momentum there must be a loss of some type of angular momentum in the total molecular system. For aromatic carbonyl compounds it is *orbital* angular momentum that is lost as *spin* angular momentum is created. This exchange of orbital and spin angular momentum is termed “spin–orbit coupling” (SOC) and it is the mechanism which drives the $^1S_1 \rightarrow ^3T_1$ transition.^[17] Because an orbital transition has a specific spatial relationship to the molecular framework, it is plausible to assume that the spin momentum that is created to produce $^3T_1$ will also have a specific orientation to the molecular framework. This orientation can be described in terms of the spin being oriented along one of three Cartesian axes which refer to the molecular frame. When dealing with n, π^* transitions of ketones, to a good approximation, the local symmetry about the C=O bond is not very sensitive to substituents so that an axis along the C=O bond can serve as a reference (z -axis). Furthermore, the SOC for carbonyl compounds can be viewed as a one-center orbital interaction localized at the oxygen atom. This implies that the SOC is largely determined by the local symmetry of the C=O group. It can be shown^[5] from such considerations that the SOC along a z -axis which corresponds to the C=O bond is strongest for a $\pi \rightarrow n$ interaction. This z -axis is also termed the spin quantization axis.

The most favorable orbital situation for SOC for a ketone is shown schematically in Scheme 7. The greatest degree of orbital angular momentum is generated along the C=O bond near the oxygen atom when $n \rightarrow \pi$ orbital motion occurs, for example, during molecular vibrations. Coupling of the orbital motion of a π electron with the motion of the spin of the electron in the n orbital “flips” the spin of the n electron and creates a triplet state and spin along the z -axis. In terms of state mixing, such an interaction corresponds to mixing of a $^1S_1(n, \pi^*)$ state with a $^3T_1(\pi, \pi^*)$ state. For aromatic ketones there is generally an energetically accessible $^3T_1(\pi, \pi^*)$ state which lies close to the chemically reactive n, π^* state.

The basic idea in the spin–orbit coupling mechanism is that the orbital angular momentum must change by one unit at the same time that the spin angular momentum changes by one unit so that the total change in angular momentum is zero. This idea is depicted schematically in Scheme 7, of which the



Scheme 7. SOC-induced $S_1 \rightarrow T_1$ ISC of a ketone possessing a lowest energy $1S_1(n, \pi^*)$ state. See text for further discussion.

left hand side shows the $1S_1(n, \pi^*)$ state. Orbital motion corresponding to the $n \rightarrow \pi_y$ rotation generates orbital angular momentum about the z -axis. This orbital motion couples to the spin motion of the electron in the n orbital, so that there can be a decrease in the orbital angular momentum from 0 to -1 (or 1 to 0) and an increase in the spin momentum from 0 to 1. This effect corresponds to a singlet to triplet transition and an intersystem crossing from $1S_1$ to $3T_1$. Total angular momentum is conserved and the spin selection rules are obeyed. The mixing is strongest when the full π_y electron is transferred to the n orbital (El-Sayed's Rule).^[17] When this happens a $T_1(\pi, \pi^*)$ state is produced.

2.2.2. Sublevel Selectivity of ISC—ESP Creation

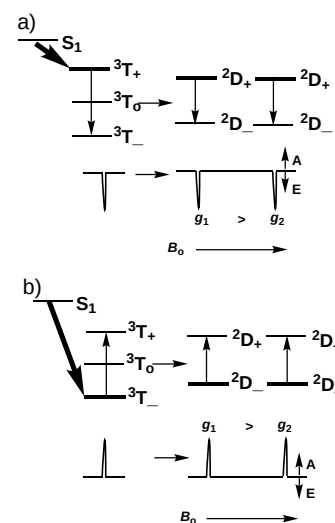
Intersystem crossing (ISC) from an excited singlet to a triplet state always takes place in accord with “spin selection rules” that operate in the molecule. If ISC occurs in the magnetic field of an EPR spectrometer we can imagine the applied field being so strong that it couples with the spin vector in the molecule and causes the spin vector to line up with the laboratory magnetic frame. In this case, the resulting triplet will be formed predominately in one of the triplet sublevels, $3T_+$, $3T_0$, or $3T_-$, (see Scheme 4) with the total electron spin oriented along one of the principle molecular axes of the ketone.

According to this model the orbital motion which is directed along the z -axis ($C=O$ direction) is transformed selectively into spin motion along the z -axis, that is, as orbital angular momentum is selectively destroyed along the z -axis, spin angular momentum is created selectively along the z -axis (shown in Scheme 7 as two arrows pointing along the z -axis). From detailed calculations for aryl alkyl ketones, this simple model predicts that the highest energy magnetic sublevel $3T_+$ (using the high field notation) will be selectively populated by ISC from $1S_1$ to $3T_1$, thus generating ESP in this level. Depending on the detailed structure and local symmetry of the carbonyl group, either $3T_+$ or $3T_-$ can be selectively

populated by ISC. This rather remarkable prediction is readily tested by experimental examination of the TREPR of $3T_1$ states.

2.3. EPR Spectroscopy of T_1 States—Some General Considerations

From the discussion of selective spin sublevel population by ISC it may be concluded that if an EPR measurement could be made immediately after the intersystem crossing step occurred in a carbonyl compound and before spin lattice relaxation brings the spin-polarized triplet to the Boltzmann distribution, the observation would be an emissive signal from $3T_+$ to $3T_0$ or an enhanced absorption from $3T_-$ to $3T_0$. Scheme 8 shows schematically the sublevel-selective ISC from



Scheme 8. Schematic drawing of the magnetic level diagram for producing ESP through the $1S_1 \rightarrow 3T_1 \rightarrow 2D+2D$ process (triplet mechanism, TM). The ISC selectively occurs to the $3T_+$ level (a) or to the T_- level (b), which leads to emissive (E) and absorptive (A) polarization, respectively, for the $\Delta m=2$ transition (at 77 K) and emissive and absorptive polarization, respectively, for the radicals produced by α -cleavage (at room temperature). See text for discussion.

$1S_1$ to $3T_1$ for a case in which the $3T_+$ sublevel (Scheme 8a) or the $3T_-$ sublevel (Scheme 8b) is populated selectively. In practice, the experimental TREPR spectrum of a molecular triplet is very complex for several reasons. The first complication is the existence of strong dipole–dipole interactions between electron spins, which are coupled to the molecular frame and are, therefore, very strong in a molecular triplet state. Furthermore, in an EPR spectrometer these interactions are modulated by the tumbling of the triplet species in a magnetic field. This modulation, in turn, causes a fast spin-lattice relaxation of the triplet sublevels. At room temperature the rate of spin-lattice relaxation of the triplet sublevel is of the order of 1 ns for nonviscous solutions.^[19]

Since the time resolution of TREPR operating in the continuous wave (CW) mode is of the order of 100 ns, this technique cannot be used to probe the population of the selective triplet sublevel directly in fluid solutions. In addition,

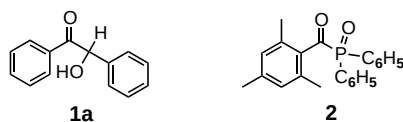
fast relaxation causes extensive line broadening in all magnetic resonance measurements. This line broadening (which causes poor signal-to-noise in the measurements), coupled with a short lifetime of the triplet sublevels makes the direct measurement of the $^3T_1$ state in solution by TREPR very difficult, even for spin-polarized triplet species. Only in a few cases has the TREPR of molecular triplets been observed in nonviscous liquids.^[20, 21]

In order to observe the predicted selective population of triplet sublevels by EPR, the measurement must be made before spin-lattice relaxation occurs and the sublevels attain the Boltzmann population. Immobilization of a molecular triplet in a transparent glass at 77 K (or lower) removes the problem of a short spin relaxation. However, there is a price to be paid for extending the triplet lifetime. In a rigid matrix, a triplet state consists of molecules randomly distributed in a large number of different orientations which are interrogated during a measurement. This situation leads to a very broad signal spanning over 4000 G for organic molecules, whereas the typical width for an EPR spectrum of a carbon-centered free radical is 100 G. In spite of these complications, when $^3T_+$ or $^3T_-$ are produced spin polarized as a result of sublevel selective ISC, the TREPR spectrum can readily be observed in rigid matrices that eliminate rotation and slow down spin-lattice relaxation. Furthermore, the magnetic parameters of the triplet state and the sublevel population ratios may be conveniently determined by comparing the observed spectrum with computer simulated spectra, and the matter of whether $^3T_+$ or $^3T_-$ is selectively populated can be resolved.

In actual experimental cases of ketones it has been found that the EPR transitions between $^3T_-$ and $^3T_+$ (so-called $\Delta m = 2$ transitions, see Scheme 8) are readily observed because of mixing of $^3T_0$ with the adjacent sublevels. This is important because the position of the $\Delta m = 2$ transition turns out to be relatively insensitive to the orientation of the triplet in the magnetic field, so a relatively sharp transition is observed.

2.3.1. Experimental Examples of TREPR of T_1 —ESP of T_1 by ISC

Figure 1 shows examples of the entire TREPR spectra of benzoin (**1a**)^[22] and diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (**2**) at 77 K^[23] and the associated simulated



spectra. At this temperature spin-lattice relaxation of the sublevels is of the order of a microsecond or longer. A $^3T_1$ state is produced with a nanosecond laser and then a TREPR spectrum is accumulated by boxcar integration in a microsecond or less.

The entire TREPR spectra of Figure 1 are spin polarized. The $\Delta m = 2$ transitions appear at about 1300 gauss in both spectra. In the case of **1a** the $\Delta m = 2$ transition is emissive, corresponding to a polarized $^3T_+ \rightarrow ^3T_-$ EPR transition, which

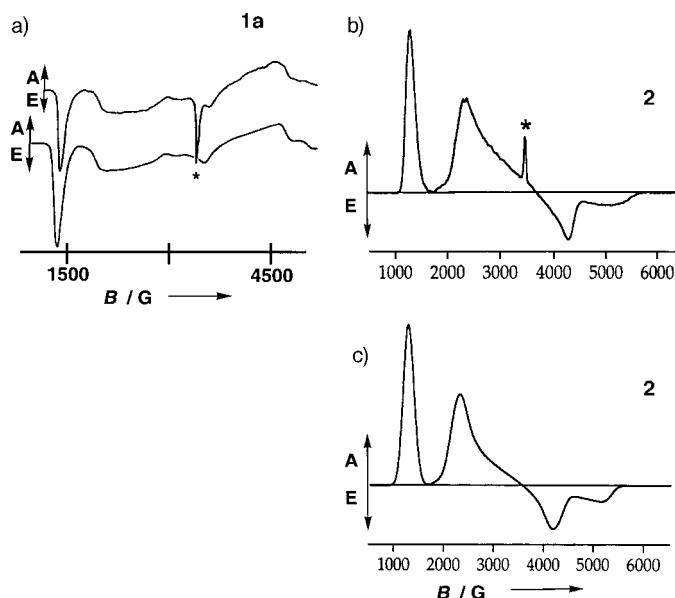


Figure 1. TREPR spectra of benzoin (**1a**) at 77 K (a; top: experimental, bottom: simulated) and diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (**2**; b: experimental, c: simulated). The sharp line indicated by the asterisk (*) arises from a carbon-centered radical produced by photolysis.

is consistent with the selective population of the $^3T_+$ state by ISC from $^1S_1$ (Scheme 8a). On the other hand, in the case of **2**, the $\Delta m = 2$ transition is in enhanced absorption, corresponding to a polarized $^3T_- \rightarrow ^3T_+$ transition Scheme 8b. Thus, we have two carbonyl compounds capable of undergoing α -cleavage to produce ESP in different phases: For **1a**, ISC selectively populates $^3T_+$ (emissive phase), and for ketone **2** ISC selectively populates $^3T_-$ (enhanced absorptive phase). The excellent agreement of simulated and experimental spectra (Figure 1a, lower spectrum, Figure 1c) confirm the assignments of the selective overpopulations.

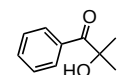
Although the ESP generated in the $^1S_1 \rightarrow ^3T_1$ ISC step for **1a** or **2** is not directly detectable by the TREPR technique at room temperature in fluid solution, it is reasonable to ask whether the selective population of triplet sublevels occurs under these conditions. In the next section we shall see how the TREPR technique answers this question indirectly but unambiguously, and how the ESP generated in the ISC step may be exploited by this technique to further examine the paradigm of Scheme 6.

2.3.2. Correlation of the TREPR of Molecular Triplets with Primary Photochemical Processes—The Triplet Mechanism of ESP

We have seen how a photophysical process, the intersystem crossing from a singlet state to a triplet state, can be investigated by TREPR spectroscopy at 77 K. Thus, $^3T_1(\mathbf{1a})$ and $^3T_1(\mathbf{2})$ are “born” spin polarized in $^3T_+$ and $^3T_-$, respectively, and that ESP is created by the ISC transition. We now follow the magnetic level correlation diagram of Scheme 4 and note the consequences of the spin-selection rules for elementary primary photochemical α -cleavage. If the polarization found by TREPR (Figure 1) at 77 K is carried over at room temperature, and if α -cleavage can occur within

the spin-lattice relaxation time of the triplet state (ca. 1 ns),^[19] then **1a** and related ketones should produce electron-spin polarized $^2\text{ACO}^\#$ and $^2\text{B}^\#$ radicals that can be observed in emission, and **2** (and related compounds) should produce electron spin polarized radicals that can be observed in absorption because angular momentum must be conserved (the superscript # signifies a polarized electronic spin). The general schemes for these two possibilities is shown in Scheme 8a and b, respectively, while the experimental results obtained in fluid solution at room temperature are shown in Figures 2 and 3.^[24]

In the case of **1b**, an emissively (E) polarized benzoyl radical (a broad triplet with $g = 2.001$, indicated by the arrow in Figure 2a) and an emissively polarized ketyl radical (seven lines, with $g = 2.003$) are observed in the TREPR spectrum. All of the lines are in net emission (E), consistent with the selective ISC of $^1\text{S}_1$ to $^3\text{T}_1$ to form $^3\text{T}_+$ followed by rapid α -cleavage of $^3\text{T}_+$ to form $^2\text{D}_+$. The triplet mechanism (TM) is the term given to the process in which spin polarization is produced through a primary photochemical reaction of a triplet excited state whose sublevels are polarized by ISC.



1b

In the case of **2**, an absorptively (A) polarized benzoyl radical (a broad triplet with $g = 2.001$, indicated by the arrow in Figure 3a) and an absorptively polarized phosphorous centered radical (a doublet, with $g = 2.003$) are observed in the TREPR spectrum. The polarization produced by the photolysis of **2** contrasts with the polarization produced in the photolysis of **1b**, that is, all of the lines in the TREPR spectrum of **2** are in net absorption (A), which is consistent with ISC of $^1\text{S}_1$ to $^3\text{T}_1$ to form $^3\text{T}_-$ selectively, followed by rapid α -cleavage of $^3\text{T}_-$ to form D_- .

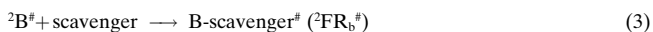
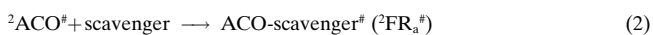
A careful inspection of Figures 2a and 3a reveals that the polarization is not exactly symmetrical at low field (left) and high field (right). This is a common situation that arises from other polarization mechanisms occurring in addition to the TM, which should generate a completely symmetrical polarization pattern. One of these other mechanisms that can produce spin polarization, the radical pair mechanism (RPM), will be discussed in Section 2.5.

Thus, we have two exemplars of α -cleavage of carbonyl compounds that demonstrate the ability of the TREPR technique to track ESP produced by sublevel-selective ISC through to the free radicals produced by the primary photochemical process. The combination of ESP and TREPR provides a means of unambiguous direct observation and identification of the ^2FR species produced by a primary photochemical process through measurement of hfc and g -values. In addition, ESP-TREPR spectra provide a means of indirectly determining the spin characteristics of the $^*\text{R}$ species that underwent cleavage through the observation of the phase of the ESP. The observation of TM ESP confirms the paradigm expectation that the primary photochemical process occurs from a triplet state (step 3 in Scheme 2 and 6).

We next consider how TREPR and ESP can be used to track secondary reactions involving radical scavenging and to elucidate the intermolecular interactions of the partners of a dynamic radical pair.

2.4. TREPR Investigations of ESP Transfer from Primary Spin Polarized Reactive Radicals Produced by α -Cleavage to Secondary Scavenging Reactions—Reactive ESP Transfer

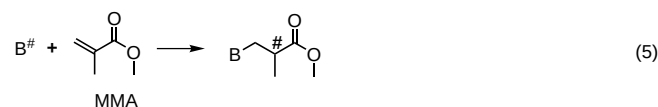
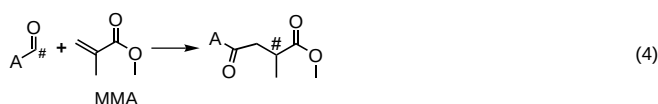
We have seen above in Section 2.3 that the polarization created in spin-selective ISC from $^1\text{S}_1$ to $^3\text{T}_1$ is carried over to produce polarized $^2\text{ACO}^\#$ and $^2\text{B}^\#$ radicals formed in the α -cleavage of $\text{AC}(\text{O})\text{B}^*$. In these cases the polarization generated in a specific sublevel of a parent $^3\text{T}_1$ serves firstly to label the daughter radicals produced by a photochemical event, secondly to enhance the signal-to-noise of detection of the daughter radicals, and thirdly to provide definitive evidence on the origin of the precursor of the daughter radicals. In many photochemical reactions the radicals produced in the primary photochemical process are scavenged by reactions with molecules (secondary processes) to produce secondary radicals [Eqs. (2) and (3)].



It is natural to inquire whether the expected spin-selective scavenging reactions that produce spin-polarized secondary radicals can be detected by TREPR spectroscopy. The requirement for observing polarized radicals from scavenging reactions is that the rate of the scavenging reaction is faster than the spin-lattice relaxation of the parent polarized free radical. Since the spin-lattice relaxation of carbon-centered free radicals^[1] is of the order of 1–10 μs , the scavenging reaction must be of this order of magnitude or faster. Thus, in favorable cases, ESP may serve as a “tracer” or a very subtle and noninvasive “label” of radical reactions. The process of transfer of polarization from a $^2\text{FR}^\#$ radical to a new radical by reaction is termed reactive ESP transfer (ESPT). Since the spin-selection rule for ESP is expected to operate in the elementary step of a reaction of a radical with a molecule, the phase of the polarization of the primary ^2FR radical is expected to be transferred to the secondary radical produced by scavenging [Eqs. (2) and (3)]. Of course reactions of two ^2FR radicals produce diamagnetic molecules (P in Scheme 6) which are not detected by EPR.

2.4.1. Examples of a TREPR Investigation of Reactive ESPT—Secondary Reactions of Free Radicals Produced in Primary Photochemical Processes

Carbon-centered radicals produced by photochemical α -cleavage of carbonyl compounds such as **1b** and **2** are scavenged by ethylenes in the photoinitiation step in free radical polymerization. The photolysis of **1b** and **2** produces polarized $^2\text{ACO}^\#$ and $^2\text{B}^\#$ radicals, and if the free radical can add to the olefinic monomer within the spin relaxation time of the polarized radical then the addition of these radicals to ethylenes is expected to generate spin-polarized adducts. For example, Equations (4) and (5) show the addition of the fragments from α -cleavage of a polarized ketone to methyl methacrylate (MMA), an important monomer in photoin-



duced free radical polymerization. The issue to be considered by TREPR spectroscopy is whether the polarization of the $^2\text{ACO}^{\bullet}$ and $^2\text{B}^{\bullet}$ species that is produced by TM is carried over to the new radicals formed by the scavenging of the radicals. The expectation from spin-selection rules is that since the parent radicals produced in the photolysis of **1b** (Figure 2a) are emissively polarized, the scavenged radicals produced from **1b** will also be emissively polarized. Similarly, since the parent radicals produced from the photolysis of **2** are produced in enhanced absorption, the scavenged radicals produced from **2** will also be absorptively polarized. These expectations are confirmed in Figures 2 and 3.

Figure 2^[24] shows the TREPR spectrum produced by photolysis of **1b** in acetonitrile in the absence (Figure 2a) and presence (Figure 2b, c) of MMA. A simulated spectrum (Figure 2d) for the adducts of $^2\text{ACO}^{\bullet}$ and $^2\text{B}^{\bullet}$ to MMA (of which both spectra are essentially identical) is also shown.

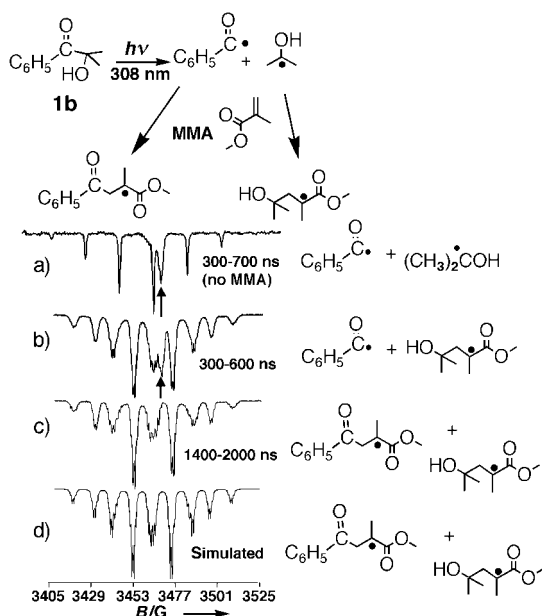


Figure 2. ESP-TREPR spectrum resulting from the photolysis of **1b** in acetonitrile (a), or in MMA 300–600 ns (b), 1.4–2 μs after the laser flash (c) as well as the simulated spectrum of the radical adduct with MMA (d). The arrow points to the signal derived from the $^2\text{ACO}^{\bullet}$ radical.

From the simulation it is clear that the radicals present after 2 μs arise from the addition of ACO and B to MMA. In addition, the observed E polarization of the radical produced by scavenging is the same as that of the primary parent precursor. The observation of transfer of emissive ESP from the primary radicals to the secondary radicals confirms that the spin angular momentum is conserved in the addition step.

In addition to confirming the spin-sublevel selectivity of the addition of radicals to ethylenes, there is further mechanistic information that can be extracted from the experimental spectra. For example, from Figure 2b it is noted that the addition of the $^2\text{B}^{\bullet}$ radical is complete within a few hundred nanoseconds. This observation implies that the rate constant for addition of $^2\text{B}^{\bullet}$ to MMA is of the order of 10^6 – $10^7 \text{ M}^{-1} \text{ s}^{-1}$, a value consistent with the value of $10^7 \text{ M}^{-1} \text{ s}^{-1}$ reported in the literature.^[25] It is also noted that the addition of the $^2\text{ACO}^{\bullet}$ radical to MMA is about an order of magnitude slower, since some of the polarized $^2\text{ACO}^{\bullet}$ radical has still yet to react after about 500 ns (arrow in Figure 2b). However, the signal corresponding to $^2\text{ACO}^{\bullet}$ is gone after approximately 1500 ns.

The rate constant for propagation of MMA is approximately $10^2 \text{ M}^{-1} \text{ s}^{-1}$, which is much slower than for the addition of either radical to MMA monomer, so that even in MMA solvent (ca. 10M) it is expected that the second propagation step will be too slow to detect by TREPR spectroscopy at the integration times employed. Thus, the observed radical produced by addition of polarized radicals is expected to be only the first adduct produced by scavenging of the primary radicals, but this cannot be demonstrated with this experiment since the tertiary radical and other oligomeric radicals produced by polymerization would be expected to have a spectrum essentially identical to the secondary radical.

We now consider the spectrum produced by MMA scavenging (Figure 3a–c) of the polarized radicals produced by photolysis of **2**. A simulation of the spectrum expected if the polarized radicals $^2(\text{C}_6\text{H}_5)_2\text{P=O}^{\bullet}$ add to MMA is shown in Figure 3d. First, we note directly from the spectra that $^2\text{ArCO}^{\bullet}$ adds more slowly to MMA than $^2(\text{C}_6\text{H}_5)_2\text{P=O}^{\bullet}$, since the signals at high and low field arising from $^2(\text{C}_6\text{H}_5)_2\text{P=O}^{\bullet}$ are completely gone after about 300–700 ns, whereas the signal for $^2\text{ArCO}^{\bullet}$ persists (Figure 3b). This observation is consistent with the higher reactivity of $^2(\text{C}_6\text{H}_5)_2\text{P=O}^{\bullet}$ than $^2\text{ArCO}^{\bullet}$ toward MMA. We note also that a comparison of Figures 2b and 3b implies that if acyl radicals are produced from two independent sources of different polarization, their chemical reactivity is unaffected.

Since the phosphorous hfc can be observed in the first adduct to MMA, it was of interest to determine if, at the longest times of observation, the TREPR technique can be used to observe transfer of polarization to the scavenging of the secondary radical by MMA. In fact, this was not observed, presumably the rate of propagation of polymerization of MMA is too slow that the second addition occurs after loss of polarization of the secondary radical. However, polarization transfer has been tracked through two addition steps in the case of the addition of $^2(\text{C}_6\text{H}_5)_2\text{P=O}$ to isoprene.^[26, 27] The first radical formed upon addition to isoprene shows a clear phosphorous hfc. At later times the phosphorous hfc disappears as the initially formed radical adds to a second monomer. In this case the addition of the first-formed radical to a second monomer is sufficiently fast to occur before the spin polarization has disappeared.

Thus, through the exemplar paradigm of the photochemical α -cleavage of carbonyl compounds with specific examples of **1b** and **2**, we have shown how the TREPR technique is an excellent tool for experimentally observing 1) the creation of

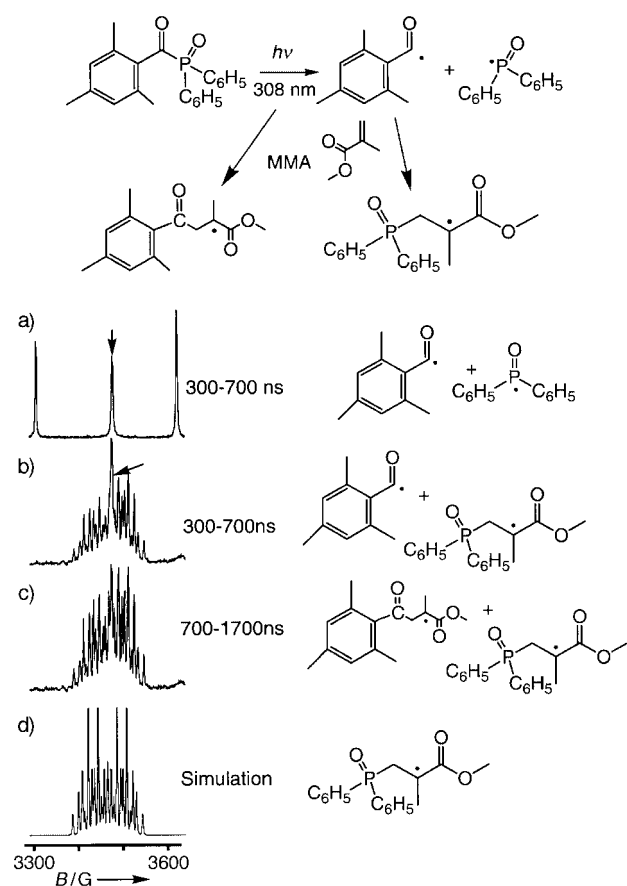


Figure 3. TREPR spectrum resulting from the photolysis of **2** in acetonitrile in the absence (a) and presence (b, c) of MMA. a), b) 300–700 ns after laser excitation; c) 700–1700 ns after laser excitation; d) simulation of the spectrum expected from the addition of $(\text{C}_6\text{H}_5)_2\text{P}=\text{O}$ to MMA. The arrow points to the signal derived from the $^2\text{ACO}^\#$ radical.

spin polarization ($^1\text{S}_1 \rightarrow ^3\text{T}_1^\#$), 2) reactive ESPT through a primary photochemical process ($^3\text{T}_1 \rightarrow ^2\text{D}^\# + ^2\text{D}^\#$), and 3) reactive ESPT through secondary scavenging reactions ($^2\text{D}_1^\# + \text{scavenger} \rightarrow ^2\text{D}_2^\#$). Next we will show how the TREPR technique can provide information on nonreactive supramolecular intermolecular interactions and collisions between paramagnetic species.

2.4.2. Examples of Nonreactive ESP Transfer Through Intermolecular Interactions of Paramagnetic Species

The working paradigm for organic photochemistry (Scheme 2, box) teaches that, in addition to the combination reactions of free radicals, the dynamic free radical pair involves nonreactive collisions followed by repeated reencounters between free radicals. This is a rather striking feature of the paradigm, since radical–radical reactions between carbon-centered radicals are usually diffusion controlled. Here again the spin-selection rules come into play. Since the primary geminate radical pair (^3RP) produced by α -cleavage is a triplet it cannot undergo radical–radical combination reactions, because these reactions produce molecules in a singlet state in an elementary step. However, upon separation of ^3RP , two free radicals (^2FR) are produced which undergo spin relaxation and then reencounter. The reencountering

pairs produce triplet and singlet pairs based purely on spin statistics in a 3:1 ratio. The latter are reactive toward combination reactions and the former are unreactive, and following the collision, the ^3RP separate to form free radicals again. The TREPR technique provides a means of investigating the nonreactive collisions of reactants through nonreactive ESP transfer (ESPT).

The basic concept behind nonreactive ESPT experiments is that upon a collision between an unpolarized radical ($^2\text{FR}^\bullet$) and a polarized radical ($^2\text{FR}^\#$), two possible results may occur as a result of supramolecular interactions: 1) a reactive radical–radical collision (for ^1RP pairs) to produce a diamagnetic combination product $\text{P}(^1\text{S}_0)$, which is EPR “silent” or 2) a nonreactive radical–radical collision (if the pair is ^3RP) followed by separation into free radicals.^[28] During the nonreactive collision, the electron exchange interaction J between the radicals is significant and spin polarization can be transferred from the initially polarized radical to the nonpolarized partner in the pair. Upon separation, the originally unpolarized partner will emerge polarized [Eq. (6)] and the process of nonreactive ESPT can be detected in the ESP-TREPR spectra.



These ideas lead to the conclusion that polarized $^2\text{ACO}^\#$ and $^2\text{B}^\#$ radicals produced by photolysis of $\text{AC}(\text{O})\text{B}$ can consequently produce polarization by a nonreactive, electron-exchange mechanism through encounters with any nonpolarized radicals. For example, it is expected from spin-conservation rules that encounters of a nonpolarized free radical with $^2\text{ACO}^\#$ and $^2\text{B}^\#$ produced from **1b** will produce an emissive radical (as expected from the results with **1a**) as the result of nonreactive ESPT and that $^2\text{ACO}^\#$ and $^2\text{B}^\#$ produced from **2** will produce an absorptively polarized radical as the result of nonreactive ESPT.

Experimental examples of nonreactive ESPT are given in Figure 4. The tack employed is to add TEMPO, a stable,

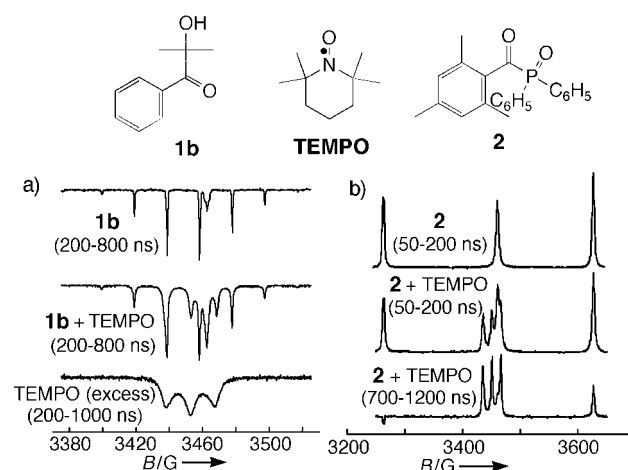
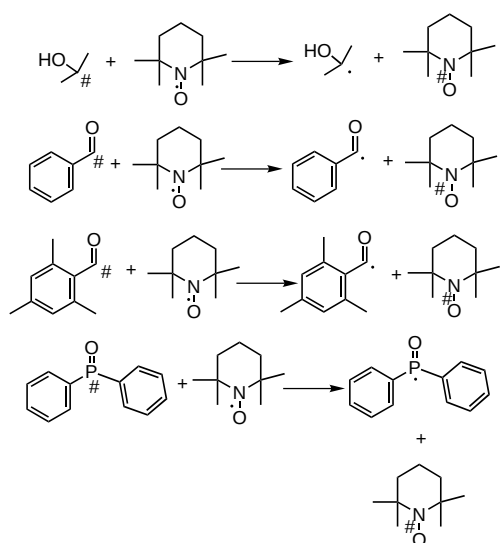


Figure 4. ESP-TREPR spectra demonstrating the nonreactive ESPT. a) An example of the photolysis of **1b** in ethyl acetate. b) An example of the photolysis of **2** in ethyl acetate. A comparison of the spectra in (a) shows it has similar features as the CW-EPR spectrum of TEMPO. The broadening of the signal stems from spin–spin interactions arising from the high concentrations employed.

unpolarized, free radical nitroxide to the sample at a concentration that is too low to be detected by TREPR spectroscopy. If the polarized radicals $^2\text{ACO}^\#$ and $^2\text{B}^\#$ that are produced by photolysis of **1b** or **2** encounter TEMPO, collisions will either produce a singlet radical pair (^1RP) that will lead to a diamagnetic product $\text{P}(^1\text{S}_0)$, or produce a triplet radical pair (^3RP), which will separate to regenerate free radicals. Since the electron-exchange interaction is significant in ^3RP , some of the polarization of $^2\text{ACO}^\#$ and $^2\text{B}^\#$, will be transferred to TEMPO upon separation of the radicals (Scheme 9). Photolysis of **1b** and **2** in the presence of nitroxides leads to the ESP-TREPR spectra shown in Figure 4a and 4b, respectively. It is clear that the expectation of selectivity of polarization transfer is confirmed by the TREPR measurements: Photolysis of **1b** in the presence of TEMPO results in a net emissive spectrum of TEMPO and the photolysis of **2** in the presence of TEMPO leads to a net enhanced absorption spectrum.



Scheme 9. Nonreactive ESPT between the primary polarized radicals $^2\text{ACO}^\#/\text{B}^\#$ and TEMPO in the photolysis of **1b** (first and second reaction) and **2** (third and fourth reaction).

It should be emphasized that the transfer of polarization from one radical to another is the result of supramolecular interactions, that is, noncovalent interactions between reactive species. Thus, the TREPR technique is an excellent tool for examining such supramolecular interactions.

2.5. Polarization Sorting—TREPR Evidence for Nonreactive Collisions of Free Radicals: The Radical Pair Mechanism of ESP

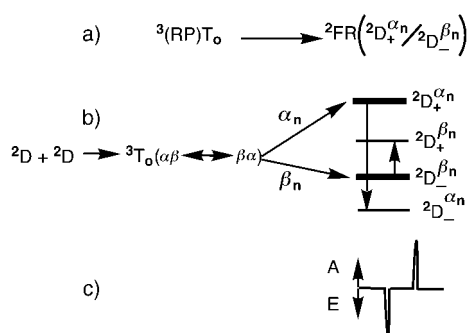
A second mechanism exists for the dynamic radical pair (Scheme 2) to produce ESP in nonreactive encounters and collisions of radicals and thus serves as a second probe of supramolecular interactions of radicals with one another. The ESP arises during the dynamic radical pair lifetime as the result of magnetic interactions between initially nonpolarized pairs of radicals in the presence of an applied magnetic

field.^[1, 3, 4] The ESP is said to be generated through the radical pair mechanism (RPM) and involves an electron exchange induced mixing of magnetic states during the encounter. Only the mixing of $^1\text{S}_0$ and $^3\text{T}_0$ is responsible for the RPM because $^3\text{T}_+$ and $^3\text{T}_-$ split from $^3\text{T}_0$ (large energy gap) as the magnetic field increases in magnitude. Since the rates of interconversion between the triplet sublevels and singlet level depend on the energy gap, these two triplet sublevels are essentially cut off from ISC by the Zeeman interaction.

For carbon-centered radicals, either differences in the g -factors of the radicals (Δg) or hyperfine coupling can cause the $^1\text{S}_0$ - $^3\text{T}_0$ mixing which leads to spin polarization. The effect of Δg is usually smaller than the hyperfine interactions for organic radicals, so that the latter typically dominate the polarization effects in the RPM. Note that for $^1\text{S}_0$ - $^3\text{T}_0$ mixing, the number of up and down spins remains constant in the presence of a strong magnetic field.

During an encounter and separation step of a dynamic geminate radical pair, spins of the two partners of the pair are alternately under the influence of the electron exchange interaction (during collision of the pair) and the hyperfine interaction (when the pair is separated by one or more solvent molecules). When hyperfine interactions are responsible for the mixing it is reasonable to expect that some hyperfine states can enhance the mixing faster than others and that these states will be more populated in the free radicals of the pair when separation occurs. The spin states of the two ^2FR that reencounter are mixtures of ^1S and ^3T states. However, these states polarize as if they were triplets, because the pairs with dominant singlet character react efficiently and are thus removed from the paramagnetic pool and leave behind radical pairs that have dominant triplet character. The current theory of polarization of free radicals requires that a pair must first encounter, then separate to a distance for which the value of J is near zero, and then return to form a colliding pair.^[1, 3, 4] This polarization selection occurs upon radical reencounter under the influence of J . The creation of ESP through the RPM depends upon the subsequent action of the electron-exchange interaction J , which introduces a “phase shift” into the pair wave function and accumulates more α spin in one radical and more β spin in the other radical. Both ^1S and $^3\text{T}_0$ contain equal amounts of α and β spins (vector description of S and T in Scheme 4 bottom), so that mixing cannot create any excess of up or down spins, and any excess of α spins must be balanced by an excess of β spins. Experimentally, the mixing of some hyperfine states of the radicals usually exhibits an excess of absorption (A); other hyperfine states exhibit an emission (E).

The typical result for radicals of the type $^2\text{ACO}^\#$ and $^2\text{B}^\#$ of the exemplar is usually (assuming the usual negative sign of the exchange interaction, which means that S has a lower energy than T) the production of a RPM-polarized spectrum in which the line(s) at low field (lower field than g) of a doublet are in emission and the high field line(s) (higher field than g) are in enhanced absorption. When a spectrum has these characteristics it is said to exhibit an E/A polarization pattern. The energy level diagram that produces such polarization by RPM is shown schematically in Scheme 10 for one of the radicals of the pair. In this example, it is assumed that



Scheme 10. Schematic energy level diagram for the RPM for polarization. In the case shown, the energy levels are not to scale, J is negative, and the hfc constant is positive. a) A ^3RP that diffuses away from each other to yield two ^2FR ; b) energy level diagram—for simplicity, only one radical coupled to a single spin $\frac{1}{2}$ nucleus is illustrated; c) typical TREPR spectrum derived from the RPM including enhanced emission and an enhanced absorption (E/A) pattern for one of the radicals.

during the mixing the $^2D_-$ state, which is hyperfine coupled to the β_n nuclear spin, is overpopulated and the $^2D_+$ state, which is coupled to the α_n nuclear spin, is also overpopulated. From the energy level diagram it can be seen that such overpopulations lead to an emissive (E) allowed transition from the $^2D_+^{\alpha}$ level and an enhanced absorption (A) allowed transition from the $^2D_-^{\beta}$ level. The higher energy $^2D_+^{\alpha}$ transition occurs at a lower magnetic field in a TREPR experiment.

It is important to keep in mind that the net polarization of the radical ensemble is zero, that is, the number of $\alpha(\uparrow)$ and $\beta(\downarrow)$ spins remains identical to that of the Boltzmann distribution so that the integrated intensity of the RPM to the spectrum is zero before spin relaxation. However, the radicals are polarized far away from the Boltzmann distribution, and the system can be examined by TREPR.

The timescale of production of polarization by the RPM is usually longer than the that by TM, which is expected to be of the order of nanoseconds and is limited by the rate of α -cleavage. The development of RPM polarization requires separation of a radical pair followed by diffusive escape and subsequent encounters. Experimentally, the timescale for the build-up of RPM polarization in carbon-centered radicals is of the order of 100–1000 μs .^[29]

As an example of the RPM, we consider the photolysis of **2**. The ESP-TREPR spectrum 0.1–0.2 μs after laser excitation (Figure 5a) is in total enhanced absorption, whereas at 2–5 μs after excitation (Figure 5b) the spectrum transforms into an E/A pattern. This arises from the RPM, which occurs at longer times because of the requirement of separation and reencounters. This is a nice example showing that if α -cleavage is fast enough the TM determines the observed TREPR at short times, but at longer times polarization by the RPM comes into play. We note that in this case the TREPR method allows direct investigation of steps 3 and 4–6 of the dynamic radical pair of Scheme 6.

An important feature of RPM is that it represents a spin reorganization among the magnetic sublevels—not a spin creation as occurs in $^1S_1 \rightarrow ^3T_1$ intersystem crossing nor a spin transfer as occurs in reactive and nonreactive interactions

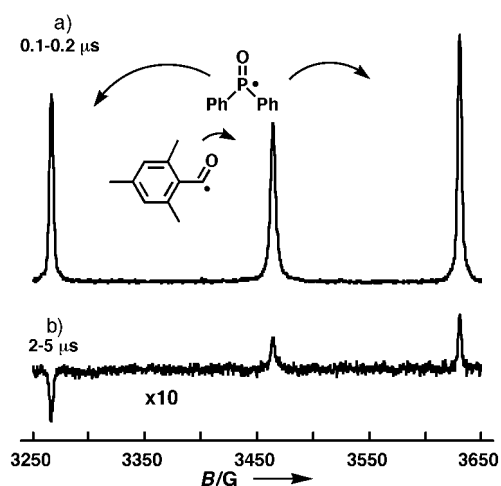


Figure 5. ESP-TREPR spectrum of diphenyl(2,4,6-trimethylbenzoyl)-phosphane oxide (**2**) in ethyl acetate at two times after the laser pulse. The lines at highest and lowest fields are derived from the phosphorus radical, whereas the central line results from trimethylbenzoyl radical.

between radicals. Another important feature is that the RPM depends on the effects of an external field on the spin dynamics of the radical pair. The RPM is more general than the TM in that it does not require the formation of a triplet (free radical encounters which produce ^3RP are sufficient) and does not depend on the triplet spin lattice relaxation time. The photolysis of phenyl (*tert*-butyl)phenylketone provides an interesting example for which no TM polarization is observed by TREPR spectroscopy,^[30] presumably because in this case the α -cleavage is so slow (ca. $10^7 s^{-1}$)^[17] that equilibration of the triplet sublevels occurs before cleavage and the primary radicals are unpolarized. However, the unpolarized radicals separate and, upon reencountering, produce the expected RPM E/A polarization.

3. Supramolecular Chemistry and Supramolecular Photochemistry

Supramolecular chemistry refers to the structures, dynamics, and transformations of molecules that are “connected” by intermolecular interactions and intermolecular noncovalent “bonds”.^[31, 32] Many supramolecular systems may be characterized as guest@host systems for which a small “guest” molecule is adsorbed and noncovalently bonded to a large “host” molecular or aggregate of small molecules. While many of the properties of the guest and host molecules remain intact in the supramolecular system, the guest@host system will have properties that can be ascribed to more than just the sum of its parts.^[33] Supramolecular photochemistry is concerned with the effect of absorption of light on supramolecular systems. One of the key questions deals with the empirical criteria for a guest@host system such that it can be usefully termed a supramolecular system. After the molecular properties of a system have been defined theoretically or experimentally, a system is most readily classified as supramolecular when the system exhibits measured properties that are measurably different from the “sum” of the molecular components.^[34]

3.1. Supramolecular Spin Chemistry

Let us examine this question of supramolecularity in the framework of the paradigm of Scheme 2 by focusing on the entities ^3RP (or ^1RP) and $^2\text{FR}+^2\text{FR}$. We can describe ^3RP (or ^1RP) as a “supramolecular” entity as the result of its noncovalent intermolecular spin characteristics and interactions. These interactions are missing in free radicals. How do we examine this supramolecular aspect of ^3RP (or ^1RP) experimentally? An essential difference between ^3RP and $^2\text{FR}+^2\text{FR}$ is the intermolecular noncovalent electron exchange interaction J , which causes the electron spins to be correlated, that is, ^3RP and ^1RP are spin-correlated radical pairs that possess a finite value of J , whereas $^2\text{FR}+^2\text{FR}$ constitute two spin uncorrelated, separate radicals with no intermolecular interactions and therefore possess $J = 0$. In this example, an important issue is the distance between the two radical centers, since the value of J is expected to decrease exponentially with separation between the radical centers. Most importantly, the molecular nature of the radicals remains unchanged, but the reactivity of both radicals is altered by their separation, since the singlet or triplet character depends on distance. This alteration arises from the noncovalent spin interactions between the radicals when they are in proximity to each other. We can see from this analysis that if the magnitude of the electron exchange interaction between two radicals can be measured, then this measurement would provide one of the most subtle empirical criteria for defining the supramolecularity of a radical pair. Indeed, TREPR spectroscopy is one of the few methods that allows the measurement of J -values for ^3RP (^1RP is EPR “silent”) and consequently allows for the experimental examination of the supramolecular features of this reactive intermediate.

As mentioned above, many supramolecular structures can be described as “guest@host” systems. Of interest to this article are those supramolecular systems for which the host may be described as a liquid supramolecular “cage” for a guest. In particular, we are concerned with systems for which ^3RP is the guest. Such a system is described as $^3\text{RP@cage}$, which implies that we are concerned with situations involving a spin-correlated radical pair in a liquid cage. We now make a brief excursion to discuss the concept of liquid cages because the concepts are analogous to those describing supramolecular cages.

3.1.1. The Cage Effect—From the Gas Phase to the Molecular Solvent Cage to the Supramolecular Cage

In a seminal paper Franck and Rabinowitch coined the term “cage effect”, to make a distinction between features of a photochemical cleavage reaction in the gas phase and those in liquids.^[35] The absorption of a photon was considered to excite a molecule to a “dissociative” energy surface (Figure 6). The dissociative surface was considered to be repulsive so that some critical bond of the molecule begins to stretch and then break to produce two radicals (indicated by arrows in Figure 6, top). In the gas phase there are no forces to constrain the radical fragments (no solvent “walls” to contain

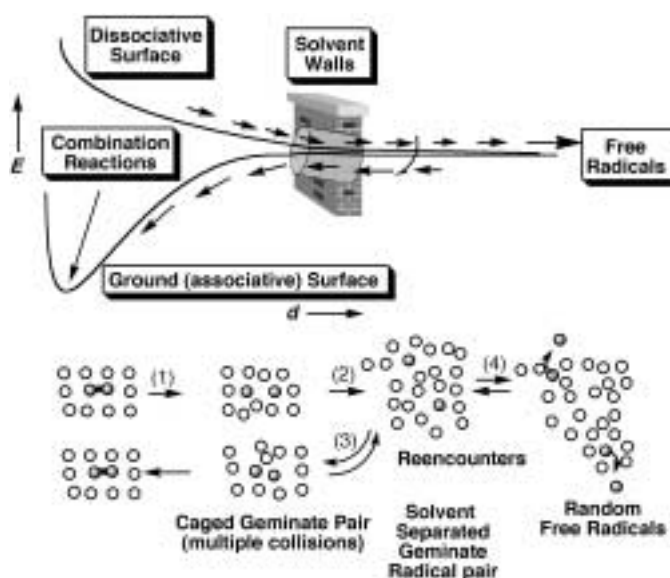


Figure 6. Energy surface and mechanical description of the cage effect. The lower energy curve represents an attractive, ground-state molecule. This molecule is photochemically excited to the dissociative state. As the distance d between the two radical centers increases, the relative energy decreases. At some point, the ^3RP may cross over to the ground state (^1RP) surface and ultimately recombine. The solvent walls may prevent complete separation and encourage multiple collisions. If the radicals diffuse beyond the initial solvent cage, $^2\text{FR}+^2\text{FR}$ are formed. See text for further discussion.

the radicals), therefore the radical pair dissociates efficiently into two free radicals. However, when a pair of reactive radicals are formed by such a bond cleavage in an inert liquid, the solvent molecules serve as a “cage” around the geminate (“born together”) radical pair (Figure 6, bottom). The authors suggested that the probability of escape of a reactive pair of fragments produced by photodissociation is lower in solution compared to the gas phase because of the action of the solvent cage about the pair. The solvent is expected to remove energy and serve as a hindrance or “solvent wall” to separation. Both features will enhance the probability of the recombination. The enhancement in the probability of recombination of a pair of radicals produced by dissociation in a liquid compared to in the gas phase has been termed the “cage” effect produced by solvent molecules in a liquid. However, the solvent walls in a liquid are not impermeable to the radicals, but rather are more or less “porous”. The “cage effect”, or the probability of recombination of the geminate pairs, will depend on the porosity of the solvent walls.

In a second seminal paper on the cage effect, Rabinowitch and Wood simulated the cage effect in liquids by measuring the behavior of a mechanical container consisting of a series of agitated balls and a control button which was constantly being struck by the balls. Only certain balls were made of conducting material and “collisional hits” were observed from these balls.^[36] The “collisions” of molecules in a solvent were “simulated” by this mechanical device. From this device the authors concluded that collisions between molecules that are nearest neighbors in a solvent cage occur in sets.

In a third seminal paper on the cage effect, Noyes pointed out that purely from random walk statistics, the very fact that

a pair of radicals is “born” together in a solvent cage provides them with a certain probability of geminate reaction even after the geminate pair has left the primary solvent cage.^[37] Noyes suggested that random diffusive displacement of the pair in the primary cage will be followed by a “reencounter” of the geminate pair in a secondary solvent cage. At this point, there is a certain probability of reaction between the secondary geminate pairs. The Noyes model suggests that the definition of a solvent cage is a region of space in a homogeneous liquid for which the probability of recombination reactions of a geminate radical pair is much higher than the probability of reaction with radicals of pairs generated from other dissociations or with scavengers.

The ideas of these seminal papers are summarized schematically at the bottom of Figure 6. The cleavage of a bond leads to the geminate radical pair in the solvent cage (step 1). The geminate pair undergoes multiple collisions before one or more solvent molecules separates the pair (step 2). The separated pair then either undergoes a reencounter to reform a caged pair (step 3, rare in homogeneous nonviscous liquids) or continues to separate to become free radicals (step 4) that undergo combination reactions with other free radicals generated from separate dissociations. The cage effect is also described, and it is of interest to compare it to the “dynamic radical pair” described in Scheme 2.

3.1.2. The Role of Spin on the Cage Effect—The Molecular Versus the Supramolecular Cage

We now reconsider the idea of the cage effect in terms of a modern paradigm of supramolecular photochemistry which includes the spin of the radicals produced by a photochemical dissociation. We shall show how TREPR spectroscopy can assist in investigating the properties of a triplet geminate radical pair existing in a molecular or a supramolecular cage. Figure 7 expands on the classical ideas of the solvent cage shown in Figure 6 and attempts to show the interrelationships

of electronic energy surfaces, spin interconversions, and the supramolecular cage effect.

In Figure 7 the stereochemistry of the electron spin structure is represented in terms of a conventional vector notation (compare with Scheme 4, bottom). In this notation the two spin vectors are strongly coupled (strong electron exchange interaction, large J -value) when the radical pair is in a collision complex, and the spin vectors are weakly coupled (small J -value) when the radical pair is separated by one or more solvent molecules. When the pair is separated by several solvent molecules, $J = 0$ and the probability of reencounter of the geminate pair in a nonviscous homogeneous solution is also equal to 0, that is, the pair is no longer a geminate pair possessing spin correlation, but a spin uncorrelated pair of free radicals.

When the geminate pair is in a collision complex (termed a contact pair), the exchange interaction is so large that the configuration of the spins is retained during collisions. This result implies that ISC is unlikely to occur in a collision complex because the exchange force for maintaining spin stereochemistry is much more powerful than any available magnetic forces capable of reorienting the spin stereochemistry. However, when the pair has separated in space to several angstroms, the exchange interaction decreases to a value that is small compared to the weak, but available, magnetic interactions, such that the stereochemistry of the spins $^3T_0$ and $^1S_0$ can be “mixed”. Thus, for a recombination reaction to occur starting from a primary triplet geminate pair, the inert triplet geminate pair must separate from a collision complex, singlet character must be introduced, and the pair must reencounter to form a reactive collision complex with singlet character.

In ordinary nonviscous organic solvents the probability of a geminate pair reencountering is statistically very small. Most of the geminate pairs never reencounter and free radicals are formed (compare with the molecular photochemistry paradigm in Scheme 2). A supramolecular liquid environment may be considered as one for which reencounters are enhanced by the “reflecting” walls of a supramolecular boundary (shown schematically as a porous physical wall in Figure 6 and as dashed lines in Figure 7). In the same way that the walls of a solvent cage “reflect” a primary geminate pair into multiple collisions before exiting from the solvent cage, the walls of a supramolecular cage “reflect” a geminate pair into multiple reencounters before allowing exit from the supramolecular cage. However, the important point is that, compared to homogeneous solutions, the supramolecular wall can be much more effective in reflecting radicals back into reencounters and collision complexes. The size of the supercage as well as the chemical characteristics of the solvent walls and of the guest radicals will all be factors in determining the cage effect and radical–radical interactions. Furthermore, the size of the supramolecular cage can be varied, which implies that the distance at which the radicals can separate can also be varied experimentally. Since J depends on the separation of the radicals, J will vary with the size of the supramolecular cage. In Section 3.4.1 we will consider micelles as models of supramolecular cages for radical pairs. However, first we need to consider the influence of J and the supramolecular

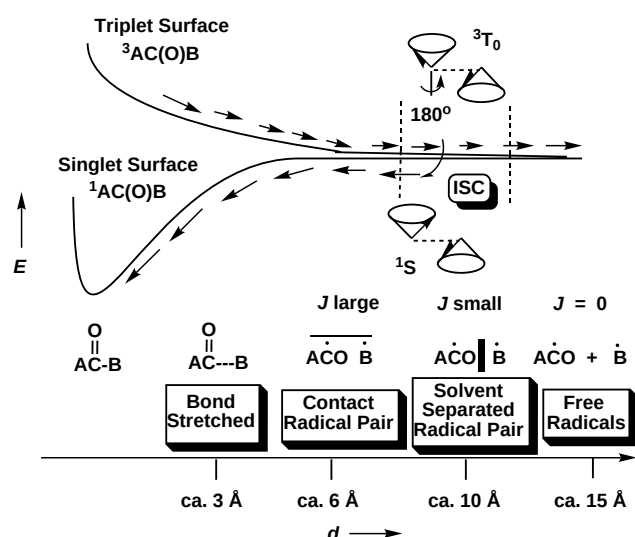


Figure 7. Energy surface description of the cage effect with supramolecular aspects and spin aspects included. The strong electron exchange interaction occurs when the radicals are in close proximity. See text for further discussion.

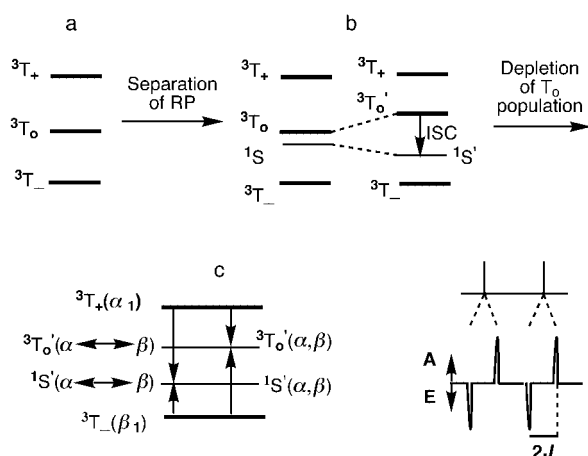
interactions of a radical pair in a supercage on the ESP-TREPR spectrum.

3.2. TREPR of Spin-Correlated Radical Pairs—Investigation of the Intermolecular Exchange Interaction

In the paradigm of Scheme 2 the entity ^3RP , a spin-correlated triplet geminate radical pair, was considered as a paramagnetic entity and a reactive intermediate that was required to be produced by spin-selection rules as the result of a primary photochemical process from a triplet excited state. We may now ask if TREPR spectroscopy can provide experimental evidence for the existence of such an entity. The lifetime of ^3RP (the lifetime of a radical pair in a solvent cage) in homogeneous solutions is less than a nanosecond, which is shorter than the time resolution of the TREPR method. However, the lifetime of ^3RP in a supramolecular cage (for example, a micelle) can be manipulated systematically, for example, by changing the size or nature of the supercage. In this manner, the residence time of the pair in a supramolecular cage can be varied from nanoseconds to milliseconds. The direct investigation ^3RP within the time resolution of the TREPR technique thus becomes possible by incorporating the pair in a supramolecular cage. From these considerations, we conclude that it should be possible to employ the TREPR technique to investigate geminate radical pairs if the pairs are spin polarized. We now present an analysis of a polarization mechanism that is characteristic for spin-correlated radical pairs, and which allows a TREPR characterization of these species and so definitively allow them to be differentiated from polarized free radicals.^[38, 39]

3.3. Magnetic Level Diagram for a Spin-Correlated Radical Pair

Scheme 11 shows a qualitative, schematic description of the magnetic energy diagram for a spin-correlated radical pair (in a solvent cage or a supramolecular cage). We shall use a concrete model as an example of how the spectrum of ^3RP



Scheme 11. Schematic energy level diagram of a spin-correlated radical pair and the mechanism of ESP of such a pair. See text for discussion.

becomes polarized in a very interesting manner and how the measurement of a ESP-TREPR spectrum provides information on the average value of the exchange interaction between the radicals. In the model it is assumed that the $^3T_1 \rightarrow ^3\text{RP}$ process (step 3 of Scheme 6) leads to equal population (indicated by identical thickness of the levels in Scheme 11 a) of all three triplet sublevels, $^3T_+$, $^3T_0$, and $^3T_-$. Further, this model implies that the TM is absent. Again using the exemplar of α -cleavage, the radicals are in close contact immediately after cleavage so that the value of J is high. Under these conditions the $^3T_0$ state is split in energy from the other two triplet levels and ^1S is split very far from any of the T levels (the S state is not shown in Scheme 11 a because of the large energy separation from the T levels under these conditions).

However, as the radical pair separates in the cage or supercage, the value of J decreases (Figure 7). We consider specifically the situations for which J is of the order of the energy gap between the triplet sublevels, that is, the energy of ^1S is lower than that of $^3T_0$ but higher than that of $^3T_-$ (Scheme 11 b). Because of their proximity, the $^3T_0$ - ^1S states begin to mix, and as a result the $^3T_0$ level will be selectively depleted as ISC occurs and ^1RP are formed and removed rapidly by reaction (Scheme 11 b). However, $^3T_+$ and $^3T_-$ do not mix efficiently with ^1S because they are energetically separated in energy. The net effect is that $^3T_+$ and $^3T_-$ become overpopulated (spin polarized) relative to $^3T_0$ (shown schematically in Scheme 11 c as the thinner lines for the energy levels for the $^3T_0$ and ^1S state). Thus, if an EPR spectrum of the radical pair is taken before spin relaxation, an emissive spectrum will occur from transitions from $^3T_+$ (Scheme 11 c, α_1 transitions) and an enhanced absorption spectrum (Scheme 11 c, β_1 transitions) will occur as the result of transitions from $^3T_-$.

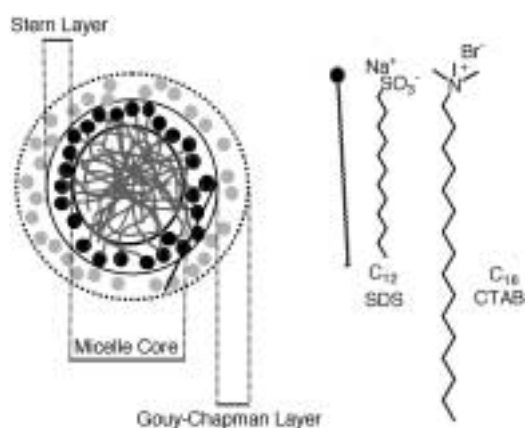
Since mixing between $^3T_0$ and ^1S occurs, each state is no longer pure singlet or pure triplet and must be classified as "mixed" states $^3T_0'$ and $^1S'$. As a result, transitions occur from $^3T_+$ and $^3T_-$ to both $^3T_0'$ and $^1S'$ (Scheme 11 c). The net result is two emissive transitions [$^3T_+(\alpha_1) \rightarrow ^3T_0'(\alpha, \beta)$ and $^3T_+(\alpha_1) \rightarrow ^1S'(\alpha, \beta)$] and two absorptive transitions [$^3T_-(\beta_1) \rightarrow ^3T_0'(\alpha, \beta)$ and $^3T_-(\beta_1) \rightarrow ^1S'(\alpha, \beta)$]. From this simplified model the experimental ESP-TREPR spectrum will appear as shown in Scheme 11 c. Note the remarkable and very characteristic feature predicted by the analysis: each hyperfine component of the polarized spectrum, which normally would be a single line, is observed as an E/A doublet split by $2J$. The analysis suggests that if an ESP-TREPR spectrum can be observed the value of the supramolecular interactions, as estimated by J , can be extracted directly from the separation of the E/A doublets in the spectrum. It is worthwhile noting that this analysis holds true in cases where the linewidth of the peak is smaller than the splitting (that is, when the EPR lines are sharp compared to a broad splitting).

For the purposes of this account the main conclusions of the analysis of the energy diagram are the expectation that ^3RP in contrast to ^2FR will possess hyperfine components that are E/A and that the energy difference corresponding to the separation of the E/A lines will be related to the exchange

interaction. We now describe the properties of micelles that make them excellent models for supramolecular cages for radical pairs and present some experimental examples of ESP-TREPR spectra of ^3RP produced in micelles by photochemical α -cleavage of ketones.

3.4. A Structural Model of Micelles—Micelles as Supramolecular Cages

Micelles are self-assemblies that have lifetimes in the vicinity of milliseconds and monomer exchange in the microsecond time domain. Micelles are commonly formed in aqueous solutions from surfactants that have a hydrocarbon chain and a hydrophilic headgroup.^[40] The headgroup can be cationic, anionic or non-ionic (Scheme 12). The surfactants aggregate as a result of the hydrophobic effect in such a way



Scheme 12. Left: Schematic representation of a micelle. The black circle represents the ionic portion of the headgroup (for example, SO_3^-), while the long tail depicts the hydrophobic, alkyl chain. Right: Sodium dodecyl sulfate (SDS, C_{12}) and cetyltrimethylammonium bromide (CTAB, C_{16}) each have about 60 monomer units per micelle and are typical anionic and cationic micelle-forming surfactants, respectively.

that the hydrocarbon portions of the surfactant structure avoid water by forming a hydrophobic core consisting of entangled hydrocarbon chains. While the core of the micelle is “oily” and will solubilize organic guests, a certain amount of water penetration is expected.^[41] This is an important consideration, because if one is trying to characterize a geminate pair in a micelle by TREPR spectroscopy, observation of the radical species may be occurring on a timescale where monomer exchange, or even complete dissolution of the self-assembly has occurred. ESP-TREPR spectra can be monitored on a nanosecond to microsecond timescale and thus provides an excellent method to observe the dynamics of the radical species within the micelle. On average, micelles are nonspherical and their shape can be modified with a change in the ionic strength, temperature, and pH value. These systemic properties can be changed with ease, so it is quite possible to investigate the transfer of electron spin polarization under a variety of micellar conditions and hence different levels of environmental constraint.

The supramolecular aspects of micelles enable us to divide the host into several potential binding sites (see Scheme 12).

The Gouy–Chapman layer is the region in which the anions reside in a solution of sodium dodecyl sulfate (SDS). In this region, it is feasible for a cationic guest to bind to the anionic headgroup. A second binding site may be envisioned in the region between the headgroups (Stern layer), which is fairly polar, and is accessible to the bulk water. Yet another binding site can be the hydrophobic “core” inside the micelles, where one expects guest hydrocarbons and aromatic compounds to be located as a result of the hydrophobic effect. The constraints and diffusional dynamics imposed on molecules in these various micellar regions is one of particular importance in supramolecular chemistry, and to ESP. For example, guest radical pairs will have decreased ability to form free radicals as a consequence of an increased propensity for recombination because of the presence of the supercage. We will now show that the combination of electron polarization and the TREPR technique can be exploited to investigate weak intermolecular interactions, such as the electron exchange responsible for ESPT, between radicals in supramolecular systems.

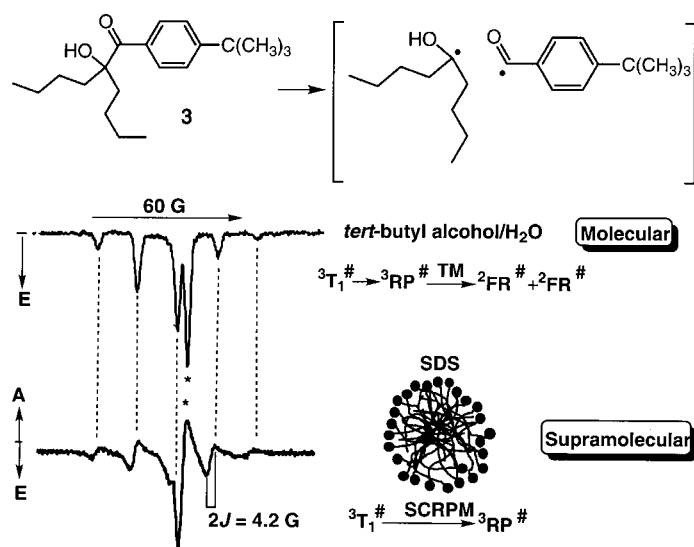
According to the paradigm, a primary photochemical reaction such as α -cleavage of a triplet ketone $^3\text{AC}(\text{O})\text{B}$ (Scheme 6), produces a geminate spin correlated radical pair $^3(\text{ACO}\cdot\cdot\text{B})$ inside the solvent cage. The solvent cage has a lifetime of the order of picoseconds for common nonviscous solvents. This lifetime is far too short to be measured directly by the TREPR technique. However, incorporation within a micelle enhances the lifetime of the geminate pair within the micellar supercage by several orders of magnitude.^[29, 42–44] The radical pair as a guest and the “supercage” as a host ($^3\text{RP@supercage}$) form a long-lived supramolecular structure capable of direct detection by the TREPR technique. We can now move to a discussion of the use of ESP-TREPR studies to characterize a micellar supramolecular assembly.

3.4.1. TREPR of ^3RP in Micelles—Models of Supramolecular Systems

We now examine the TREPR spectra of ^3RP produced by photochemical α -cleavage of ketones in micelles. The structural and dynamic properties of a supramolecular structure will depend on the characteristics of both the guest (^3RP) and host (micelle). Thus, the characteristics of both the micelle structure and the radical pair are expected to determine the features of the TREPR spectra. For example, increased micelle hydrophobicity, or increased micelle size will induce a longer residence time for the RP in the micelle. Low hydrophobicity or small size results in faster exit from the micelle. Thus, the ESP-TREPR spectrum of a ^3RP in a small micelle will be similar to that for α -cleavage in homogeneous solution, while a longer lifetime in the micelle will consequently lead to more interaction between the spins and in particular, differences in the exchange interaction will be apparent. In exceptionally large micelles, one could obtain ESP-TREPR spectra similar to those obtained in homogeneous solution because the radicals will be able to separate to a distance where the spins no longer interact. We now consider some experimental examples of the use of TREPR

spectroscopy to examine spin-correlated radical pairs produced in micelles directly.

Scheme 13 shows the TREPR spectrum produced by the photolysis of ketone **3** in an aqueous solution containing *tert*-butanol (top) and in an aqueous solution containing SDS micelles (bottom).^[42] The spectrum in homogeneous solution



Scheme 13. TREPR spectra obtained 450–500 ns after laser flash photolysis of 1.5 mM ketone **3** at 266 nm in a water/*tert*-butyl alcohol (1/1 w/w) mixture and in an aqueous solution of SDS (micelle concentration 1 mM). The signals from the benzoyl radical are marked with an asterisk. The simulation of the supramolecular radical pair is shown in Scheme 15.

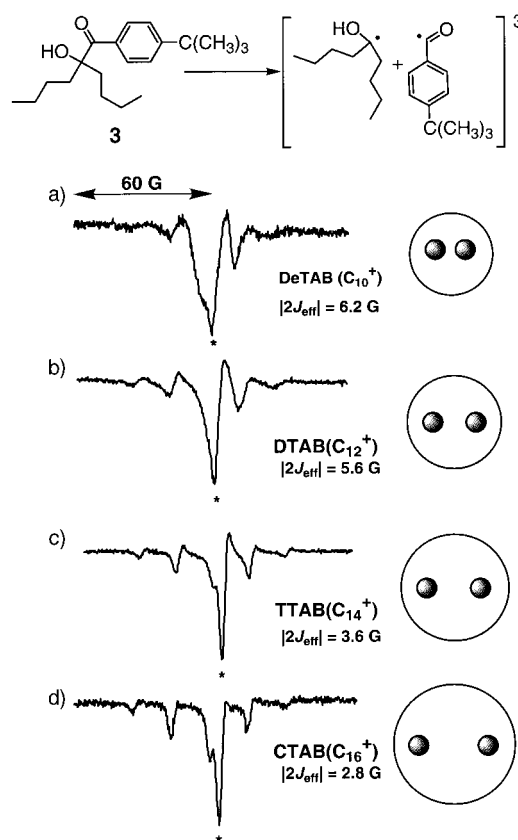
(Scheme 13, top) has the appearance of a pair of polarized free radicals whose ESP is produced by the triplet mechanism (compare to Figure 2a, Section 2.4.1), but in micellar solution, for time scales up to and beyond a microsecond, the spectrum has a very different appearance (Scheme 13, bottom). Each emissive hyperfine line peak in the homogeneous solution spectrum (Scheme 13, top) is matched with a peak that is “split” into E-part at low field and an A-part at high field in the micellar spectrum. This E/A splitting of the hyperfine lines is exactly the spectrum expected for a spin-correlated radical pair ^3RP (see Section 3.3 and Scheme 11). Accordingly, in a supramolecular system $^3\text{RP@micelle}$, TREPR spectroscopy provides direct detection and allows characterization of a spin-correlated radical pair ^3RP , which is an important intermediate expected from the paradigm.

According to the paradigm the splittings of the hyperfine lines are the result of electron spin–electron spin interactions, so that the separation of these splittings are directly related to the electron exchange interaction J between the partners of the pair in these systems. Thus, from TREPR experiments, one can directly measure the level of intermolecular interactions between two unpaired electrons through measurement of the electron exchange interaction. We will now show that this interaction is a function of supramolecular structure and dynamics of the guest@host system.

3.4.2. TREPR and the Supramolecular Structure of Radical Pairs in Micelles

Let us now consider how TREPR spectroscopy can provide articulation of the paradigm of supramolecular structure of model guest@host systems through a systematic analysis of spin-correlated radical pairs (SCRPs) in micelles. The strength of the “bonding” intermolecular interactions responsible for stabilization of the SCRPs is expected to depend on both the structure of the components of the pair and on the structure of the micelle. For example, the average proximity of the partners will depend on the micellar size, the hydrophobicity of the core, as well as the size and the hydrophobicity of the partners of the pair. In molecular chemistry, this is akin to the covalent bonding of atoms in a molecule that characteristics of which depend on the component atoms. The magnitude of the electron exchange interaction J depends on the degree of orbital overlap between the two radical centers and consequently the proximity of the partners of the pair. Since the value of J can be extracted from the TREPR spectrum, the technique offers an excellent opportunity to investigate some of the more subtle structural features of a supramolecular system such as SCRPs.

As examples of supramolecular structural effects on the radical pair, Scheme 14 shows the TREPR spectra resulting from a systematic investigation of the photolysis of ketone **3** in a series of micelles formed from a series of cationic micelles of alkyltrimethylammonium bromides (C_{10} to C_{16}).^[42] The shape



Scheme 14. TREPR spectra obtained during the photolysis of **3** in a series of micellar solutions of alkyltrimethylammonium bromides. The line marked with the asterisk is attributed to the benzoyl radical. For further information see the text.

of the TREPR spectra and the values of J extracted from the spectra show a clear dependence on the alkyl chain length: J drops from 6.2 G for the smallest micelle (C_{10}) to 2.8 G for the largest micelle (C_{16}). In fact, the spectrum for the longest chain (Scheme 14d) is almost purely emissive, with only a small SCRP component. It is worth noting that the lack of the observation of the SCRP mechanism in the case of the largest micelles might be a result of the short lifetime of the radical pair. These same trends were seen using the same hydrophobic precursor of the radical pair placed in anionic micelles formed from a series of alkyl sulfates of different chain length.

The above results demonstrate how ESP-TREPR spectroscopy, through extraction of J -values, can reveal how the size of the micellar host can control the average proximity of the partners of the guest radical pair. Since a radical pair in a micelle is a supramolecular structure, we expect the guest structure will also influence the proximity of the partner and the value of J .

Scheme 15 shows the TREPR spectra of a series of radical pairs **4a–d** of differing structures produced by α -cleavage of related ketones in SDS micelles. The electronic and magnetic properties of each of the pairs are very similar. However a major difference in the characteristics of the pairs in SDS micelles is their supramolecular structure. Assuming that hydrophobicity will be a dominant feature in determining the supramolecular stability of the $^3\text{RP@micelle}$ structure, the lifetime of the fragments of the pair is expected to correlate with the number of hydrocarbon fragments in the pair. In the

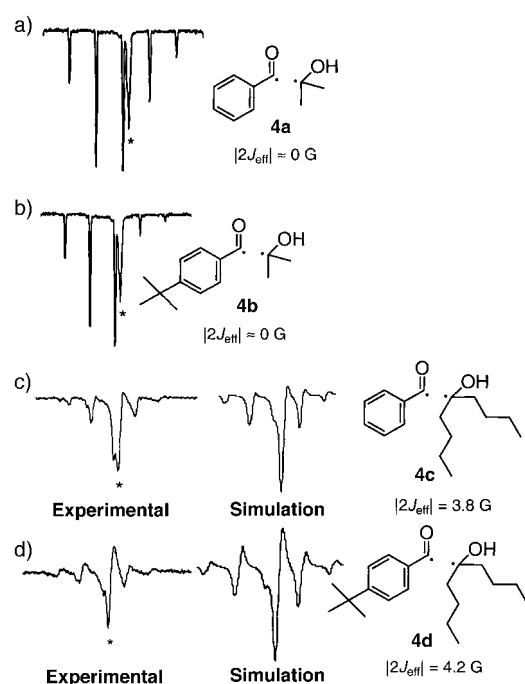
case of pairs **4a** and **4b** the ESP-TREPR spectrum in SDS micelles shows essentially no SCRP component ($J \approx 0$ G) and the spectrum appears analogous to that observed in homogeneous solution (compare to Scheme 13a). However, the TREPR spectra for the pairs **4c** and **4d** show significant SCRP contribution and the values of J extracted from the spectra are 3.8 and 4.2 G, respectively.

It is expected that the hydrophobic character of a radical pair will be important in determining the supramolecular structure and dynamics of the pair in a given micelle. For the radical pairs **4a** and **4b**, both of which consist of one small hydrophilic C_3 ketyl fragment, the rate of escape from the micelle of this small fragment is evidently faster than the time resolution of the TREPR measurement. The ESP-TREPR spectrum observed is essentially the same as that for homogeneous solution (Scheme 15a, b) and the system does not appear to have supramolecular spin properties. However, for radical pairs **4c** and **4d**, which contain two relatively hydrophobic fragments, the residence time of the pair in the micelle is within the time resolution of the TREPR experiment and the ESP-TREPR of SCRP's are observed (Scheme 15c, d). Thus, the supramolecular characteristics of the $^3\text{RP@micelle}$ are directly observed. One can observe directly from the experimental results that exit of one of the radical partners from the micelle destroys the "supramolecularity" of the system. In general, the function of the parts is the same as the function of the whole system when only one ^2FR is located within the micelle. However, the presence of an exchange interaction when two ^2FR are located in the hydrophobic core ensures that the overall reactivity of the system changes. Thus, this experiment clearly demonstrates that spin chemistry, electron interaction, and TREPR spectroscopy can determine the "extent of supramolecularity" of a given system with spin.

The variation in the supramolecular structure of a radical pair in a micelle as a function of supramolecular hydrophobicity is analogous to the "molecular system" of a biradical whose radical centers are separated by flexible covalent linkages. In this case shorter chains result in a greater degree of orbital overlap between the radical centers. In both situations, the exchange interaction is increased by the closer proximity of the radical species. Indeed, the ESP-TREPR spectra of biradicals display the expected dependence of J on the chain length.^[11]

4. Other Applications of the TREPR Technique and ESP

A goal of this account is to inform the interested reader that TREPR spectroscopy is a powerful technique for the investigation of paramagnetic species (triplets, radical pairs, free radicals) produced in many photochemical reactions. For the sake of clarity the presentation concentrated on a single exemplar paradigm, the α -cleavage of carbonyl compounds. However, the TREPR technique has been employed to investigate all of the important primary photochemical processes of ketones^[5] including those producing biradicals.^[2, 11, 38, 39] In addition to the photoreactions of ketones,



Scheme 15. ESP-TREPR spectra of radical pairs **4a–d** in a micellar SDS solution recorded about 500 ns after laser flash excitation. For radical pairs **4a** and **4b** the experimental spectra are shown. Both the experimental and simulated spectra are shown for **4c** and **4d**. The lines attributed to the benzoyl radical are marked with asterisks. The remainder of the spectrum is attributed to the ketyl radical partner of the pair. The experimental spectra can be well simulated and the value of $2J$ is extracted from this process. Note that the value of the electron exchange interaction increases as the relative hydrophobicity (as measured by the number of hydrocarbon fragments) of the pair increases.

many other organic substrates^[1–6, 28, 45, 46] have also been investigated by this technique. The TREPR technique and ESP have also been powerful tools for the investigation of problems particularly of interest in the area of photosynthesis, for example, to provide direct evidence for a specific electron acceptor in photosystem I,^[47] and elucidation of donor–acceptor pairs in the bacterial photosynthetic reaction center.^[48, 53, 54] A recent study in photosynthetic models has revealed structural information using a zinc porphyrin with a *para* arrangement of *N,N,N',N'*-tetraalkyl-*para*-phenylenediamine and naphthoquinone units.^[49] Other studies dealing with the photosynthetic reaction center demonstrate that additional kinetics and structural information can be obtained by operating the EPR spectrometer at various frequencies.^[50, 51] The energy and electron-transfer processes of the fractions of the photosynthetic reaction center have been used in the form of liquid crystals.^[52] Other groups have used these spin techniques to understand the interaction between electrons at close ranges in processes involving polarized electronic spin within supramolecular systems, such as micelles^[55] and cyclodextrins.^[56] These are just a few of the examples that demonstrate how important and vital the ESP-TREPR technique and other techniques based on TREPR are in helping to unravel the mystery of how electrons interact with each other and how solar energy gets converted into chemical energy.

5. Summar

This account has attempted to bring together, in a qualitative and conceptual manner, the power of employing TREPR to exploit electron spin polarization (ESP) that is created, transferred, and sorted in many photochemical processes. The ESP that is created serves as a mechanistic tool for the investigation of the paramagnetic species involved in the exemplar paradigm for the α -cleavage reactions of carbonyl compounds and can be examined by TREPR spectroscopy. The exchange interaction is involved in the transfer of spin polarization in nonreactive collisions between triplet radical pairs, in the generation of polarization by the RPM, as well as in the splitting of energy levels leading to polarization and the characteristic transitions of spin-correlated radical pairs. For this reason, geminate triplet radical pairs are excellent models for the investigation of supramolecular noncovalent interactions. The observation of the ESP-TREPR spectrum of triplet radical pairs in supercages such as micelles allows the direct investigation of supramolecular RP@micelle systems in detail through the extraction of *J*-values from the E/A splittings of the hyperfine lines in the TREPR spectra. In fact, it has been shown that the TREPR experiment can directly interrogate spin chemistry and electron interactions. Consequently, the TREPR technique can also determine the “extent of supramolecularity” for a system with spin.

Abbreviations

#	polarized, unpaired electron spin
$\vec{B}_0$	magnetic field

D	doublet electronic state (also free radical, ² FR)
EPR	electron paramagnetic resonance
ESP	electron spin polarization
ESPT	electron spin polarization transfer
² FR	free radical
g_e	<i>g</i> -factor (2.0023 for free electron, g_e)
hfc	hyperfine coupling
HOMO	highest occupied (ground state) molecular orbital
I	reactive intermediate
ISC	intersystem crossing
<i>J</i>	electron exchange interaction
k_B	Boltzmann constant
LUMO	lowest unoccupied (ground state) orbital
μ	magnetic moment
P	product
R	reactant
*R	excited state of reactant
RP	radical pair
¹ RP	singlet radical pair
³ RP	triplet radical pair
³ RP@cage	Host–guest complex involving a ³ RP and a cage host system
RPM	radical pair mechanism
<i>S</i>	spin of an electron
¹ S ₀	ground state singlet
¹ S ₁	excited singlet state
SCRIP	spin correlated radical pair
SOMO	singly occupied orbital
SOC	spin–orbit coupling
³ T ₁	excited triplet state
TM	triplet mechanism
TREPR	time-resolved electron paramagnetic resonance

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How Capillary Electrophoresis Sequenced the Human Genome**

Norman J. Dovichi* and Jianzhong Zhang

1. Introduction to DNA Sequencing

Now that the sequence of the human genome is essentially complete, it is appropriate to review how we have come to this point in the history of science. In 1953, Watson and Crick reported the structure of DNA, which confirmed the central role of DNA for storage of genetic information.^[1] A few years later, the genetic foundation that describes the translation of DNA to proteins was fully known.^[2]

The relationship between the genetic and protein sequences was a powerful motivation for the development of methods to determine DNA sequences. Not only would DNA sequences provide insight into mutation and evolution, but they could also provide a shortcut to the determination of protein sequences. Until 1976, the determination of DNA sequences involved laborious, classical chemical methods. Sanger et al.'s "plus and minus" method was the first step in sequencing chemistry, which allowed the determination of the 5375 base sequence of the bacteriophage Φ X174 in 1977.^[3] Two much more elegant sequencing methods were reported in 1977, the dideoxy chain-terminating method of Sanger and co-workers (Sanger's method) and Maxim and Gilbert's chemical degradation method.^[4, 5] Sanger and Gilbert shared the 1980 Nobel Prize in Chemistry for their development of analytical methods for DNA sequencing.

Biologists pursued DNA sequencing with great enthusiasm, and a cottage industry developed for the manual determination of the sequences of viruses, genes, and genetic markers. GenBank was started in 1981 as a public depository for DNA sequence information.^[6, 7] Figure 1 presents the number of bases recorded in GenBank since its inception, along with several important milestones in sequencing history. From 1984

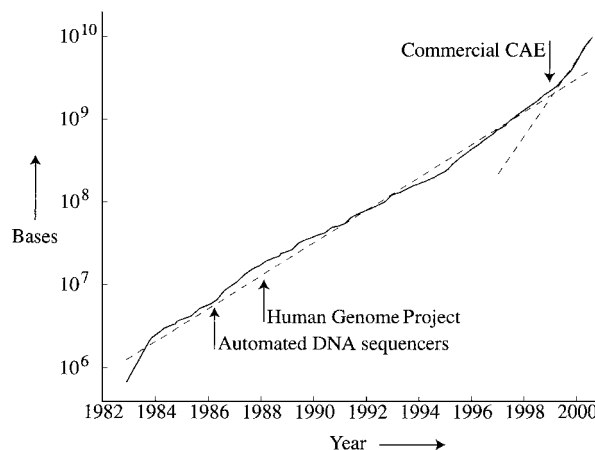


Figure 1. Number of bases of DNA sequences deposited in GenBank. The dashed lines are least-square fits to the logarithm of the linear data; the data increased exponentially with a two-year doubling rate until 1999. Since then, GenBank has increased in size with a six-month doubling rate.

to 1999, the rate at which data were deposited into GenBank increased exponentially, with a doubling period of roughly two years.

Instruments for automated capillary-array electrophoresis (CAE) became available in 1999, which precipitated an explosive increase in the rate at which DNA sequences were reported in GenBank, reaching a doubling rate of six months during the year 2000. These instruments were used in large factories with robotic sample handling and huge bioinformatics capabilities. The industrialization of DNA sequencing has had a breathtaking impact on biology. More DNA sequences were deposited in GenBank over the past two weeks than reported during the whole of 1995. When the GenBank data are combined with sequences held in commercial databases, it is likely that the amount of DNA sequences known at the end of 2000 will be ten times larger than that known at the start of the year.

The development of capillary-array DNA sequencers and their installation in sequencing factories has changed biology forever. In this Essay, we trace the history of the sequencer that has made this revolution possible.

2. Sanger's DNA Sequencing

Most researchers used Maxim and Gilbert's sequencing method during the early 1980s, primarily because reagents

[*] Prof. N. J. Dovichi,^[+] Dr. J. Zhang
Department of Chemistry
University of Alberta
Edmonton, Alberta T6G 2G2 (Canada)
Fax: (+1) 780-492-8231
E-mail: norm.dovichi@ualberta.ca

[+] After January 1, 2001:
Department of Chemistry
University of Washington
Seattle, WA 98195-1700 (USA)
Fax: (+1) 206-685-8665
E-mail: dovichi@chem.washington.edu

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were not widely available for Sanger's method. However, the commercialization of dideoxynucleotides and the development of automated DNA synthesizers made Sanger's sequencing method practical and virtually all DNA sequencing relies on this method today.

In the vocabulary of DNA sequencing, the piece of DNA with the unknown sequence is called the template. To sequence a single-stranded template using Sanger's method, an approximately 20-base primer is synthesized which is complementary to a specific region of the template. The primer is mixed with the template, where it hybridizes forming a short region of double-stranded DNA that is recognized as an initiation site for chain extension by a DNA polymerase. This enzyme extends the primer sequence, adding new nucleotide triphosphates to the 3' end of the primer and synthesizing a DNA fragment that is complementary to the template.

The key to Sanger's sequencing reaction is the addition of a small amount of dideoxyadenosine triphosphate to the reaction mixture. This reagent has a triphosphate group and it can be incorporated into the growing strand. However, the reagent is missing a hydroxyl group at the 3' position of the sugar; no nucleotides can be added to the dideoxynucleotide and the growing strand is terminated upon its incorporation. On completion of the reaction, a population of products is generated. All products start at the primer, are complementary to the template sequence, and terminate upon incorporation of a dideoxyadenosine (ddA), but they are of different lengths depending upon the position where the ddA was incorporated. These reaction products are called a sequencing ladder. Similar sequencing ladders are generated from the template with dideoxycytidine, dideoxyguanosine, and dideoxythymidine. This synthesis remains essentially unchanged from Sanger's original work, although improved enzymes are used today.

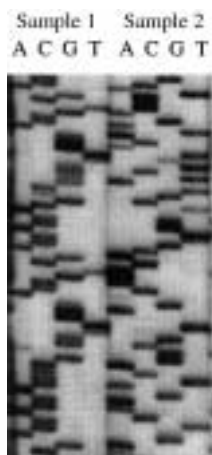


Figure 2. Autoradiogram generated from a DNA sequencing gel. Two samples were subjected to the Sanger sequencing reaction. Each sample generated four sequencing ladders that were separated on adjacent lanes of the polyacrylamide gel slab.

In Sanger's original method, the sequencing ladders were separated, based on their size, in four adjacent lanes of a polyacrylamide gel. Radioactive phosphorus was used in the reaction mixtures so the end base of the sequence was radiolabeled. The fragments were detected by autoradiography, where film was placed over the gel and the radioactive decay of the labeled DNA created dark bands on the film (Figure 2). These autoradiograms were inspected by a skilled technician who would determine the DNA sequence based on the pattern of bands and manually enter the data into a computer. Roughly 12 hours were required for the electrophoresis, another 12 hours for the autoradiography, and many hours to read the sequence, which was an error-prone and mind-numbing exercise.

Figure 2 shows the image from a small portion of a sequencing gel. Two samples are analyzed in adjacent lanes in this figure; the original gel analyzed 12 samples. Roughly 50 bases worth of sequence are shown in the figure; the entire gel would generate a few hundred bases of sequence for each sample. The smallest fragments migrated fastest and appear at the bottom of the gel. The sequence of sample 1 begins with CGACCACCACCCCGAGGTT.

2.1. Automated DNA Sequencing

In 1986, Smith, Hood, and co-workers reported an important improvement in Sanger's method whereby the radioactive labels, autoradiographic detection, and manual data interpretation were replaced with fluorescent labels, laser-induced fluorescence detection, and computer-based data analysis.^[8] In their method, a primer was synthesized, split into four batches, and each batch was labeled with one of four different fluorescent dyes. Each dye-labeled primer was used in a sequencing reaction with one of the dideoxynucleotides. These reaction products were pooled and analyzed in a single lane of a sequencing gel. A four-color laser-induced fluorescence detector monitored the DNA fragments as they migrated to the bottom of the gel; the fluorescence signature was used to identify the terminal nucleotide. This technology was commercialized by Applied Biosystems in 1987.

Figure 3 presents raw data generated by this sequencing protocol; the fluorescence from each DNA fragment generates a peak with a particular spectral signature. The spectrum from each dye is rather broad and generates a signal in several spectral channels. A simple matrix inversion procedure is used to convert the spectral data into dye-space data, and this dye information is then used to determine the identity of each sequencing fragment.

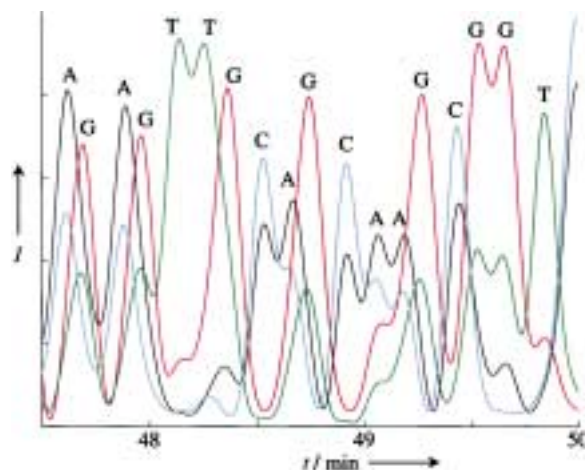


Figure 3. Four-color DNA sequencing data for bases 376–393 of M13mp18. Four different fluorescent labels were used to tag the primer, which was then used in the Sanger sequencing reaction. After the four sequencing ladders were synthesized, the reaction products were pooled and separated in a single electrophoresis capillary. A laser-induced fluorescence detector monitored the fluorescence signal in four spectral channels, which are plotted as the four-color traces. I = fluorescence.

Prober et al. at DuPont developed an automated sequencer that used more elegant chemistry based on a set of fluorescently labeled dideoxynucleotides;^[9] these labeled dideoxynucleotides were combined and used in a single sequencing reaction, which simplifies the synthesis of the DNA sequencing ladders. The fragments were separated in a single lane of sequencing gel and identified by the fluorescence signature of the dye-labeled dideoxynucleotide. DuPont commercialized this technology for a brief period but ultimately sold the license to Applied Biosystems.

Ansorge et al. developed a simpler system based on a single fluorescent label and separation of fragments in four lanes of an automated sequencer. This technology was commercialized by Pharmacia.^[10]

The introduction of these automated DNA sequencers was an important event in biotechnology. A group of visionary biologists realized that this new technology would allow the completion of very large DNA sequencing efforts. The Human Genome Project was initiated in 1988,^[11] two years after Smith, Hood et al.'s report of an automated DNA sequencer. The audacious goal of the genome project was to determine the primary structure of a set of molecules with a combined molecular weight of 2×10^{12} Da and to complete this task by 2005 with the commercial, automated sequencers. The National Institutes of Health of the United States of America has provided the lion's share of public funding for the Human Genome Project, which has grown into an international effort of global proportions. The Wellcome Foundation in Great Britain has been notable in their support of the Human Genome Project. Since 1999, Celera has become an important private-sector company active in very large-scale sequencing efforts.

3. High-Throughput DNA Sequencing

Conventional DNA separations are performed using glass plates that are coated with an approximately 200 μm thick layer of cross-linked polyacrylamide gel. The preparation of these gels is tedious and manually intensive. The plates are separated, scraped to clean off old polymer, and reassembled with appropriate spacers. An acrylamide monomer mixture is prepared and poured between the plates to polymerize; this mixture contains an aqueous buffer and 8 M urea. Once polymerization is complete, the slab of gel is placed in the electrophoresis apparatus, sample is pipetted into wells formed at the top of the gel, electrodes are attached, and the DNA sequencing fragments are separated. Between 16 and 96 samples are separated simultaneously on a single gel.

The thickness of the slab of gel limits the speed with which DNA can be separated. The poor heat dissipation of the thick gel limits the electric field used for DNA separation. At high fields, the temperature rise due to Joule heating causes the sequencing gel to distort and ultimately boil, which destroys the separation. The modest electric field used in conventional slab-gel electrophoresis results in relatively tedious separation of the sequencing fragments.

3.1. Capillary Electrophoresis for DNA Sequencing

The foundation for capillary electrophoresis was laid by Hjerten in the 1960s,^[12] Everaerts and co-workers in the late 1970s,^[13] and Jorgenson and Lukacs in the early 1980s.^[14, 15] Jorgenson and Lukacs' work was particularly important because they treated electrophoresis as an instrumental technique and introduced capillary electrophoresis to the analytical chemistry community.

Capillary electrophoresis is performed with capillaries that are typically 50 μm in inner diameter, 150 to 350 μm in outer diameter, and about 30 cm in length. The capillaries are made from high-purity fused silica which is similar to that used in the manufacture of fiber optics.

Instrumentation for capillary electrophoresis is quite simple (Figure 4). The capillary holds a sieving medium, which allows separation of the DNA fragments based on their size. The sample is injected into the capillary by dipping the capillary

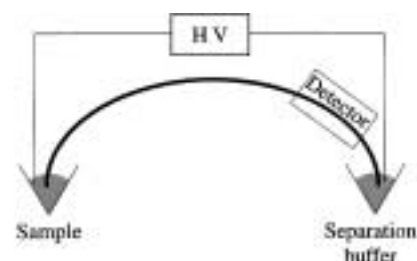


Figure 4. Single-capillary electrophoresis instrument. A fused-silica capillary is used for the separation. One end of the capillary is dipped into the sample or buffer reservoir, while the other end passes through a detector before being placed in a buffer-filled reservoir. High voltage (HV) is applied through platinum electrodes. The high voltage end of the capillary is held in a safety interlock equipped chamber.

and an electrode into a sample solution and briefly applying electric current through the capillary so that DNA fragments migrate onto the tip of the capillary. Once injection is complete, the sample is replaced with running buffer and electric field is reapplied to drive the DNA fragments through the capillary. A laser-induced fluorescence detector near the end of the capillary records the fluorescence signal in four different spectral channels to resolve the fluorescence signature from the four dyes.

The small inner diameter of the capillary reduces Joule heating to negligible levels and allows the use of extremely high electric fields for very rapid separations of DNA sequencing fragments.^[16] However, the phenomenon of biased reptation with orientation is observed at high electric field and degrades the separation of longer sequencing fragments.^[17] The electric fields employed in capillary electrophoresis represent a two-fold improvement over the best slab-gel instruments.

Despite the ability to use high electric field for electrophoresis, the most important property of capillaries is that they are highly flexible and easily incorporated into an automated instrument. It is the ease of automation, rather than fundamental thermal properties, that has resulted in the successful development of advanced sequencing instruments.

The technician time necessary to operate a slab-gel sequencer was the major bottleneck in large-scale DNA sequencing. Now, a single technician can service dozens of instruments, by just loading microtiter plates containing the sequencing fragments in each machine and occasionally replenishing reagents.

The first capillary electrophoresis DNA sequencer that used flexible, fused-silica capillaries, which ultimately became the standard for DNA sequencing, was reported in 1990 by Swerdlow when he was a graduate student in Gesteland's group at the University of Utah.^[18]

That year, Swerdlow moved to our laboratory at the University of Alberta as a visiting student. During his time in our lab, we published the first description of the use of a sheath-flow cuvette detector for DNA sequencing by capillary electrophoresis (Figure 5).^[19] This cuvette, modified from a

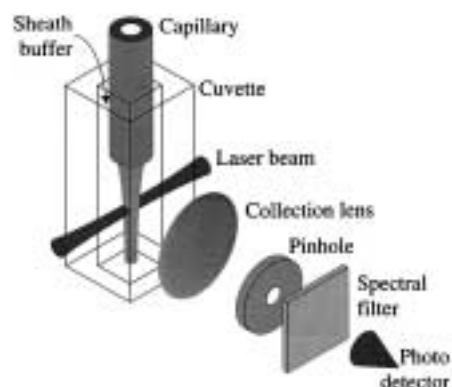


Figure 5. Single-capillary sheath-flow cuvette. A fused-silica capillary is placed inside of a square quartz flow chamber with high optical quality windows. Sheath fluid is pumped in the space between the capillary and the window, and this fluid draws the analyte into a thin stream in the center of the flow chamber. A laser beam is focused beneath the capillary tip on the sample stream. Fluorescence is collected by a lens, spectrally filtered, imaged onto an aperture, and detected with a photomultiplier tube.

flow cytometer, proves to be an extraordinarily efficient fluorescence detector. The cuvette has an approximately 175 μm square flow chamber that passes through an approximately 1 mm square piece of quartz. The separation capillary fits snugly in the flow chamber. Buffer is pumped at a very low rate in the space between the capillary and the cuvette window, creating a stream that draws DNA sequencing fragments as a thin filament in the center of the flow chamber. Due to the excellent optical quality of the cuvette windows, light scatter is virtually eliminated as a source of background signal in fluorescence measurements. When used with carefully designed collection optics and a low power helium–neon excitation laser, the cuvette has achieved detection of single molecules of β -phycoerythrin after separation by capillary electrophoresis.^[20]

In 1993, Karger and co-workers at Northeastern University realized that cross-linked polyacrylamide was an inappropriate matrix for DNA sequencing by capillary electrophoresis because the capillary must be discarded after each use. Instead, he reported the use of non-cross-linked polyacrylamide for the separation.^[21] This matrix can be pumped from

the capillary after each use and replaced with fresh material without having to replace the capillary itself.

The non-cross-linked material has relatively low viscosity, so that it may be pumped into the capillary under modest pressures. However, there was concern that the viscosity of the matrix would drop at higher temperatures and that electroosmosis would pump the matrix from the capillary. The use of high temperatures for separation is important because DNA denatures at elevated temperature. Intrastrand hydrogen bonding forms hairpin structures in the DNA that give rise to anomalous migration patterns during electrophoresis that degrade sequencing accuracy.

We studied the effect of temperature on the separation of DNA sequencing fragments in cross-linked and non-cross-linked polyacrylamide under high electric fields in capillary electrophoresis.^[22, 23] In 1995, we reported the first separation of DNA sequencing fragments at 60 °C with non-cross-linked polymer capillary electrophoresis.^[24] This temperature was sufficiently high to eliminate sequencing artifacts, thereby generating a high-quality sequence. We also demonstrated that the sequencing read length increased with temperature, which opened the door to practical, accurate long read length separation of DNA sequencing fragments.

3.2. Capillary Array Electrophoresis

While the performance of capillary electrophoresis is superior to that of conventional slab-gel electrophoresis, a single-capillary instrument does not offer significant advantages over a multi-lane slab-gel system. By separating many samples simultaneously, the slab-gel system produces much more data than is possible from a single-capillary instrument.

It is necessary to operate an array of capillaries to obtain throughput that is comparable with conventional electrophoresis systems. Zagurski and McCormick presented the first report of capillary array electrophoresis (CAE) for DNA sequencing in 1990.^[25] That instrument's detector scanned across the capillaries, recording the fluorescence signal sequentially from each capillary. This general approach of scanning a detector across the capillary array has been duplicated by other groups. However, this scanning method suffers from limited duty cycle: while one capillary is interrogated by the detector, DNA is also migrating in the other capillaries so that most DNA is not detected. Limited duty cycle can be severe. For example, each capillary in a 96 capillary array is probed for 1 % of the time; 99 % of the DNA is lost without detection.^[26]

The more successful capillary-array sequencers rely on continuous monitoring of each capillary. Two instruments are of particular interest, one from Kambara and co-workers at Hitachi and the other from this group.^[27–32] Both instruments rely on a sheath-flow cuvette to simultaneously monitor fluorescence from a linear array of capillaries (Figure 6). Sheath fluid is pumped through the interstitial space between the capillaries and entrains the DNA sequencing fragments as discrete streams, with one stream per capillary. A laser beam is focused into the cuvette and skims beneath the capillary tips (Figure 4), thus exciting fluorescence from all of the capil-

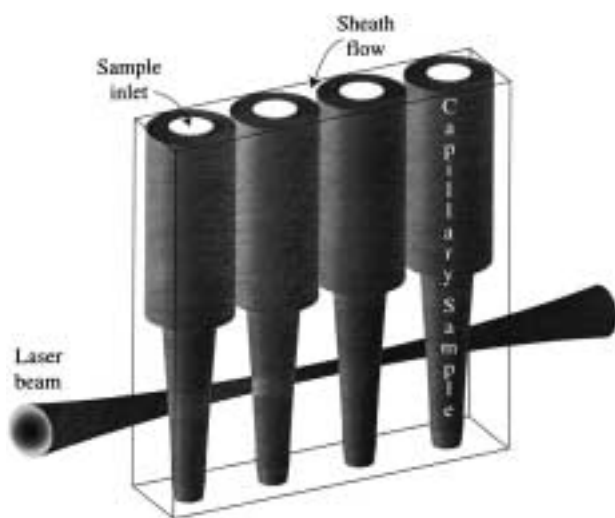


Figure 6. Capillary linear-array sheath-flow cuvette. A linear array of fused-silica capillaries is placed inside of a rectangular glass flow chamber. Sheath fluid draws the analyte into a thin stream in the center of the flow chamber, with one stream produced down-stream from each capillary. A laser beam is focused beneath the capillary tips on the sample streams. Fluorescence is collected by a lens, spectrally filtered, and detected with either an array of photodiodes or a CCD camera.

laries simultaneously. When observed from the front, the detector generates a set of fluorescent spots, with one spot beneath each capillary and with the spots separated by the capillary outer diameter. This detection cuvette is efficient in its use of laser light. As the sheath fluid is highly transparent, there is no disruption of the laser beam as it traverses the cuvette; one low-power laser beam is used to simultaneously illuminate samples migrating in all capillaries. An optical system images the fluorescence from each capillary onto a CCD camera or an array of photodiodes. In this way, fluorescence from each capillary is monitored continuously and the instrument's duty cycle approaches 100%.

The sheath-flow cuvette CAE instrument has been licensed to Applied Biosystems and is commercialized as the "Model 3700 DNA sequencer". The instrument operates with 96 capillaries for separation and has eight spare capillaries. A robot rapidly transfers samples from a 96-well microtiter plate to an injection block, where samples are loaded onto the capillaries. Sample loading, sieving-matrix replacement, electrophoresis, data collection, and base calling are all automated, so that the instrument can run for 24 hours without intervention.

It has been claimed that these CAE instruments represent a tenfold increase in sequencing throughput compared with automated slab-gel sequencers.^[33] This claim is probably an underestimate. The fourfold increase in the sequencing rate over the past year (Figure 1) is due to the output of a couple of thousand automated sequencers that replaced tens of thousands of automated slab-gel electrophoresis instruments.

Conclusions

Advances in DNA sequencing include the development of large-scale shotgun sequencing,^[34] highly automated DNA

sequencers, and powerful data-processing algorithms.^[35] This Essay focuses on the latest generation of highly automated DNA sequencers, which is based on CAE.

The Human Genome Project is the most ambitious and important effort in the history of biology. It has provided the complete genetic blueprint for human life, and will provide important insights into human health and development. The vast majority of this sequence was determined with high-throughput DNA sequencers based on CAE with a sheath-flow detector.

We are at the threshold of a new era in the biological sciences, which is based on genomic information that was unimaginable two decades ago. In 1980, the sequencing of a small gene could be the foundation of a complete PhD thesis. Due to the development of powerful analytical instrumentation, it is now possible to complete the sequence of a prokaryote in a few weeks and the sequence of a vertebrate in less than a year. This revolution in the biological sciences is possible only because of advances in analytical instrumentation for DNA sequencing.

Acknowledgements

It may be instructive to document the funding history for this work. Between 1989 and 1992, we submitted five proposals to local, national, and international funding agencies for the support of this project; all were rejected. Our first successful proposal for DNA sequencing was supported by the Department of Energy (USA) Human Genome Program and we held this funding from 1992–1998. Additional funding was received from SCIEX, a division of MDS, Inc. from 1995–1998. That funding was matched by the Natural Sciences and Engineering Research Council for the same period. Funding was also received from the Canadian Bacterial Diseases Network and the Canadian Genetic Diseases Network starting in 1994. The CBDN funding was recently terminated due to lack of commercial success. We gratefully acknowledge our sole source of funding for our current work in DNA analysis, the Canadian Genetic Diseases Network.

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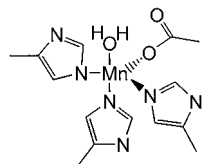


The Pharmaceutical Potential of Manganese-Based Superoxide Dismutase Mimics

Roland Krämer*

A considerable part of O_2 metabolized by the human organism is converted by one-electron reduction to the highly reactive superoxide radical anion, $O_2^{\cdot-}$. Sources of superoxide include “leaks” in the cytochrome c oxidase mediated O_2 -to- H_2O transformation pathway in mitochondria, various autooxidation reactions (e.g. of glutathione in red blood cells) but also the controlled production of $O_2^{\cdot-}$ by membrane-bound NAD(P)H oxidase in phagocytes to support immune defense against bacterial and fungal infections.

Endogenous overproduction of $O_2^{\cdot-}$ (and a number of highly reactive oxidizing agents thus formed) combined with reduced ability to eliminate radicals (= oxidative stress) clearly has the potential to inflict considerable damage on biological systems.^[1] In particular, the protonated form HO_2 ($pK_a=4.8$) is a very potent initiator for the selective autooxidation of lipid membrane components at the allylic position of unsaturated fatty acids and is also an active agent for the abstraction of ribose hydrogen atoms in DNA leading to extremely mutagenic DNA damage.



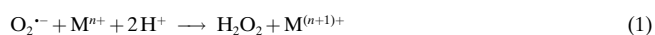
Scheme 1. Mn^{II} coordination sphere in manganese superoxide dismutase from human mitochondria.^[7]

The presence of superoxide in biological systems was first revealed by the discovery in 1969 of superoxide dismutase (SOD) enzymes by McCord and Fridovich.^[2] In healthy organisms radical accumulation is suppressed by these enzymes which contain, in the active site, either a manganese ion (Mn -SOD, Scheme 1, present in mitochondria) or a dinuclear Cu/Zn unit (Cu/Zn -SOD, present in the cytosol and extracellular space). The second-order rate

constant for the spontaneous disproportionation of $O_2^{\cdot-}$ to O_2 and H_2O_2 is $5 \times 10^5 M^{-1} s^{-1}$ at $21^\circ C$ and pH 7.4. This corresponds to a half-life of about 0.2 seconds at an initial $O_2^{\cdot-}$ concentration of $10^{-5} M$ ($t_{1/2}$ is proportional to $1/[O_2^{\cdot-}]$), sufficient to effect serious damage to cell components. The SOD enzymes dramatically accelerate $O_2^{\cdot-}$ decay

with second-order catalytic rate constants of up to $2 \times 10^9 M^{-1} s^{-1}$ for the Cu/Zn -SOD system (diffusion controlled reaction).

In the reduced state the metal (Mn^{II} or Cu^I) converts superoxide into H_2O_2 by a one electron reduction [Eq. (1)], followed by oxidation of a second equivalent $O_2^{\cdot-}$ to O_2 by the Mn^{III} or Cu^{II} center, respectively [Eq. (2)]. The less aggressive H_2O_2 is destroyed or metabolized by other enzymes such as catalase.



Net reaction:



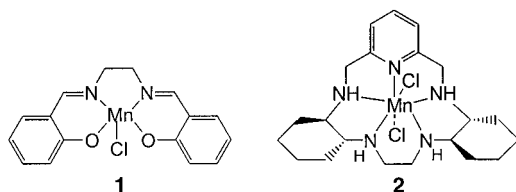
Oxidations mediated by superoxide are currently believed to participate in the pathogenesis of a number of important degenerative diseases.

The typical course of myocardial or cerebral infarct includes transient ischemia (stopping of the blood flow) of tissue areas followed by reperfusion with oxygenated blood. Since during the hypoxic events the cell is continuing to build reducing equivalents, reperfusion results in high levels of superoxide which are responsible to a great extent for cell death and tissue damage. In autoimmune conditions oxidants produced by activated neutrophils at sites of inflammation can have serious consequences for the organism, for example, joint damage in arthritis. Severe neurological disorders, such as amyotrophic lateral sclerosis (ALS), Parkinson's and Alzheimer's disease may be related to nerve cell damage caused by superoxide. Some types of cancer are thought to arise from oncogene (tumor forming) mutations caused by oxidative damage to DNA.

Application of SOD enzymes to animals has clearly shown beneficial effects in some of the above mentioned diseases. Cu/Zn -SOD preparations (trade names: palosein, orotein) are available for the treatment of inflammatory diseases in dogs and horses. In spite of encouraging results in animal models, SOD is not yet used for the treatment of human disease, a result of complications in clinical trials, in particular immunogenic (allergic) responses.

[*] R. Krämer
Anorganisch-Chemisches Institut
Im Neuenheimer Feld 270
69120 Heidelberg (Germany)
Fax: (+49) 6221-548599
E-mail: roland.kraemer@urz.uni-heidelberg.de

Low molecular weight mimics could have significant advantages over SOD enzymes such as lack of immunogenic response, longer half-life in the blood, improved access to cells and intercellular space, potential for oral delivery, and low costs. The effects of metal-based SOD analogues on biological systems have been investigated for two decades,^[3–5] but the requirements for a mimic that could be used as a human pharmaceutical agent are high: high chemical and metabolic stability, SOD enzyme like activity and specificity under physiological conditions, low toxicity, and favorable biodistribution. In this respect, the manganese complexes **1** and, in particular, **2** (discovered by the research group of D. Salvemini and P. Riley at MetaPhore Pharmaceuticals^[6]) are among the most promising candidates. Complex **2** was designed on the basis of molecular modeling considerations. These studies predicted that, of a class of complexes derived from the 1,4,7,10,13-pentaazacyclopentadecane framework, the properties for **2** were most favorable.



Complex **2** is thermodynamically stable, the constant for its formation from the macrocycle and Mn^{II} ions in water is >17 ($\lg K$). After intravenous injection in rats it is taken up into the heart, lungs, brain, liver, and kidneys and is excreted intact. The second-order rate constant for catalytic breakdown of superoxide is $2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ at pH 6 and 21°C and is comparable to that of manganese SOD enzymes. This is a rare example for a low molecular weight enzyme mimic that displays structural similarities to the enzyme active site ($\text{Mn}^{\text{II}}/\text{Mn}^{\text{III}}$ redox cycle, nitrogen donors) and at the same time approaches the catalytic rate of the enzyme under physiological conditions. Complex **2** is selective for superoxide and does not react with NO and H_2O_2 .

The anti-inflammatory effect of **2** was tested in rats in which paw edema (swelling) was provoked by a local injection of carrageenan.^[6] The increase of paw volume, a consequence of inflammatory response, as well as the infiltration by neutrophil blood cells was almost completely blocked by administering complex **2** (10 mg kg^{-1}) 30 min before the carrageenan injection.

In another rat experiment certain arteries were occluded to generate prolonged ischemia of intestinal tissue. Subsequent reperfusion results in neutrophil infiltration of the intestine, increased plasma levels of lipid peroxidation products and cytokines, severe hypotension, and finally in circulatory shock. The consequence is a high mortality rate within the first two hours. All these effects are significantly reduced when 15 min before reperfusion infusions of **2** (1 mg kg^{-1} rat) are given, with survival rates of 90% after 4 hours. Thus superoxide radical anions appear to be one of the mediators for the above mentioned events.

In summary the potent SOD mimic **2** which is about 60 times smaller than the natural enzyme has strong anti-inflammatory and cytoprotective effects in rats. It is a powerful pharmacological tool to explore the selective role of superoxide in physiological processes. The potential of SOD mimics such as **2** for the treatment of a broad range of inflammatory and cardiovascular diseases and their efficiency compared with other antioxidants has to be explored by clinical studies.

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How Can Metallic Ferromagnetism Be Explained?

Itinerant Ferromagnetism of the Transition Metals: Our Present Understanding

Gernot Stollhoff*

In a recent review, Landrum and Dronskowski (LD)^[1] gave an introduction to the itinerant ferromagnetism (that is the ferromagnetism arising from the delocalized electrons) of the transition metals covering *ab initio* as well as model calculations. They presented a new chemical picture by reinterpreting standard density functional (DF) results. However already in their introduction they deviate from the basic understanding of the itinerant ferromagnetism of transition metals within the frame of DF theory. Also the subsequent discussion by LD of results from the Hubbard model is inadequate. Important contributions to this field were not cited, and the comparison of density functional and model results for the transition metals was absent. No wonder that the chemical picture of itinerant ferromagnetism proposed by the authors is inadequate and irrelevant. To be able to judge the proposal of the authors, a basic view of our present understanding of the itinerant ferromagnetism is needed as gained from DF as well as model calculations.

Itinerant ferromagnetism is a consequence of electronic interactions. Without the specific influence of the interactions, the electrons would very much prefer to occupy the lowest-lying single-particle states, and be maximally delocalized. Such a delocalization causes maximal charge fluctuations on a local or atomic level. These fluctuations cost Coulomb interaction energy. Usually such charge fluctuations are reduced by correlations that correct them independently on individual atoms, but sometimes a state with a coherent long-range order that also reduces such fluctuations is energetically more favorable. Itinerant ferromagnetism is such a case. It is particularly interesting since the ferromagnetically ordered electrons are still partly delocalized.

Thus, itinerant ferromagnetism is characterized by a competition between delocalization (and band or kinetic energy) and localization (and interaction energy). A single property like the newly claimed antibonding nature of particular electrons can therefore not be the sole explanation of ferromagnetism. To demonstrate the competition underlying

ferromagnetism, it is worthwhile to separate the total energy of the electronic ground state as a function of its magnetic moment M_0 into two parts, one part describing the loss in delocalization or kinetic energy of the underlying single particle ground state, integrating over incremental contributions Δ , and the other, the gain in interaction energy, integrating over I . The ground state energy E_G is thus described by Equation (1). The function I as a parameter independent of M , has been introduced by Stoner^[2] M was later generalized by Wohlfarth.^[3] Magnetism occurs when $I(0) \geq \Delta(0)$, and the ground state moment is given by the condition $I(M_0) = \Delta(M_0)$.

$$E_G(M_0) = E_G(0) + \frac{1}{4} \int_0^{M_0} \Delta(M) dM^2 - \frac{1}{4} \int_0^{M_0} I(M) dM^2 \quad (1)$$

Equation (1) is exact. All unknown effects are simply transferred into the unknown function $I(M)$. Such a separation is justified if the detailed differences between the individual transition metals are covered by the first term, and if $I(M)$ is largely independent of individual details. This is the case for the local density approximation (LDA) of the DF theory. The ground state energy of a magnetic state obtained in LDA can be easily split in analogy to Equation (1).^[4] Here, $I(M)$ reduces to a quantity I_{LDA} that is almost independent of M and of the lattice parameters, and also shows a smooth dependency on the atomic number.^[4] In LDA, the differences in ferromagnetism for different solids originate mostly from the first term $\Delta(M)$. This term is dominated by the details of the density of state per spin $n(E)$ of the nonmagnetic ground state which may be obtained from a LDA calculation. When all secondary changes to the band structure besides a refilling of the bands (a good zero-order approximation) are ignored then Equation (2) holds for $\Delta(0)$, and $\Delta(M)$ averages over the inverse densities of state at the Fermi energy of the shifted bands. This result is plausible because the higher the density of state, the less band energy is lost for the redistribution.

$$\Delta(0) = \frac{1}{n(E_F)} \quad (2)$$

These findings indicate that in the frame of LDA, details of the density of states around the Fermi energy play the

[*] G. Stollhoff
Max-Planck-Institut für Festkörperforschung
70569 Stuttgart (Germany)
Fax: (+49) 711-6-89-1010

dominant role, and that ferromagnetism can only be expected if the density of state at the Fermi energy is high enough for the nonmagnetic state. It plays no role whatsoever whether this density of state is connected to bonding or antibonding parts of the bands, or to corresponding parts of partial distributions.

The LDA results explain the ferromagnetism of the transition metals and of their alloys astonishingly well.^[4,5] They are deficient only for partial properties, a few of which will be mentioned below. On the other hand, LDA fails for itinerant antiferromagnetism, and for the (partially itinerant) magnetism of the transition metal compounds. By definition, success and failure are connected to the quantity I_{LDA} which is an approximation to the true $I(M)$. I_{LDA} is a DF construct solely based on the properties of the homogeneous electron gas, while the true $I(M)$ is expected to be related to effective atomic interactions. Atomic orbitals are well pronounced in the transition metals, and the fluctuations causing ferromagnetism are consequently fluctuations on the atomic scale. The homogeneous electron gas on which the LDA is based contains no information whatsoever about atomic orbitals.

A detailed understanding of the function $I(M)$ can be gained from calculations where atomic interactions appear explicitly. For the ferromagnetic transition metals, such calculations were not yet performed on an ab initio level. However, on a model level, such an investigation was made. Here the energy bands obtained, for example, from LDA calculations are used for a single-particle model Hamiltonian to which effective atomic interactions are added. The resulting generalized Hubbard Hamiltonian is then fully treated.

Such a model approach to ferromagnetism was first performed by Stoner for a single band system with the density of state per spin, $n(E)$. He treated the interaction in a mean field approximation (MFA). If one assumes an atomic interaction U then in MFA it leads to Equation (3) for the

$$I_{\text{MFA}}(1) = U \quad (3)$$

MFA-Stoner parameter $I_{\text{MFA}}(1)$ of the single band and the so called Stoner criterion for the onset of ferromagnetism reads $Un(E_F) > 1$. In the MFA, no correlations are included in the computation. The only way to avoid the repulsive interactions is through magnetic ordering. In reality, however, electrons can correlate in the nonmagnetic case. This reduces the interaction costs, even without order, and strongly diminishes the Stoner parameter which is then smaller than U . When assuming a structureless density of state (i.e. $n(E) = W^{-1}$; W = band width), then from the above Stoner criterion it follows that ferromagnetism can occur in MFA only for $U/W > 1$. However, MFA is a poor approximation for such a ratio of U/W , and correlations are important in this range and are expected to shift the ferromagnetic transition to very much larger ratios if they do not eliminate it all together. This turns the single-band ferromagnetism into a strong-correlation problem, and makes the original separation of the total energy of Equation (1) questionable. Ferromagnetism in not strongly correlated single-band systems can only be expected

with the Fermi energy in the vicinity of a density of state maximum. This would imply small magnetic moments. As far as the theoretical coverage of single-band itinerant ferromagnetism is concerned, I am aware of a single unequivocal derivation of a ferromagnetic ground state for a (one-dimensional) single-band Hamiltonian.^[6]

Happily, all this changes for systems with sufficient degenerate bands N , like the transition metals ($N=5$). In such a case, the atomic interactions are best described by Slater parameters. The two most relevant ones are U and J . They lead to interactions of $U+2J$, $U-J$, and U for electrons in the same orbital, and in different orbitals with same or different spins, respectively, and to an exchange interaction J .^[7,8] The U of the single-band Hamiltonian operator used above is here replaced by $U+2J$. In terms of these interaction parameters, the MFA Stoner parameter for the N band system reads as Equation (4). The prefactor $1/N$ compensates a

$$I_{\text{MF}}(N) = \frac{1}{N} (U + (N+1)J) \quad (4)$$

similar prefactor in the inverse density of state (for a constant density of state, $n(E) = N/W$). Thus the exchange interaction J between degenerate orbitals strongly favors itinerant ferromagnetism. When assuming a ratio U/J of about five, then even with correlation corrections added, ferromagnetism can be expected for ratios $U/W < 1$, that is, in a domain where a weak correlation expansion is sufficient, and where the Stoner separation of the energy in Equation (1) is still meaningful.

For all existing compounds in the solid state, effective atomic interactions U are expected to be limited to $U < 10$ eV. The above simple model consequently indicates that itinerant ferromagnetism with large moments can only be expected for densely packed solids with narrow-band contributions, like those built by d or f orbitals, or for molecular crystals with narrow bands. But ferromagnetism with small moments might occur in the vicinity of every sufficiently strong density of state maximum.

In the correlation context, the N -fold degeneracy has another advantage. While in a single band the charge fluctuation per atom is covered by a single correlation operator on that atom, in an N -fold degenerate band, the N fluctuations arising in MFA are covered by $N(N+1)/2$ density correlation operators on that atom. The latter describe the correlations either on the individual atomic orbitals or between pairs of them on the same atom. These correlation channels mutually screen each other thus less burden is put on the individual operators and the validity range of a weak correlation expansion is significantly extended.^[7] Because of this screening, we could perform correlation calculations that satisfactorily converge for five-band Hubbard–Hamiltonian operators. In the relevant interaction-parameter range, the validity of the used weak correlation expansion was verified.^[7] For the correlation calculations, a model variant of the local ansatz (LA) was used. The LA is a method for the ab initio correlation treatment of large molecules and solids (for the most recent publication, see ^[9]). The LA is related to quantum chemistry methods but differs by the efficient use of local (not orthogonal) correlation operators. In contrast to the LDA, it

does not suffer from any homogeneous electron gas approximation.

The required interaction parameters of the model were chosen so that the correct ground state moment was obtained. The required values were reasonable,^[8] and their variation with the atomic number could be understood.^[10] Roughly, the following relationships hold: $W \approx 5$ eV, $U \approx 3.5$ eV, $J \approx 0.7$ eV.^[8] It turned out that the same interaction parameters were needed, among other things, for a successful description of the X-ray core spectroscopy,^[11] anisotropic exchange splitting for Ni,^[12, 8] satellite structures^[12, 13] and photoemission line broadening.^[14] Also many-body features in the Compton profiles could be explained.^[15] The Compton experiments represent a definite proof for the validity of such a weak correlation scenario.

The LA treatment of the model was fully satisfactory. This method can be regarded as the canonical approach to the treatment of the ground state of the five-band model for transition metals. The LA calculations were significantly easier to perform than the much more recent attempts to handle single-band or two-band models.^[16, 17] The experience gained from the latter calculations is, because of the discrepancies in the degeneracies, of no value for understanding the transition metals themselves.

Very recently, we managed to derive model interaction parameters from the full Hamiltonian with the help of the LA (for the method, see ref. [10]). Relevant results have so far been obtained for Cu in high-temperature superconductors,^[9] and for V.^[18] These are in the range of the values obtained from our model calculations described above. This indicates that not only the single-particle features of the model Hamiltonian but also its interaction parameters can be closely tied to the full Hamiltonian operator. While J roughly corresponds to the atomic exchange interaction, the values of U differ greatly from the unscreened interactions U_0 for single atoms, which are in the range of 20 eV. This is because of a necessary convolution of the long-range Coulomb interaction into a local term (in zero-order, $U \approx U_0 - V$, where V is the largest neighboring interaction). In the past it had been argued that the dominant reduction is because of the screening by the 4s,4p electrons.^[19] Such a screening is also present but its effect on the interaction strength is relatively small.^[9, 18] Again, we found that the interaction parameters U for delocalized electrons are basically disconnected from interactions in the atomic limit.^[10]

Since the weak correlation approximation applies, and consequently the Stoner approach of Equation (1) is still meaningful, it makes sense to condense the interaction contributions into the Stoner parameter (now called I_{LA}), and to compare the it to I_{LDA} . The true model Stoner parameter I_{LA} is typically half as large as I_{MFA} (given in Equation (4)) for the same Hamiltonian operator.^[20] Half of the contributions to I_{LA} originate from the exchange contributions (J), and these are also roughly half as large as those obtained in the MFA.^[20] In contrast to I_{LDA} and I_{MFA} , I_{LA} is not independent of bandfilling; it is strongly reduced by correlations near half filling. This is plausible since near half filling, antiferromagnetism is expected, and ferromagnetic ordering would not lead to any energy gain as compared with

incoherent correlations on the individual atoms. The preference of antiferromagnetism around half filling had also been found from short-range correlation studies for these models.^[21] I_{LA} is also largely M dependent. This is because of magnetic or Hund's rule correlations that are very efficient even without magnetic order.^[22] They reduce I_{LA} considerably more for $M=0$ than for the maximal moment. This M dependence is very important in the computation of the Stoner transition temperature. A constant (not M dependent) Stoner parameter leads to a Stoner magnetic transition temperature that is much too high. This was always interpreted as a need for dominant fluctuation corrections, either resulting from order parameter fluctuations, or from disordered local moments. In reality this deviation is caused mainly by the M dependence of the Stoner parameter. With the M dependence properly included, the Stoner transition temperature reduces, and the difference of this (order parameter) mean field temperature from the true transition temperature seems to be more reasonable.^[20]

All this demonstrates that I_{LA} displays deviations from I_{LDA} that make sense. We even found an interpretation that explains many LDA deficiencies for the transition metals, and matches I_{LDA} to a particular approximate treatment of the atomic interactions. We had found many properties significantly influenced by U , regardless of whether they originated from exchange anisotropies or from correlation corrections. Whenever such effects showed up, LDA calculations had shortcomings for the same properties.^[7, 8, 20] These findings can be condensed into the conclusion that LDA seems to overscreen the Coulomb interaction and is best related to our results by setting $U=0$. This leaves only J as a possible origin of I_{LDA} . A connection between both is actually gained from single atom calculations. When applied to an atom with a partially filled shell, the LDA without symmetry breaking seems to reproduce an energy that equally averages over all possible electronic states while only the symmetry broken result represents the Hund's rule ground state. This indicates that LDA is able to recognize the atomic exchange interaction J_{atom} but treats it in MFA, that is it accommodates it only when the symmetry is explicitly broken. Essentially the same happens when a solid is calculated. Consequently, the LDA can be interpreted as handling the two atomic interactions U and J very differently. The first quantity seems to be completely neglected while the second is accommodated in MFA. For I_{LDA} , we propose the Equation (5).^[20, 23] Support for

$$I_{\text{LDA}} = \frac{N+1}{N} J_{\text{atom}} \quad (5)$$

this interpretation comes from another LDA deficiency. In LDA, not only ground states with a globally broken symmetry but also with a locally broken symmetry are obtained. The latter states are often used as a basis for disputed disordered local moment theories. Such a local symmetry breaking is a MFA artifact and would never occur in a correct correlation calculation.^[21]

In our interpretation, the success of LDA for the ferromagnetism of the transition metals arises from the chance compensation of two errors. The first is the complete omission of U from the Stoner parameter, and the second is the MFA

for J . From our experience it seems as if for $N=5$, that is, in the case of the transition metals, both errors almost exactly compensate each other. This interpretation is supported by the failure of LDA when antiferromagnetism is concerned. There, U apparently plays a relatively larger role. Degeneracies greater than five do not occur in nature, however, our interpretation predicts difficulties of the LDA with ferromagnetism for degeneracies smaller than five.

With the help of this unified interpretation of the itinerant ferromagnetism, we may now investigate the interpretation by LD. LD have based their picture on LDA calculations, but never introduced or explained I_{LDA} . Therefore, their interpretation is not consistent. The second relevant feature is the density of state around the Fermi energy. The authors did not use this density of state but elaborated on more general antibonding properties of electrons close to the Fermi energy, properties that naturally include the density of state. It will now be demonstrated for the specific ferromagnetic metals that besides I only the density of state itself plays a role, and that the new antibonding features from LD are not needed. The following discussion represents standard knowledge that has been available for few decades, except from a small correction resulting from our connection of I_{LDA} and J_{atom} . The discussion is qualitative, but still contains the essential features of the full quantitative treatment. For a qualitative treatment I refer to the citations given below, and to a large number of LDA publications.

Let us begin with the difference between fcc and bcc-Fe. The fcc Fe is not magnetic. In this case, I or I_{LDA} are apparently a little bit smaller than the inverse 3d density of state. Except for a few spikes, the fcc 3d-density of state is essentially a constant (see Figure 22 in ref. [1]). However, the same is not true for bcc-Fe. The bcc density of state has a pronounced minimum with two peaks around it (see Figure 17 in ref. [1]), and the Fermi energy of the nonmagnetic state lies in the middle of one of the maximums. Because of the higher density of state, the Stoner criterion is fulfilled for the bcc case which has the same I_{LDA} as the fcc case, and the system is ferromagnetic. However, bcc-Fe is not completely ferromagnetic. Even this can be understood in the Stoner picture. With increasing moment M , the Fermi energies for the new majority band and for the minority band move out of the maximum of the density of state. Beyond the ground state moment M_0 , the Stoner criterion is no longer fulfilled, and a complete magnetization is not allowed.

The next topic concerns the transition from fcc-Fe to -Co and -Ni. All cases show the same (rather constant) density of state so that the different positions of the Fermi energies are not expected to be responsible for the onset of magnetism. However, because of the stronger localization of the 3d orbitals for the heavier elements, the band width W shrinks and the density of state increases when going to Ni, while the exchange interaction J and also the interaction U rise, therefore the Stoner parameter increases. Consequently, the Stoner criterion can be fulfilled, and Co and Ni are ferromagnetic.

The same arguments apply when going to the 4d and 5d elements Pd and Pt. Again the density of state is similar to that of Ni, but the band width W is larger, and correspondingly

the interactions J and U , and thus I are smaller. Therefore, the Stoner criterion can no longer be fulfilled. The origin of the decrease of J is, by the way, not a result of stronger screening but because the 4d and 5d atomic orbitals are more diffuse (a consequence of the enlarged core and the Fermi exclusion). J is basically a bare, unscreened matrix element. The decrease of U with rising principle atomic quantum numbers has been discussed in detail for the series of elements C to Sn.^[24]

For all the explanations given above only the detailed densities of state and a little knowledge of I were used. There is no need for seemingly new information like the crystal-orbital Hamilton population (COHP) of LD in the context of itinerant ferromagnetism. Further conclusions drawn from the COHP picture are wrong. The negative energies of the local 3d COHPs have nothing to do with magnetism itself. In my opinion, they might be connected to the charge transfer from the 4s,4p orbitals to 3d. There is even an indirect relation between this charge transfer and magnetism (causing the inverse magneto volume effect for Ni^[9]), but this is out of the range of the LDA that was used by LD.^[9] Additionally, the narrowing of the majority band and the widening of the minority band do not cause ferromagnetism but instead represent an attempt to reduce a little the band-energy losses originating from the ferromagnetic redistribution.

To conclude, itinerant ferromagnetism is caused by atomic interactions, and is strongly supported by the same exchange interactions J between degenerate atomic orbitals explain the Hund's rule order in atoms. Ferromagnetism may occur only if the band width of the electrons involved is sufficiently small, as was predicted by Stoner half a century ago. The LDA is successful for the transition metals because the Stoner theory applies and because apparently two errors in the Stoner parameter I_{LDA} almost compensate one another. Our LA model computations have demonstrated that the Stoner theory can be applied because the 3d electrons of the transition metals are weakly correlated, a result of mutual screening.

A long time ago Van Vleck proposed a theory for the itinerant ferromagnetism of the transition metals^[25] that is complementary to the Stoner theory. His theory is based on the existence of localized magnetic moments, based on the strong correlation limit. This theory was shown to be false by experiments, by LDA calculations, and also by our work. Nevertheless, proposals similar in spirit to the theory of van Vleck have reappeared in very recent work on Hubbard models, always because of a false choice of degeneracy for the used models. LD have proposed to submit the old work of van Vleck to Occams razor (see citation 13 in ref. [1]). I feel that the speculation of van Vleck was not unreasonable in his time. I would rather propose to apply this tool to the most recent contributions of LD in the field of itinerant ferromagnetism. Landrum and Dronskowski should have known better.

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Reply

Gregory A. Landrum and Richard Dronskowski*

In the preceding paper,^[1] many-body theorist Gernot Stollhoff criticizes our recent review of a chemistry-oriented approach to understanding metallic ferromagnetism.^[2] Although we heartily welcome a lively debate between chemists and physicists on the subject, Stollhoff's critique, in our view, misses the deciding point.

Within the largest part of his text, Stollhoff goes to great lengths to speak in favor of the Hubbard ansatz and related theories based on W/U parameters. He also presents his own mini review explaining ferromagnetism in metallic species. For an, admittedly quite concise, treatment of Hubbard models, the reader is referred to Sections 2.3, 3.1, and especially 5.1 of our review.^[2] As we have already said, Stollhoff's theoretical approach is far from being the only theory in existence. Stollhoff questions both density functional theory (DFT) as well as the local density approximation (LDA), and promotes his own computational approach. Stollhoff obviously has severe problems with accepting DFT in general, but that is another matter. With respect to our own theory, we do not demand that the method we are using to construct chemical explanations be 100 percent "accurate" (whatever that means) although the LDA is, as he admits, totally precise in its predictions with respect to ferromagnetism and the transition metals.

It is true that our results are based on density functional (mostly LDA) calculations; while the LDA performs astonishingly well in most cases, there are deficiencies that we summarized in Sections 5.2 and A.1. Two years ago an independent review on the successes and failures of DFT was presented from the perspective of the physics community.^[3] However, the presence of antibonding states at the Fermi level is observed, which we view as the ferromagnetic

"fingerprint", in *both* LDA and gradient corrected calculations (see Section A.1)! This much at least is not an artifact of the LDA. It was not in fact our intention to "sell" DFT or LDA or any other numerical theory as the optimum solution. On the contrary, as we demonstrated in Appendix B, a much more primitive theory, the extended Hückel (!) is able to reproduce the ferromagnetic fingerprint, provided that the method is asked the right questions. Thus, we have demonstrated that the characteristic effect is theoretically "good-natured" and does not need an excessively sophisticated treatment. Also, our approach is orbital oriented and may thus be easily subjected to a chemical interpretation. It is hard for us to say whether equations (1)–(6) of Stollhoff's article^[1] contain chemically valuable information, and we leave this decision to the experimentalists.

Towards the end Stollhoff touches our contribution directly. When talking about fcc- and bcc-Fe, Stollhoff actually uses our results but does not present new information. With respect to his discussion of Co and Ni, he does not consider the changing position of the Fermi energy within the fcc density of states since our explanation allows more immediate insight than his W/U reasoning. In fact, the crystal-orbital Hamilton population (COHP) result in Figure 21 of ref. [2] qualitatively demonstrates the different strengths of ferromagnetism in Fe, Co, and Ni! Concerning Stollhoff's remarks on the heavier transition metals, he does not realize the analogy between main group and transition metal chemistry as far as differential shielding is concerned. He writes about the situation with Pd and Pt: "The origin of the decrease of the J is, by the way, not a result of stronger screening but because the 4d and 5d atomic orbitals are more diffuse (a consequence of the enlarged core)." Exactly, the 4d and 5d atomic orbitals are more diffuse, and the core is enlarged. This was the basis of our argument. We did not attempt to explain the decrease of J , we wanted to avoid any mention whatsoever of J in our treatment of the heavier transition metals.

On the other hand, Stollhoff does not seem to understand the origin of the negative COHP at the Fermi energy although

[*] R. Dronskowski, G. A. Landrum
Institut für Anorganische Chemie
Rheinisch-Westfälische Technische Hochschule
Prof.-Pirlet-Strasse 1, 52056 Aachen (Germany)
Fax: (+49) 241-8888-288
E-mail: drons@HAL9000.ac.rwth-aachen.de

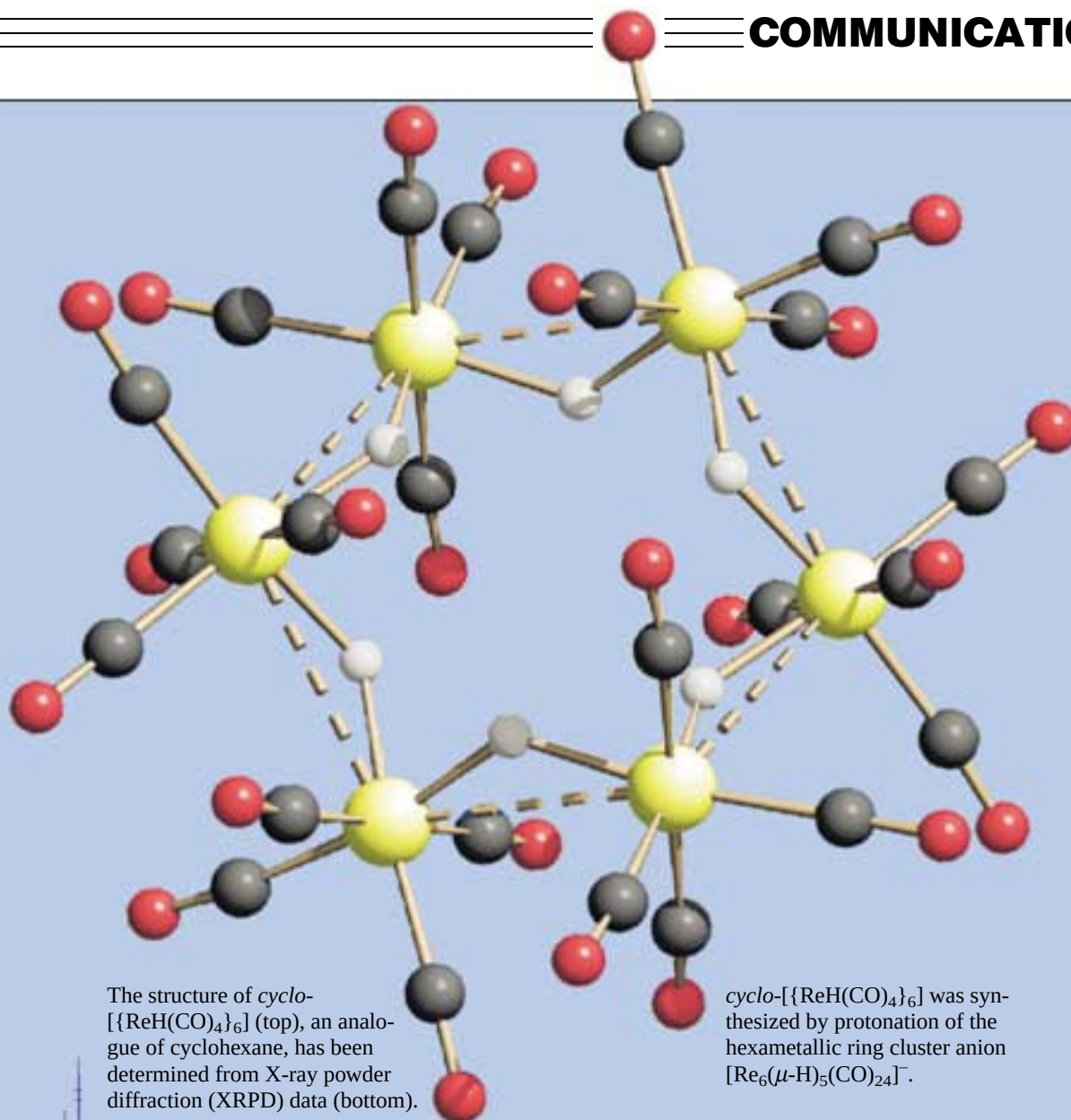
we have pictorially indicated its origin from the t_{2g} orbitals in Figures 14 and 16 of ref. [2]. The message contained in Stollhoff's remark "The negative energies of the local 3d COHPs have nothing to do with magnetism itself. In my opinion, they might be connected to the charge transfer from the 4s,4p orbitals to 3d." is not clear to us. Continuing his criticism of COHPs Stollhoff says: "There is no need for seemingly new information like the COHP of LD in the context of itinerant ferromagnetism." This type of statement is indefensible in any scientific paper. While interpretive tools apparently hold no value within some segments of community A, they can be well received by a significant portion of community B. Each community develops its own complexity of questions and answers. Stollhoff: "...the narrowing of the majority band and the widening of the minority band do not cause ferromagnetism but instead represent an attempt to reduce a little the band-energy losses originating from the ferromagnetic redistribution..." The changes in band width are, as we write in our review, a consequence of ferromagnetism. We do *not* call them a cause. We fail to see, however, how narrowing a band can *reduce* kinetic energy penalties. Unless our own understanding is also hampered by language barriers, broadening the minority band certainly helps out on the kinetic energy front, but narrowing the majority band results in a kinetic energy penalty.

Summarizing, a many-body theorist is arguing in favor of his method. There is nothing to say against such work, at least as long as it allows new insight and does not unnecessarily disparage other approaches. At the same time, we believe that we have the right to argue in terms of our own theory of ferromagnetism which is not only in harmony with atomic and molecular findings but can also, as we have shown, be extended to intermetallic alloys. Let us remind the reader that the purpose of our work was not to present the one, true, eternal theory of ferromagnetism, but to provide a framework by which the phenomenon could be more easily grasped by chemists and, more importantly, be used to help guide synthetic attempts. We again note that our qualitative picture

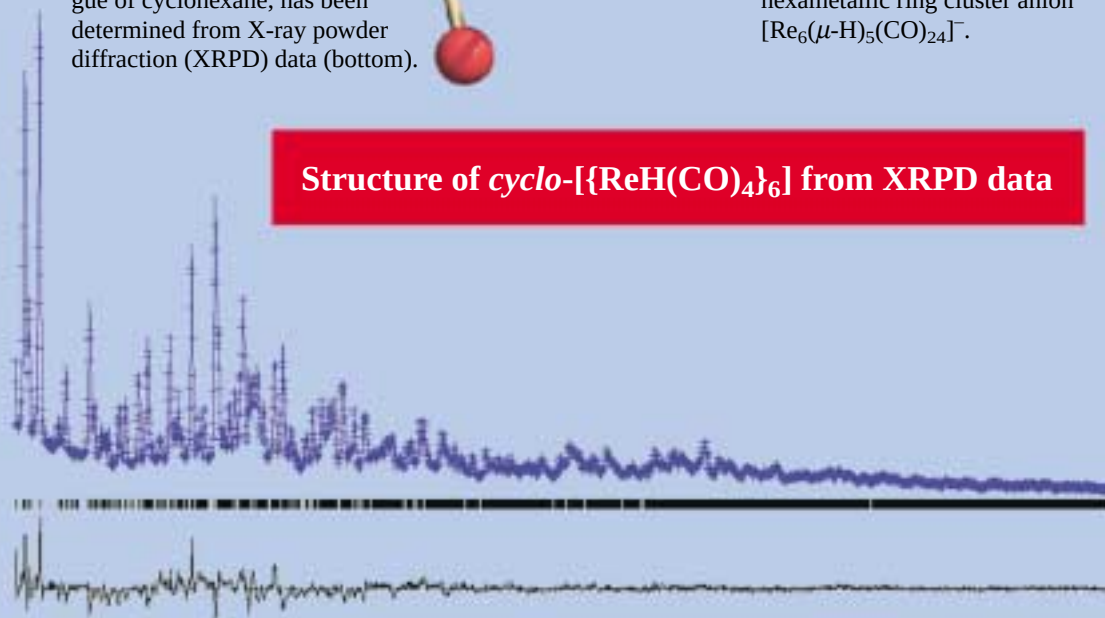
is conceptually straightforward and can be used productively: Want to increase the chances of an alloy being ferromagnetic? Increase or decrease the electron concentration to shift the Fermi energy towards those antibonding states at the top of the d block. It is probably much harder to see how to increase the Hubbard J value of a complicated alloy. Besides that, our theory is in excellent harmony with other chemical ideas such as the concept of chemical hardness, which now turns out to be a quantitative ferromagnetism criterion.

We acknowledge that it perhaps costs efforts for some colleagues to accept or at least understand our chemical theory of metallic ferromagnetism since it does not rely on the standard physics arguments. In fact, there are a number of good reasons not to follow that typical reductionist approach.^[4] On the contrary, our approach is just another demonstration that the atoms/bonds paradigm does have its justification in the quantum world, especially if it allows a simple, chemical, but still precise picture of itinerant ferromagnetism. From this perspective, our finding seems to be chemically obvious although it has been overlooked by many for a very long time because of their reductionist point of view. Finally, we believe that there is another lesson to be learned: We certainly need more bridges between theoretical physics and chemistry. Theoretical chemists have profited enormously, and still do, from solid-state quantum physics in the last two decades, and we only hope that some of our many-body friends will read more chemical journals and learn more about theoretical chemistry ideas. Stollhoff's contribution demonstrates that the first part of that has happened, and that is a start.

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Structure of *cyclo*-[$\{\text{ReH}(\text{CO})_4\}_6$] from XRPD data



Find out more on the following pages.

Complete Structural Characterization by Ab Initio X-Ray Powder Diffraction of $[\{\text{Re}(\mu\text{-H})(\text{CO})_4\}_n]$ and $[\{\text{Re}(\mu\text{-H})(\text{CO})_4\}_6]$, Inorganic Analogues of Polyethylene and Cyclohexane**

Norberto Masciocchi, Giuseppe D'Alfonso,*
Luca Garavaglia, and Angelo Sironi*

We have recently shown that anionic “initiators”, $\text{X}^- = \text{Re}(\text{CO})_5^-$ or Cl^- , can promote the oligomerization of the unsaturated (“ethylene-like”) complex $[\text{Re}_2(\mu\text{-H})_2(\text{CO})_8]$ (**1**).^[1] This process is unprecedented in organometallic chemistry and mimics the polymerization of olefins, even if only relatively short chains (up to ca. 10 metal atoms) have been obtained so far by this approach.^[2] These $\text{X}[\text{ReH}(\text{CO})_4]_{2n}^-$ oligomers can be viewed as isolobal analogues of linear alkanes, thanks to the established^[3] isolobal relationship^[4] between (singlet) methylene and the $\text{ReH}(\text{CO})_4$ fragment. We report here the characterization of a truly isolobal analogue of polyethylene, that is the $[\{\text{ReH}(\text{CO})_4\}_n]$ polymer (**2**), obtained by polymerization of $[\text{Re}_2(\mu\text{-H})_2(\text{CO})_8]$. This process occurs spontaneously, affording a white, highly insoluble precipitate, when the concentration of **1** in a donor solvent like THF exceeds 10^{-2} M, at, or below, room temperature. At lower concentrations, **1** is still unstable, but the main reaction process is the cyclodimerization to the square cluster $[\text{Re}_4(\mu\text{-H})_4(\text{CO})_{16}]$, whose synthesis and structure have been reported previously.^[5]

The last species is a member of the series of cyclic oligomers of formula $[\{\text{ReH}(\text{CO})_4\}_n]$ ($n = 3-5$).^[3, 5] which provide isolobal analogues of $(\text{CH}_2)_n$ cycloalkanes. We have now obtained the highest member of this series, namely the neutral $[\text{Re}_6(\mu\text{-H})_6(\text{CO})_{24}]$ cluster (**3**), an analogue of cyclohexane. It has been synthesized by protonation of the hexametallic ring cluster anion $[\text{Re}_6(\mu\text{-H})_5(\text{CO})_{24}]^-$,^[6] which was obtained recently through a $[4+2]$ cycloaddition between $[\text{Re}_4\text{H}_2(\mu\text{-H})_3(\text{CO})_{16}]^-$ and the $[\text{Re}_2(\text{CO})_8]$ fragment (generated from the labile $[\text{Re}_2(\text{CO})_8(\text{THF})_2]$ complex).^[7]

Unfortunately, both **2** and **3** were obtained as fine powders that were insoluble in all common solvents,^[8] thus hampering their characterization in solution and the formation of suitable single crystals for standard X-ray diffraction. Nowadays, however, this problem can be circumvented in some cases by attempting an ab initio X-ray powder diffraction (XRPD) structure determination,^[9] a methodology pioneered by us in the field of coordination chemistry, by using conventional laboratory equipment.^[10] Herein we report the full structural characterization of **2** and **3** by this approach.

$[\text{Re}_6(\mu\text{-H})_6(\text{CO})_{24}]$ (**3**) crystallizes in the monoclinic space group $P2_1/n$; according to the Patterson map, the metal skeleton consists of a ring of six rhenium atoms lying about a crystallographic inversion center. However, due to the intrinsic limitation of XRPD, we were unable to complete this starting model by Fourier methods; thus we had to rely on molecular mechanics and some previous stereochemical knowledge to obtain the whole structure. It is of particular interest that the preliminary information afforded by powder diffraction (i.e., lattice constants, space group symmetry, and “good” heavy atom locations) *uniquely determine the shape of the “cavity”* containing the molecule, and hence the molecular stereochemistry. For this reason even a rough force field can afford a “good” molecular geometry.^[11] Practically, molecular graphics (SMILE^[12]), using the known stereochemistry of $[\text{Re}_6(\mu\text{-H})_5(\text{CO})_{24}]^-$,^[6] allowed the ligand envelope to be built; lattice-constrained molecular mechanics (PACKMM/MM3^[13]) fitted the model structure into the crystal; and, eventually, a sagacious usage of constraints in the final Rietveld refinements (Figure 1) led to the ultimate stereochemistry (Figure 2), which fully confirmed the isolobal analogy with cyclohexane.

Even in the easiest cases, owing to the lack of a well-defined sequential strategy, ab initio XRPD is never trivial. This is particularly true when phase contamination and/or “uncertainties” in chemical formulation complicate the early steps of structure analysis. Indeed, the characterization of the $[\{\text{ReH}(\text{CO})_4\}_n]$ polymer was only possible when we recognized that decomposition of **1** in THF affords two polymorphs (α and β) of **2**, and when we became able to selectively produce each of them, by controlling the reaction temperature (see Experimental Section). The α -phase, which has much narrower peaks than the β -phase, was successfully indexed and eventually fully characterized by following the steps described in the Experimental Section. The resulting crystal structure confirms the polymeric nature of **2**; it crystallizes in the trigonal $P3_1$ space group and consists of homochiral 3_1 helices (parallel to c) hexagonally packed in the ab plane (Figure 3). Even though the poor crystallinity of the β -phase hampered its structural characterization, its negligible solubility, the IR spectrum (similar to that of the α phase and less structured than those of all known cyclic oligomers), and the (relative) paucity of low-angle peaks of its XRPD pattern (somewhat “similar” to that of the α -phase, Figure 4) suggest its polymeric nature, hence the above stated polymorphism. Polymer **2** is the second structurally characterized linear-chain metal carbonyl polymer, and, at variance from its predecessor *trans*- D_{4h} - $\text{Ru}(\text{CO})_4$,^[14] is constructed from *cis*- C_{2v} - $[\text{M}(\text{CO})_4]$ units.

[*] Prof. G. D'Alfonso, Dr. L. Garavaglia
Dip. di Chimica Inorganica, Metallorganica e Analitica and Centro C.N.R. CSMTBO
Università di Milano
via Venezian 21, 20133 Milano (Italy)
E-mail: dalff@csmtbo.mi.cnr.it

Prof. A. Sironi, Prof. N. Masciocchi^[+]
Dip. di Chimica Strutturale e Stereochimica Inorganica and Centro C.N.R. CSMTBO
Università di Milano
via Venezian 21, 20133 Milano (Italy)
Fax: (+39)02-70635288
E-mail: angelo@csmtbo.mi.cnr.it

[+] On leave from:
Dipartimento di Scienze Chimiche, Fisiche e Matematiche, Università dell'Insubria, via Valleggio 11, 22100 Como (Italy)

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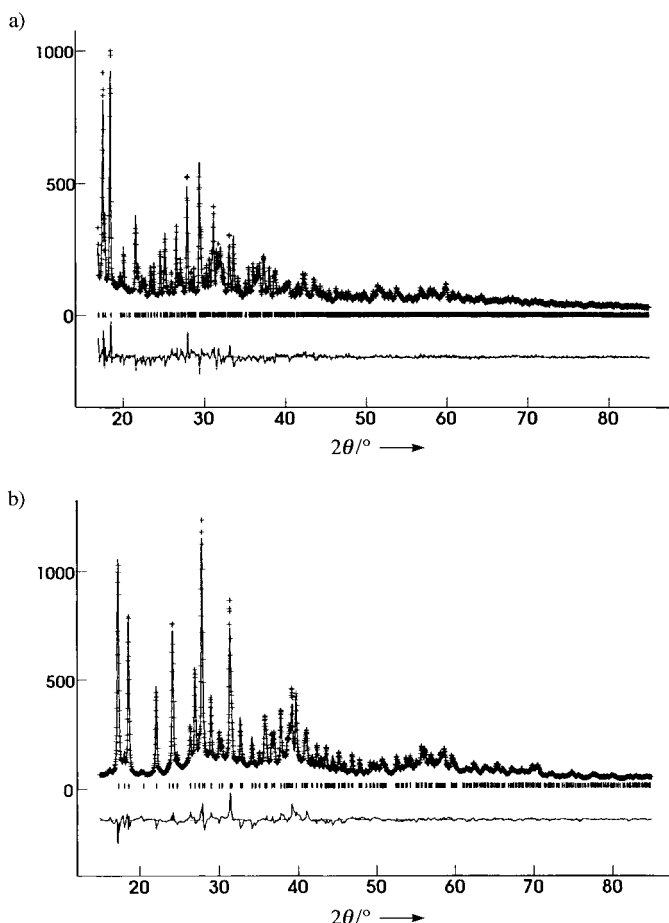


Figure 1. Rietveld refinement plots with peak markers and difference plots (bottom): a) $[\text{ReH}(\text{CO})_4]_6$ (**3**) and b) $\alpha\text{-}[\text{ReH}(\text{CO})_4]_n$ (**2**). Low-angle data have been omitted to avoid beam overflow and axial divergence effects. Intensity values are in arbitrary units.

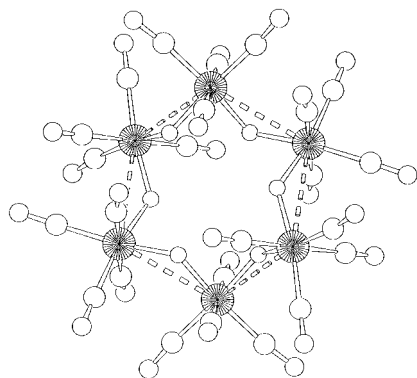


Figure 2. Molecular drawing of the $[\text{ReH}(\text{CO})_4]_6$ (**3**) species; Re atoms are cross-hatched.

In spite of their different backbones, **2** and **3** share a common structural feature, both having an “open” metal core which accommodates the hydrides. As shown by CPK models (see Supporting Information), these hydrides are not only inside the metal core, but also definitely buried within the carbonyl ligands cage, no space being left outside. Thus, it is the competition for space that pushes the less bulky (bridging) hydrogen atoms inside the “open” metal core. It might be considered odd to speak about hydrogen locations when we

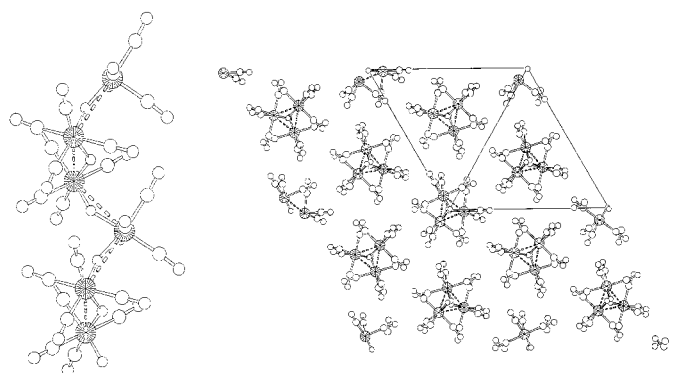


Figure 3. Sketches of the $\alpha\text{-}[\text{ReH}(\text{CO})_4]_n$ (**2**) polymer; Re atoms are cross-hatched. Left) A fragment of the 3_1 helix; right) crystal packing viewed down c .

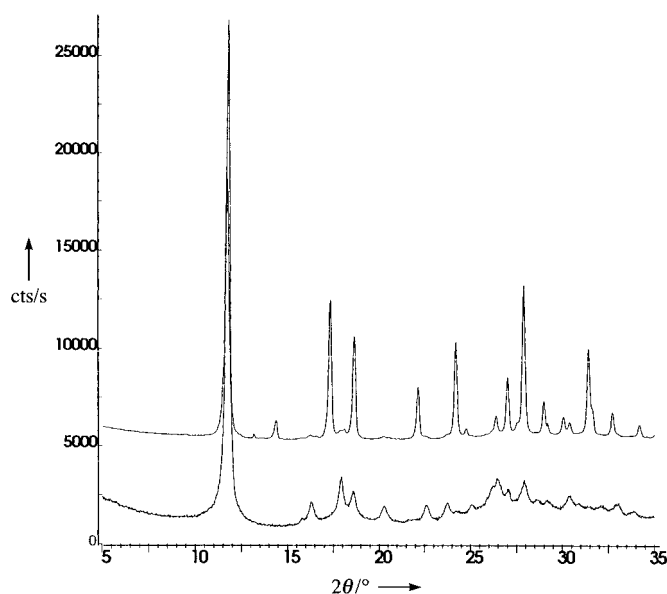


Figure 4. Raw diffraction data for the α - (top) and β - (bottom) $[\text{ReH}(\text{CO})_4]_n$ (**2**) phases.

barely see the metal centers. However, it is well known that we can derive, at the molecular level, the location of the hydrides knowing that of the other atoms.^[15] What we suggest here is that, at the crystal level (i.e. using the proper symmetry information), this is possible even starting from the metal atom positions alone. More than that, in the final stages of the refinement, carbonyl ligands contribute substantially to the fitting, and their location is no longer estimated by matching the diffraction experiment, but is actually determined from it.

A mechanistic problem still has to be solved: is the polymerization of **1** related to its anionic oligomerization,^[1] in which the donor solvent replaces the X^- ion as initiator of the process? Preliminary experiments have shown that neutral donor molecules, such as PMe_2Ph , Me_2S , NC_5H_5 , in a nondonor solvent like cyclohexane (in which **1** is quite stable), initiate the polymerization. The process, however, is much slower than in THF or acetone (days, instead of hours). Therefore the role of the donor solvents probably goes beyond that of a simple initiator and further studies of this are in progress.

Experimental Section

Synthesis of the polymer $[\text{ReH}(\text{CO})_4]_n$ (**2**, α - and β -phases): A typical procedure for obtaining the α -phase of $[\text{ReH}(\text{CO})_4]_n$ is as follows: A sample of $[\text{Re}_2(\mu\text{-H})_2(\text{CO})_8]$ (24.4 mg, 0.041 mmol) was added under N_2 to THF (2.0 mL) thermostated at 288 K. The solution (0.020 M) was maintained at this temperature for 24 h, affording a white precipitate that was isolated by centrifugation, washed with diethyl ether (5×5 mL), and dried under vacuum (10.9 mg, yield 44.6%). IR: see Figure S1 in Supporting Information. Elemental analysis (%): calcd for $\text{ReH}(\text{CO})_4$: C 16.0, H 0.33; found: C 15.8, H 0.38. The same phase was obtained by using different concentrations in the range $(1.5\text{--}2.5) \times 10^{-2}$ M at 288 K. The β -phase of $[\text{ReH}(\text{CO})_4]_n$ was obtained in the same way at 298 K (40.7% yield was obtained after 24 h from a 2.0×10^{-2} M solution). IR: see Figure S1; elemental analysis (%): C: 16.7, H: 0.47.

Synthesis of $[\text{ReH}(\text{CO})_4]_6$ (**3**): A slight excess of $\text{CF}_3\text{SO}_3\text{H}$ was added directly to the mixture formed in the reaction between $[\text{Re}_4\text{H}_2(\mu\text{-H})_3(\text{CO})_{16}]^-$ and $[\text{Re}_2(\text{CO})_8(\text{THF})_2]^{6-}$ in CH_2Cl_2 , affording a white precipitate that was isolated and washed with CH_2Cl_2 . IR (KBr): $\tilde{\nu} = 2112$ m, 2101 mw, 2045 sh, 2016 vs, 2002 vs, 1975 m, 1951 s, 1936 m, 1921 m, 1892 m.

X-ray powder diffraction studies: For both **2** and **3**, the white powders were cautiously deposited with the aid of a diethyl ether slurry on the surface of a zero background plate (Si(511), supplied by The Gem Dugout, State College, PA). Diffraction data were collected on a Philips PW1820 vertical scan diffractometer ($\text{CuK}\alpha$, $\lambda = 1.5418$ Å), equipped with parallel Soller slits in the incident beam and a secondary beam graphite monochromator. The generator was operated at 40 kV and 40 mA. Stationary sample, DS: 1.0° , RS: 0.2 mm. DS = Divergence slit, RS = receiving slit. Step scan, $\Delta 2\theta = 0.02^\circ$, $5^\circ < 2\theta < 85^\circ$, $t = 20$ s step^{-1} . Indexing, refinement, and molecular plots were performed with TREOR,^[16] GSAS,^[17] and Schakal,^[18] respectively.

According to TREOR, **3** has a monoclinic cell of approximate dimensions $a = 13.14$, $b = 12.43$, and $c = 12.56$ Å, $\beta = 96.4^\circ$ [$M(20) = 14$; $F(20)^{[19]} = 33(0.012, 52)$]. The ab initio structure solution is fully described in the text.

For **2** TREOR suggested a trigonal/hexagonal primitive cell of approximate dimensions $a = 14.93$ and $c = 6.98$ Å [$M(12) = 71$; $F(12) = 96(0.007, 17)$]. Volume considerations led to a Z value of 9, for a monomeric formula of $[\text{ReH}(\text{CO})_4]$. Since many different compatible Laue groups ($6/mmm$, $6/m$, $-3m1$, $-31m$ or -3) exist, the structure solution required several independent assumptions to be made with regard to the choice of the correct space group and the determination of the starting model. The odd Z value clearly suggests, in conjunction with the observation that *cis*- $[\text{Re}(\text{CO})_4]$ fragments cannot lie on inversion centers, a noncentrosymmetric space group. The small value for the c axis, together with the reduced "molar" volume (V/Z 151 versus 169 Å³ for nine and twelve $[\text{HRe}(\text{CO})_4]$ formula units in **2** and **3**, respectively) rules out the presence of cyclic oligomers and supports the polymeric nature of "molecules" of **2**, hence the presence of a 3_1 (or 3_2) screw axis (also confirmed by the most prominent peak in the Patterson map). Summarizing, the possible space groups are $P3_1$, $P3_121$, $P3_112$, and their enantiomorphs. Since $P3_121$ and $P3_112$ share the common subgroup $P3_1$, we decided to employ it as starting space group and to later verify the need of a higher symmetry descriptor. When run in $P3_1$, EXPO^[20] located three crystallographically independent rhenium atoms, each of which generated slightly misoriented (*ab* projection) polymers, with $\text{Re}\cdots\text{Re}$ distances in the 3.2–3.3 Å range. Raising the space group symmetry (by forcing one rhenium on a twofold axis) dramatically worsened the overall pattern matching, particularly at high angles, where lighter atoms do not substantially contribute to the scattered intensity. Finally the full molecular model was derived from the locations of the metal atoms alone by following the very same methodology described for **3**. The final structural model was then optimized by a full, although restrained,^[21] Rietveld refinement (Figure 1). Table 1 contains a summary of crystal data and final structural parameters for **2** and **3**. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-145922 and CCDC-145923. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Table 1. Summary of crystal data and structure refinement parameters for α - $[\text{ReH}(\text{CO})_4]_n$ (**2**) and $[\text{ReH}(\text{CO})_4]_6$ (**3**).

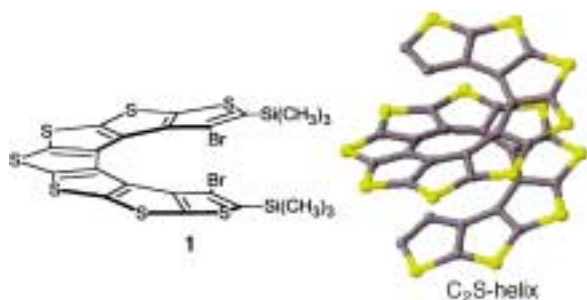
	$\text{C}_4\text{HO}_4\text{Re}$ (2)	$\text{C}_{24}\text{H}_6\text{O}_{24}\text{Re}_6$ (3)
formula weight	299.3	1795.5
crystal system	trigonal	monoclinic
space group	$P3_1$	$P2_1/n$
a [Å]	14.9733(7)	13.1182(8)
b [Å]	14.9733(7)	12.4078(8)
c [Å]	6.9984(4)	12.5694(8)
α [°]	90	90
β [°]	90	96.328(3)
γ [°]	120	90
V [Å ³]	1358.8(1)	2033.4(2)
Z	9	2
2θ range [°]	15 to 85	17 to 85
steps	3500	3400
no. of independent atoms	30	30
min FWHM [°]	0.18	0.13
R_p , wR_p	0.055, 0.071	0.063, 0.079
R_F	0.054	0.069

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Annulated Heptathiophene: A Fragment of a Carbon–Sulfur Helix**

Andrzej Rajca,* Hua Wang, Maren Pink, and Suchada Rajca

Herein we describe the iterative synthesis of a novel oligothiophene **1**, in which the thiophene rings are cross-conjugated and annulated into a helix.^[1–3] Compound **1** may also be viewed as a fragment of the unprecedented carbon–sulfur (C₂S) helicene.^[4–9]



The synthetic route to **1** consists of two iterations using 3,4-dibromothiophene and 4,4'-dibromo-5,5'-di(trimethylsilyl)di-thieno[2,3-*b*:3',2'-*d*]thiophene (**4**) as the tetrafunctionalized starting modules for the first and second iterations, respectively (Scheme 1). In each iteration, the modules are con-

nected and then annulated. As the chirality is introduced only in the final annulation step, the stereochemical problems of racemic synthesis are avoided.^[10]

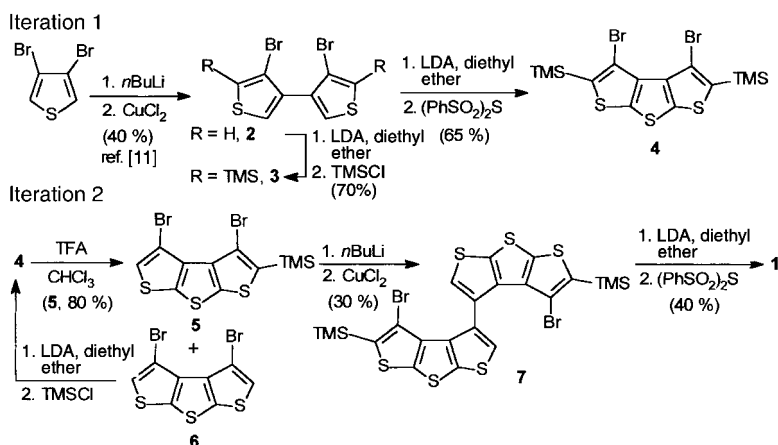
In the first iteration, 4,4'-dibromo-3,3'-bithienyl (**2**) is prepared from 3,4-dibromothiophene by a mono Li/Br exchange, as previously reported.^[11] The two most acidic α -positions in **2** are TMS-protected to give **3**. Following the LDA-mediated lithiation of the unprotected α -positions in **3**, the reaction of the dilithiated **3** with bis(phenylsulfonyl) sulfide gives the annulated product **4**.^[12]

In the second iteration, mono Li/Br exchange in **4** is not successful. Therefore, one of the TMS groups in **4** is removed to give **5** in which a modest selectivity for the mono Li/Br exchange can be attained. The incomplete conversion of **4** into a mixture of **5** and **6**, can be monitored by thin layer chromatography and NMR spectroscopy; after recycling (**6** to **4** and then to **5**), **5** is obtained in 80 % yield. Following the single Li/Br exchange on **5**, the resultant aryllithium species is oxidized with CuCl₂ to give **7**, which is subsequently annulated to give **1**.

¹H and ¹³C NMR spectra indicate the expected twofold symmetry for **1–4**, **6**, and **7**. For **3**, and **5–7**, in which at least one α -position of thiophene is not silylated, the IR spectra show additional bands between 3094 and 3114 cm^{–1} in the C–H stretch region and the ¹H NMR spectra display one singlet in the aromatic region. In the EI mass spectra of **3–5**, isotopic clusters derived from *M*⁺ and [*M*–CH₃]⁺ have comparable intensities; for **1** and **7**, the *M*⁺ regions have dominant intensity. For **1**, the *M*⁺, [*M*+2]⁺, [*M*+4]⁺ peaks have masses within less than 2.6 ppm of the calculated values and possess adequate relative amplitudes for C₂₂H₁₈Si₂Br₂.

The structures of **4** and **1** were confirmed by single-crystal X-ray analysis (Figure 1).^[13] In **4**, all the thiophene rings are approximately coplanar.^[14] The C–Br bonds are nearly parallel, with the C2...C7 distance of 3.68 Å and the Br1–C2–C7–Br2 torsion angle is 13.3°; therefore, **4** may also be viewed as a 3.7 Å spacer molecule. The structure of **1** shows the molecule has approximate twofold symmetry and includes one chloroform molecule, which is statistically disordered over two positions. The Br...Br distance is 3.90 Å and the Br atoms are pointing away from each other. The repulsion of the

facing terminal thiophene rings, and especially between the bromine atoms, causes a relatively large interplanar angle between the terminal thiophene rings, 180 – 125.9 = 54.1°. This is similar to the 58.5° for the interplanar angle between the terminal benzene rings in [6]helicene.^[5, 15] The individual thiophene rings are approximately planar with mean deviations of the least-square planes between 0.01 and 0.04 Å; the angles between the least-square planes of neighboring thiophene rings are between 7.9 and 11.2°. With the middle thiophene ring as a reference, the inner (C2, C3, C5, C7, C9, C11, C13, C15) helix climbs 2.92 Å and turns in-plane by 260°. Analogous values for [6]helicene are 3.12 Å and 317.7°, respectively.^[16] In both **1** and [6]helicene, the helix climbs



Scheme 1. Synthesis of **1**. LDA = lithium diisopropylamide, TMS = trimethylsilyl, TFA = trifluoroacetic acid.

[*] Prof. A. Rajca, Dr. H. Wang, Dr. S. Rajca
Department of Chemistry
University of Nebraska
Lincoln, NE 68588-0304 (USA)
Fax: (+1) 402-472-9402
E-mail: arajca1@unl.edu

Dr. M. Pink
X-Ray Crystallographic Laboratory
160 Kolthoff Hall, Chemistry Department
The University of Minnesota, Minneapolis, MN 55455 (USA)

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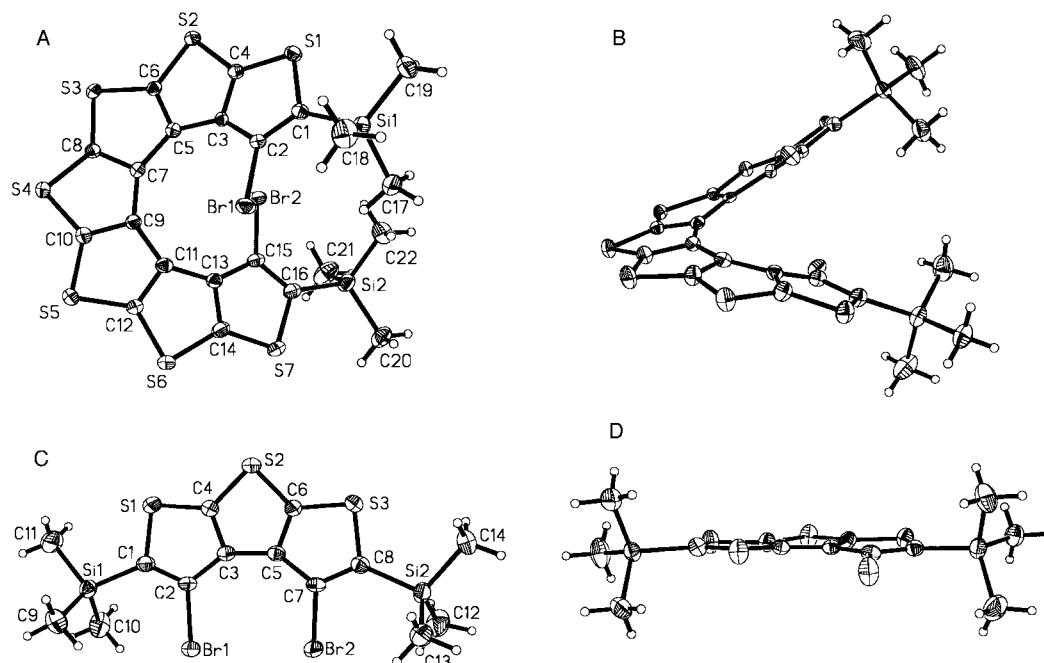


Figure 1. Molecular structure and conformation for helicene **1** and compound **4**. Carbon, bromine, silicon, and sulfur atoms are depicted with thermal ellipsoids set at the 50% probability level: A) top view for **1**, B) side view for **1**, C) top view for **4**, and D) side view for **4**.

for individual thiophene and benzene rings have similar patterns, with the smallest steps for the internal rings.^[16]

UV/Vis spectra for **1** and **4** in methylene chloride are significantly different (Figure 2).^[17] The integrated absorbance in **1** is approximately twice that of **4**. The bathochromic shift for **1** is rather substantial, considering that **1** and **4** possess

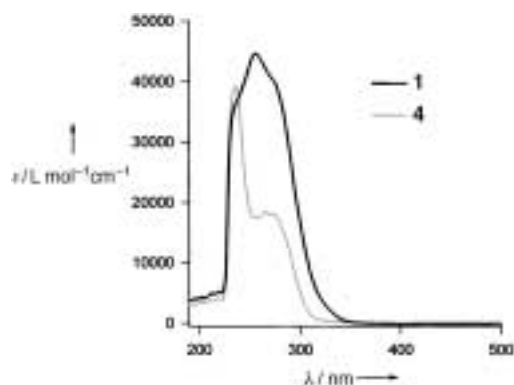


Figure 2. Electronic absorption spectra of **1** and **4** in methylene chloride at room temperature.

molecular connectivities of homologous cross-conjugated π systems.^[18] Both the helical distortion in **1** and possible conjugation through the sulfur atoms may affect the increased π -electron delocalization in **1**.

In summary, the iterative synthesis of a racemic carbon–sulfur helicene **1** was developed. This synthetic route can be applied to the synthesis of the higher homologue(s) of **1**. However, as the tetrafunctionalized module **1** is chiral, it would be more efficient to use a single enantiomer of **1** (rather than its racemate) as the starting material.^[10]

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Towards the DRED of Resin-Supported Combinatorial Libraries: A Non-Invasive Methodology Based on Bead Self-Encoding and Multispectral Imaging**

Hicham Fenniri,* Hartmut G. Hedderich, Kenneth S. Haber, Jihane Achkar, Brian Taylor, and Dor Ben-Amotz

Combinatorial synthetic methods allow for the preparation of large arrays of compounds as mixtures or individual entities. In the latter case, split synthesis has proven to be ideal in maximizing the number of compounds generated per synthetic step.^[1] While the composition of chemical libraries produced using this method is predictable with a high level of confidence, the structural elucidation of potent member(s) after an activity assay remains a challenge. To unravel the chemical nature of the active members several tactics based on encoding methodologies^[2] or deconvolutive strategies^[3] have been developed. Herein we introduce the principles and describe preliminary studies towards the implementation of an alternative approach, termed *dual recursive deconvolution* (DRED), that draws its strengths from both chemical self-encoding techniques and deconvolution strategies. DRED operates through the iterative identification of the first and last randomized positions of the active members of combinatorial libraries generated through split synthesis. The last building block can be readily obtained from pool screening after the last coupling of the split synthesis, while the first position can be “encoded” by the unique vibrational “fingerprint” of the resin beads used. Once the first and last positions are identified, the second and second to last positions are subjected to the same deconvolution process. Remarkably, this exercise would dramatically simplify the synthetic and screening efforts (Supporting Information). For instance, the preparation and DRED of a 64-million hexapeptide library would barely double the number of chemical steps required for the split synthesis of the library (246 versus 120) and would involve only 20 spectroscopically distinguishable beads (DRED beads). Because the matrix is an encoding element of the DRED strategy, libraries can be evaluated while still attached to the bead or close to the beads from which they were released.

The suitability of a near-infrared Raman imaging (NIRIM) instrument^[4] as a tool for the simultaneous identification of beads of various chemical composition was investigated. The NIRIM uses fiber-bundle image compression (FIC) technol-

[*] Prof. Dr. H. Fenniri, Dr. H. G. Hedderich, Dr. K. S. Haber, J. Achkar, B. Taylor, Prof. Dr. D. Ben-Amotz
1393 H. C. Brown Laboratory of Chemistry
Purdue University
West Lafayette, IN 47907-1393 (USA)
Fax: (+1) 765-494-0239
E-mail: hf@purdue.edu

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ogy^[5] to simultaneously collect a three dimensional (3D) Raman spectral-imaging data cube (λ -x-y) containing an optical spectrum (λ) at each spatial location (x-y) of a globally illuminated area. It should be noted that this is a real-time imaging technique as opposed to the previously reported step-scan methods,^[6] which require much longer to generate an image of the sample.

Vital to the success of the DRED method is the ability to identify resin beads based on their polymeric constituents and regardless of the chemical nature of their cargo. To address this dilemma, samples of 18 Merrifield-resin beads carrying various protected amino acids were positioned in the field of view of the NIRIM and single-bead Raman spectra were recorded between 100 cm^{-1} and 1900 cm^{-1} .^[7] Visual inspection of these spectra indicated that the spectral features were dominated by the matrix (polystyrene (PS)) and even background subtraction (unsubstituted PS beads) did not reveal the spectral features of the loaded material. Hence, Raman imaging of PS-supported compounds is insensitive to the material loaded on the beads at least up to 1.0 mmol of the amino acids studied per gram of resin.^[7] Interestingly, while the spectral features of the loaded material become detectable at much higher loadings the resin vibrations and their intensity remain essentially unaffected. An extreme illustration of this result is displayed by TentaGel beads, which are 30/70 (w/w) PS/polyethyleneglycol (PEG) graft copolymer. The Raman spectrum of TentaGel shows vibrations specific to the PEG component as well as the fingerprint transitions of PS which have not been affected by the PEG component (compare Figure 1 A and 1 C). In addition, the Raman spectra of PS and TentaGel-S-OH beads displayed unique vibrations that were used to image and identify them selectively. Figure 1 B shows a 5 $\times$ 5 FIC frames Raman image of a mixture of PS- and TentaGel-resin beads (exposure time of 45 seconds per frame, total scan time 20 minutes).^[8] The classified images (Figure 1 D–F) were readily derived from the Raman data using the spectral angle mapper (SAM)

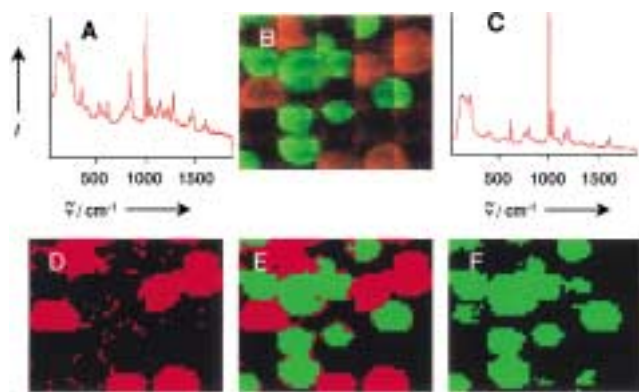


Figure 1. Experiments showing that the vibrations of the PS portion of TentaGel remains essentially unaffected by the major component (PEG) of the bead. Raman spectra of TentaGel-S-OH (A) and polystyrene (C) resin beads. 5 $\times$ 5 FIC frames Raman image (50 $\times$ 40 FIC fibers and 15 $\times$ 12 μm per single image-pixel) of a mixture of polystyrene and TentaGel-S-OH recorded with a 10 $\times$ objective, a global illumination laser power of $\approx$ 400 mW per single frame region and a single frame detector integration time of 45 seconds (B).^[8] Classification images^[9] of TentaGel-S-OH (D), polystyrene (F) and a mixture of both resin beads (E) produced from the NIRIM image B.

algorithm of the spectral-imaging software package Multi-Spec.^[9] Transitions at 1277 cm^{-1} for TentaGel-S-OH (Figure 1 D) and at 1000 cm^{-1} and 1031 cm^{-1} for PS (Figure 1 F) were used to identify the corresponding beads. Figure 1 E is an image where both PS and TentaGel beads were specifically and concomitantly identified.

To further extend our ability to reliably identify resin beads based on unique differences in their chemical nature, we have studied the following PS- and non-PS-based resins: (a) 4-bromo-PS, (b) 4-carboxy-PS, (c) PEG cross-linked Merrifield (PS) resin, (d) amino-PEGA, (e) HMBA-SPAR 50 and (f) SPAR 50. Figure 2 shows 5 $\times$ 5 frames (50 $\times$ 40 pixels) and 6 $\times$ 6 frames (60 $\times$ 48 pixels) in which all the beads were identified following the same procedure as in Figure 1. Resins a–c were chosen to establish that at least three additional PS-based resins can be readily distinguished (Figure 2 A). Resins d–f were chosen to establish the identification of polyamide-based supports (Figure 2 F), and to demonstrate that they can be readily differentiated from PS-based beads (Figure 2 C, 2 D, and 2 F–H). The images in Figure 2 were obtained by combining 1 mg of each of the resins in

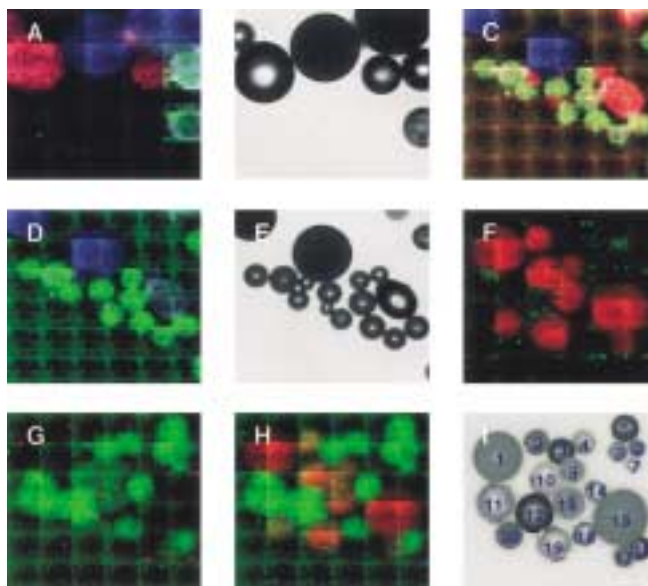


Figure 2. Series of experiments demonstrating the high reliability of multispectral imaging in identifying beads based on their polymeric constituents. The images were obtained from a statistical mixture of six different beads (see text). A) Specific NIR-Raman imaging of 4-bromo-PS (blue, 1073 cm^{-1}), 4-carboxy-PS (green, 637 cm^{-1}), and PEG cross-linked PS (red, 703 cm^{-1}). B) White-light image of the beads in A. C) Specific NIR-Raman imaging of 4-bromo-PS (blue), 4-carboxy-PS (green), and HMBA-SPAR 50 (red, 854 cm^{-1}). D) Same image as in C, but only the beads with a PS backbone are visualized. In addition, the two PS-based resins were color coded using vibrations specific to each of them (4-bromo-PS, blue, 4-carboxy-PS, green). E) White-light image of the beads in C and D. F) Specific near IR-Raman imaging of PS based resins (red, 4-bromo-PS, 4-carboxy-PS, PEG cross-linked-PS). G) Specific NIR-Raman imaging of polyamide based resins (green, amino-PEGA, HMBA-SPAR 50, SPAR 50). H) NIR-Raman image where PS- and polyamide-based resins were selectively and concomitantly identified. I) White-light image of the beads in F–H. All the beads were identified by multispectral imaging as well as by single-bead microspectroscopy (4-bromo-PS: beads No. 1 and 15; 4-carboxy-PS: beads No. 9, 10, 13, 19; PEG cross-linked-PS: beads No. 2, 17, 20; amino-PEGA: beads No. 3, 5, 12, 16; HMBA-SPAR 50: beads No. 4, 11, 14, 18; SPAR 50: beads No. 6, 7, 8).

methanol, a drop of this suspension was then deposited on a sapphire. After evaporation of the solvent the sapphire was placed in the field of view of the NIRIM. A library of single-bead NIR–Raman spectra of each of the resins was first recorded (Supporting Information), then several regions were arbitrarily selected for multispectral imaging. Since each bead appears as a collection of pixels and each pixel is a NIR–Raman spectrum of that area of the bead, comparison of these pixel spectra with the library of single-bead spectra recorded on the authentic samples confirmed the automated assignments. These results were reproducible regardless of the size and shape of the beads (Supporting Information).

The principles of dual recursive deconvolution of resin-supported combinatorial libraries have been proposed and the feasibility of the key feature of this method, the identification of the first randomized position, has been demonstrated using NIR–Raman imaging of self-encoded resin beads. The reliability of this method is very high, it has been consistently accurate over the two and a half years that we have been practicing multispectral imaging. Furthermore, any imaging technique could be applicable to the DRED method as long as the beads used display unique spectral features, and provided the loaded material does not significantly alter their spectral signature. For instance, secondary-ion mass spectrometry (SIMS)^[10] and FT-IR^[11] imaging are alternative approaches that we are investigating. Although, several commercially available and chemically distinct solid supports are eligible to explore the scope of DRED in combinatorial chemistry, inhomogeneities in their physical and chemical properties (e.g. swelling, porosity, size, reactivity) prompted the design of the DRED beads, a new class of resins for solid-phase synthesis.^[12] The added value of the DRED method is that it is a non-invasive screening technique, it does not necessarily require sophisticated equipment, it is inexpensive, and it does not involve the development of any encoding chemistry since the DRED beads will soon become commercially available.

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Polymorphism in *p*-Hydroxybenzoic Acid: The Effect of Intermolecular Hydrogen Bonding in Controlling Proton Order versus Disorder in the Carboxylic Acid Dimer Motif**

Benson M. Kariuki, Clare L. Bauer,
Kenneth D. M. Harris,* and Simon J. Teat

The phenomenon of polymorphism^[1–3] is of considerable contemporary interest in the field of organic solid-state chemistry, in part because comparisons between the properties of polymorphs provide an ideal basis on which to understand relationships between solid-state properties and crystal structure. Furthermore, in the quest to develop reliable computational techniques to predict the crystal structure(s) formed by a given type of organic molecule,^[4–8] studies of polymorphic systems provide stringent challenges for assessing the success of these methods. In general, the fact that the structures of organic molecular crystals usually arise from the interplay of several factors of comparable importance leads to intrinsic difficulties in attempting to predict and/or rationalize such structures. However, when one type of intermolecular interaction (or a small number of interactions) is dominant, a reliable rationalization of the observed molecular packing

[*] Prof. Dr. K. D. M. Harris, Dr. B. M. Kariuki, Dr. C. L. Bauer
School of Chemistry, University of Birmingham
Edgbaston, Birmingham B15 2TT (UK)
Fax: (+44) 121-414-7473
E-mail: K.D.M.Harris@bham.ac.uk
Dr. S. J. Teat
CCLRC Daresbury Laboratory
Daresbury, Warrington, Cheshire WA4 4AD (UK)

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arrangement in the crystal may be possible. In this regard, crystal structures based on strong intermolecular hydrogen bonding are often amenable to straightforward structural understanding.

The hydroxy derivatives of benzoic acid represent a potentially useful family of systems in which to explore polymorphism, as the combination of hydroxy groups and carboxylic acid groups should give rise to a variety of plausible intermolecular hydrogen bonding schemes and hence different crystal packing arrangements. Indeed, two polymorphs of *m*-hydroxybenzoic acid have been reported previously,^[9] and differ in their intermolecular hydrogen bonding arrangements. For *p*-hydroxybenzoic acid, on the other hand, only one crystal structure (hereafter referred to as form I) has been reported previously,^[10] although the possible existence of a second polymorph (which was neither isolated nor structurally characterized) was speculated^[11] on the basis of thermal analysis of samples of *p*-hydroxybenzoic acid.

Herein, we report a new polymorph (denoted form II) of *p*-hydroxybenzoic acid. Only a small number of low-quality microcrystals of this polymorph were obtained (as mixtures with other phases),^[12] and the crystal structure has been determined by exploiting synchrotron X-ray microcrystal diffraction techniques.^[13] Although the use of a microcrystal has led to a rather poorer quality of structural information^[13] than normally expected from single-crystal X-ray diffraction studies, unambiguous information on the crystal packing arrangement and other structural aspects is nevertheless obtained. Importantly, we find that form II exhibits interesting behavior with regard to the question of order versus disorder in the carboxylic acid dimer motif, contrasting markedly with the behavior of form I.

In the crystal structure^[13] of form II of *p*-hydroxybenzoic acid (Figure 1), the asymmetric unit comprises two independent molecules (denoted A and B). Each of these molecules forms an $R_2^2(8)$ centrosymmetric dimer about a crystallo-

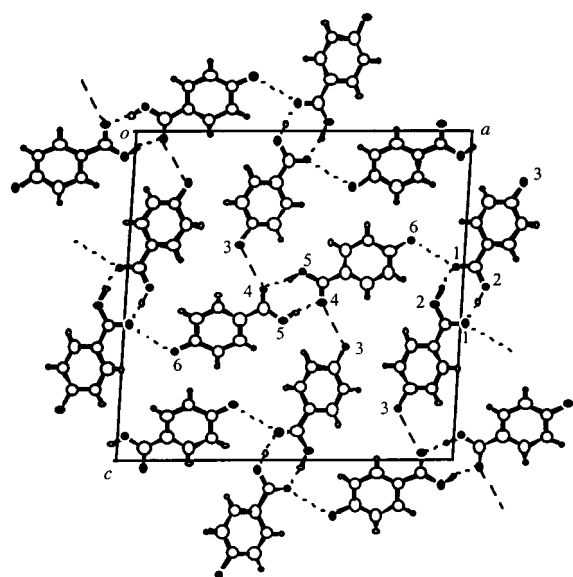


Figure 1. Crystal structure of form II of *p*-hydroxybenzoic acid viewed along the *b* axis. Dotted lines represent hydrogen bonding interactions. Molecules of type A are labeled with oxygen atoms numbered 1, 2, and 3. Molecules of type B are labeled with oxygen atoms numbered 4, 5, and 6.

graphic inversion center, a hydrogen bond pattern (involving two $O-H \cdots O=C$ hydrogen bonds) that is commonly found in carboxylic acids.^[16, 17] Clearly the two molecules in a given dimer are of the same type (A or B). For the centrosymmetric dimer involving molecules of type A, the $O(1) \cdots O(2)$ distance is 2.664(6) Å, and for that involving molecules of type B, the $O(4) \cdots O(5)$ distance is 2.617(6) Å (the atom labeling is specified in Figure 1). In both cases, these $O \cdots O$ distances are consistent with $O-H \cdots O=C$ hydrogen bonding. A hydrogen bonding interaction involving the hydroxy group of molecule B and the carboxylic acid group of molecule A is also clearly identified. In this interaction, the $O \cdots O$ distance between the hydroxy oxygen atom O(6) of molecule B and one oxygen atom O(1) of the carboxylic acid group of molecule A is 2.946(6) Å and the $O-H \cdots O$ angle is about 165° .^[18] For the carboxylic acid group of molecule B, the shortest intermolecular $O \cdots O$ distances to hydroxy groups of other molecules are 3.427(8) Å and 3.439(9) Å—these $O \cdots O$ distances involve the same oxygen atom of the carboxylic acid group of molecule B and the hydroxy groups of two different molecules of type A (related to each other by translation along the *b* axis). However, these distances are rather long to be assigned as $O-H \cdots O=C$ hydrogen bonds^[19] and other geometric characteristics^[20] also deviate from those normally observed for strong $O-H \cdots O=C$ hydrogen bonds.^[19] In this structure, there are no short intermolecular contacts between hydroxy groups.

In the space-averaged and time-averaged crystal structures determined from diffraction data, $R_2^2(8)$ carboxylic acid dimer motifs are often found to be disordered,^[16] such that the carbon–oxygen bond lengths are intermediate between those characteristic of single bonds (corresponding to $C-OH$) and double bonds (corresponding to $C=O$) and the protons are disordered (often two sites with half occupancy are located, corresponding to equal amounts of $C-O-H \cdots O=C$ and $C=O \cdots H-O-C$ situations in the average crystal structure). We now assess the evidence for order versus disorder of the carboxylic acid dimers in form II of *p*-hydroxybenzoic acid. The carboxylic acid group in molecule A is clearly not disordered. The $C-O$ bond lengths are 1.230(5) Å for O(1) and 1.333(5) Å for O(2), leading to the unambiguous assignment^[21] of O(1) as $C=O$ and O(2) as $C-OH$ in an ordered carboxylic acid group. The fact that O(1) receives a hydrogen bond from the hydroxy group in molecule B is consistent with the preference for the $C=O$ oxygen atom in an ordered carboxylic acid group (rather than the $C-OH$ oxygen atom) to act as a hydrogen-bond acceptor. Clearly the intermolecular $O-H \cdots O=C$ hydrogen bond from the hydroxy group of molecule B to the carboxylic acid group of molecule A “locks” this carboxylic acid group into an ordered arrangement. Our ability to locate the hydrogen atoms of the carboxylic acid and hydroxy groups in the crystal structure determination is discussed in reference [13].

For molecule B, the carbon–oxygen bond lengths in the carboxylic acid group are 1.273(7) Å for O(4) and 1.299(7) Å for O(5). The fact that these bond lengths in the average crystal structure are intermediate between $C-O$ single and $C=O$ double bonds suggests that this carboxylic acid group is disordered. Presumably any intermolecular interactions in-

volving the carboxylic acid group of molecule B (in particular, the rather long contacts to the hydroxy groups of molecules of type A discussed above) are insufficient to "lock" the carboxylic acid group of molecule B into an ordered arrangement.

For comparison, the crystal structure of form I of *p*-hydroxybenzoic acid^[10] also contains carboxylic acid dimers with the $R_2^2(8)$ hydrogen bond motif, but there is no direct hydrogen bonding between hydroxy groups and carboxylic acid groups (Figure 2). Instead, the hydroxy groups engage in

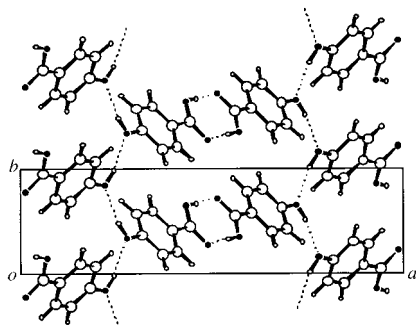


Figure 2. Crystal structure of form I of *p*-hydroxybenzoic acid (data from reference [10]).

O—H...O—H hydrogen bonds with other hydroxy groups, constructing chains that extend through the crystal.^[22] The two carbon–oxygen bond lengths in the carboxylic acid group are equal (1.266 Å) (there is one molecule in the asymmetric unit), and the hydrogen atom of the carboxylic acid group is found to be distributed (with half occupancy) between two sites. These facts clearly indicate that the carboxylic acid group is disordered.

The subject of proton disorder in carboxylic acid dimers has aroused much interest from structural and dynamic viewpoints,^[23–30] and it is clear that the two polymorphs of *p*-hydroxybenzoic acid represent an ideal basis for systematic studies of this property. Thus, from the X-ray diffraction data reported here, form I exhibits proton disorder, whereas form II has two crystallographically independent carboxylic acid dimers, one of which is clearly ordered and the other disordered.^[31] Future neutron diffraction studies, also as a function of temperature, will provide more details on structural aspects of the proton order/disorder, whereas other techniques (solid-state NMR and inelastic and quasielastic neutron scattering) will lead to an understanding of dynamic aspects. Inter alia, these variable temperature studies will allow the static versus dynamic character of the disorder to be probed, as well as the question of whether polymorphic phase transformations are observed in this system. Such future advances will be facilitated by the ability to prepare form II as a monophasic sample, ideally comprising larger single crystals than the microcrystals available in the present work.

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[12] Microcrystals (for typical crystal size, see reference [13]) of form II of *p*-hydroxybenzoic acid were obtained during preparation of inclusion compounds between deoxycholic acid and *p*-hydroxybenzoic acid, using methanol as solvent. The crystals were very small and of very poor quality.
[13] Single-crystal X-ray diffraction data were recorded for a microcrystal of form II of *p*-hydroxybenzoic acid on Station 9.8 at the Synchrotron Radiation Source (Daresbury Laboratory). This microcrystal diffraction station comprises a Siemens SMART CCD detector and goniometer system. Crystal size $0.10 \times 0.07 \times 0.05$ mm³; $T = 296(2)$ K; $\lambda = 0.68850$ Å; monoclinic, $P2_1/n$; $a = 18.66(2)$, $b = 3.860(4)$, $c = 18.82(3)$ Å, $\beta = 93.511(6)^\circ$; $V = 1353(3)$ Å³; $Z = 8$. The structure was solved by using SHELXS^[14] and refined by using SHELXL^[15] ($R_1 = 0.131$; $R_w = 0.365$). The aryl rings were refined as idealized hexagons (including hydrogen atoms). In the difference Fourier map, the strongest peak ($0.5 \text{ e } \text{\AA}^{-3}$) represented the position of the hydrogen atom of the carboxylic acid group of molecule A (the ordered carboxylic acid group—see text), and this hydrogen atom was then included in the refinement in a fixed position corresponding to standard geometry. The hydrogen atom of the carboxylic acid group of molecule B and the hydrogen atoms of the hydroxy groups were not located among the next highest peaks in the difference Fourier map. As only microcrystals of form II of *p*-hydroxybenzoic acid were available for the X-ray diffraction experiments, it is necessary to recognize that the intrinsically poorer data quality has resulted in relatively high values of R factors. Nevertheless, by maintaining the discussion and interpretation at a level commensurate with the reliability of the refined structural information, reliable conclusions on structural properties are nevertheless reached.
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[18] The geometry of the C(11)–O(6)–H...O(1)–C(7)–C(1) hydrogen bond is: O(6)...O(1) distance 2.946(6) Å; O(6)...O(1)–C(7) angle $139.6(3)^\circ$; C(11)–O(6)...O(1) angle $120.1(2)^\circ$; O(6)...O(1)–C(7)–C(1) dihedral angle $-35.6(6)^\circ$. The O(6)–H...O(1) angle, for the hydrogen atom of the hydroxy group inserted according to standard geometry, is about 165° . Note that C(11)–O(6)–H is the hydroxy group of molecule B and O(1)–C(7) is in the carboxylic acid group of molecule A, and is assigned (see text) as an O=C bond.
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112.2(4)°; C(4)–O(3)···O(4) angle 107.6(3)°; O(3)···O(4)–C(14)–C(8) dihedral angle –60.3(6)°; b) O(3)···O(4) distance 3.427(8) Å; O(3)···O(4)–C(14) angle 153.3(4)°; C(4)–O(3)···O(4) angle 123.3(4)°; O(3)···O(4)–C(14)–C(8) dihedral angle 25.5(1.3)°. Note that C(4)–O(3)–H is the hydroxy group of molecule A and O(4)–C(14) is in the carboxylic acid group of molecule B.

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Structure Determination of an Oligopeptide Directly from Powder Diffraction Data**

Emilio Tedesco, Giles W. Turner, Kenneth D. M. Harris,* Roy L. Johnston, and Benson M. Kariuki

In recent decades, single-crystal X-ray diffraction techniques have underpinned many major breakthroughs in structural biology, although it is well recognized that an intrinsic limitation of these techniques is the requirement to prepare single crystals of sufficient size, quality, and stability to allow diffraction data of sufficiently high quality to be measured. In solid-state and materials sciences, similar limitations are encountered in the scope and applicability of single-crystal diffraction methods, but much progress has been made in recent years in the use of powder diffraction data as an alternative means of obtaining structural information. Herein, we apply a new methodology for structure determination from powder diffraction data to determine the crystal structure of the oligopeptide Phe-Gly-Gly-Phe (Figure 1), which demonstrates the opportunity for future extensions to more complex systems of interest in structural biology. In this regard, we note that knowledge of the structural properties and interactions in model oligopeptide systems can yield fundamental insights towards understanding structural properties of polypeptide sequences in proteins.^[1–6]

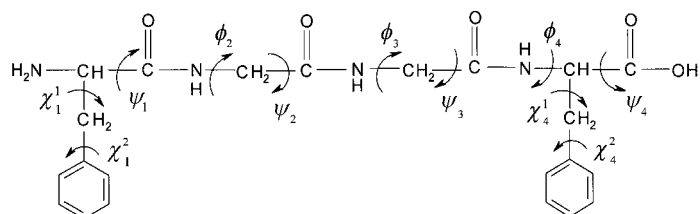


Figure 1. The molecular structure of Phe-Gly-Gly-Phe, showing the variable torsion angles in the genetic algorithm structure solution calculation.

In principle, powder diffraction data contains the same information as single-crystal diffraction data, but with the three-dimensional diffraction data compressed into one dimension. As a consequence, there is generally substantial overlap of peaks in the powder diffraction pattern, leading to severe difficulties in extracting the same quality of diffraction data (and hence structural information). Although the challenges in determining structures directly from powder diffraction data are considerable, new techniques and strat-

[*] Prof. K. D. M. Harris, Dr. E. Tedesco, G. W. Turner, Dr. R. L. Johnston, Dr. B. M. Kariuki
School of Chemistry, University of Birmingham
Edgbaston, Birmingham B15 2TT (UK)
Fax: (+44) 121-414-7473
E-mail: K.D.M.Harris@bham.ac.uk

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egies developed in recent years (reviewed in references^[7–10]) have allowed substantial progress to be made, particularly with regard to the more challenging structure solution stage of the structure determination process. It is relevant here to recall that *structure solution* involves deriving an initial (approximate) structural model directly from the experimental diffraction data, starting from no prior knowledge of the arrangement of molecules in the unit cell. If this structural model is a sufficiently good representation of the true structure, *structure refinement* can then be carried out to obtain a good quality structure. Techniques for structure solution from powder diffraction data can be subdivided into “traditional” and “direct-space” approaches.

In the *traditional* approach, the aim is to extract the intensities $I(hkl)$ of individual reflections directly from the powder diffraction pattern, and to use these $I(hkl)$ data in the types of structure solution calculation that are used for single-crystal diffraction data. Although there have been many successful applications of this strategy,^[7–9] organic molecular crystals represent a particularly challenging case. The main limitation of the traditional approach concerns the reliability of the intensities $I(hkl)$ that can be extracted from the experimental powder diffraction pattern, particularly in cases with substantial peak overlap.

In *direct-space* approaches, a large number of trial structures are generated/sampled in direct space by means of an appropriate search algorithm. The “quality” of each structure is assessed by comparing the powder diffraction pattern calculated for the structure and the experimental powder diffraction pattern, for example using the weighted profile R factor R_{wp} . As R_{wp} considers the whole digitized intensity profile, peak overlap is implicitly taken into account (and the need to extract integrated intensities $I(hkl)$ from the powder diffraction pattern is circumvented). In effect, the direct-space strategy involves searching the $R_{wp}(T)$ hypersurface to find the best structure solution (lowest R_{wp}), where T represents the set of variables that define the position, orientation, and intramolecular geometry of each molecule in the asymmetric unit (see below). Previous research has demonstrated the success of Monte Carlo^[11, 12], simulated annealing,^[13–17] and genetic algorithm (GA)^[18–22] methods for searching R factor hypersurfaces in direct-space structure solution from powder diffraction data.

In general, the limitations of direct-space structure solution concern the number and nature of the variables in the set T (defining the dimensionality and nature of the $R(T)$ hypersurface) and clearly the number of variables is larger for flexible molecules defined by a significant number of variable torsion angles. In the case of organic molecular crystals, another factor is the absence of dominant X-ray scatterers in the structure. The oligopeptide Phe-Gly-Gly-Phe studied herein represents a case of substantial molecular flexibility with no dominant X-ray scattering atoms.

In our GA approach for structure solution^[18, 19], a population of trial crystal structures is allowed to evolve subject to the types of rules and operations that govern evolutionary systems. Initially, the population comprises a set of N_p random structures. For the case with one molecule in the asymmetric unit, a given structure is specified by the coordinates $\{x, y, z\}$ of

the center of mass or a predefined pivot atom, the orientation is specified by rotation angles $\{\theta, \phi, \psi\}$ around a set of orthogonal axes, and the intramolecular geometry is specified by a set of variable torsion angles $\{\tau_1, \tau_2, \dots, \tau_n\}$. These variables represent the “genetic code” that uniquely characterizes each member of the population. The quality (“fitness”) of each structure depends on its value of R_{wp} (lower R_{wp} represents better agreement between experimental and calculated powder diffraction patterns and therefore higher fitness). The population is allowed to evolve through several generations by using the operations of mating, mutation, and natural selection. In the *mating* procedure, a given number (N_M) of pairs of structures (“parents”) are selected from the population, with the probability of selecting a given structure proportional to its fitness. New structures (“offspring”) are then generated by combining genetic information from the two parents. In the *mutation* procedure, a given number (N_X) of structures are selected at random from the population and random changes are made to parts of their genetic code to create mutant structures (the original structures from which the mutants are derived are still retained within the population). In the *natural selection* procedure, only the best structures are allowed to pass from one generation to the next. After a sufficient number of generations, the fittest member of the population (the structure with lowest R_{wp}) should be close to the correct crystal structure. In our most recent implementation of the GA method,^[23] each new structure generated during the GA calculation is subjected to local minimization of R_{wp} with respect to the structural variables in the set T , and only these minimized structures are used subsequently in the GA calculation. Introduction of local minimization in this way has been found to improve significantly the efficiency of finding the correct structure solution.

The powder X-ray diffraction pattern of Phe-Gly-Gly-Phe was recorded by using a conventional laboratory powder X-ray diffractometer.^[24] Prior to the structure solution calculation, the unit cell and space group were determined directly from the powder diffraction pattern.^[25] In the GA structure solution calculation, the molecule was defined by using standard bond lengths and angles. All peptide groups^[27] $R_1\text{-CO-NH-R}_2$ were maintained as planar units with the O-C-N-H dihedral angle fixed at 180° , and each benzene ring was constrained to be planar. All other torsion angles were varied freely (Figure 1). Thus, each structure considered in the GA calculation was defined by 17 variables $\{x, y, z, \theta, \phi, \psi, \tau_1, \tau_2, \dots, \tau_{11}\}$ (note that in Figure 1 and reference [29], a different notation for the torsion angles is used).

The GA calculation comprised 45 generations of a population of $N_p = 50$ structures. In each generation, 50 offspring ($N_M = 25$ pairs of parents) and $N_X = 10$ mutants were generated. The fitness of each structure is defined as a function of R_{wp} , and in the present case the linear fitness function $F(R_{wp}) = 1 - ((R_{wp} - R_{min}) / (R_{max} - R_{min}))$ was used, where R_{min} and R_{max} represent the minimum and maximum values of R_{wp} in the current population (R_{min} and R_{max} are updated as the population evolves under the GA). For the mating procedure between two selected parent structures, the 17 variables for a given parent were subdivided into three groups $\{x, y, z\}$, $\{\theta, \phi,$

ψ), and $\{\tau_1, \tau_2, \dots, \tau_{11}\}$. The three groups from each parent were distributed between the two offspring with no restriction on which combination of groups may come from each parent. In carrying out the mutation procedure on a selected structure, eight variables were selected at random, and a new random value was assigned to each of the selected variables (i.e. static mutation^[18, 19]). The evolution of R_{wp} during the GA calculation is shown in Figure 2, and demonstrates clearly that the quality of the population improves as the population evolves.

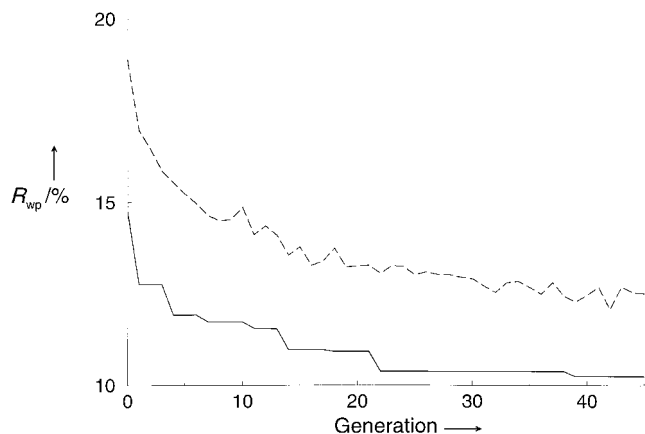


Figure 2. Evolutionary progress plot showing the evolution of R_{wp} for the best structure in the population (solid line) and the average R_{wp} for the structures in the population (dashed line) as a function of generation number in the GA structure solution calculation of Phe-Gly-Gly-Phe. Note that the values of R_{wp} refer to structures following local minimization.

The best structure solution (with lowest R_{wp} in the final generation) was taken as the starting structural model for Rietveld refinement using the GSAS program package.^[28] All atoms (with hydrogen atoms introduced into the structural model in calculated positions) were included in the refinement, with standard geometric restraints applied to the bond lengths and bond angles. Two isotropic displacement parameters were refined—a common parameter for the non-hydrogen atoms of the CH_2Ph groups, and a common parameter for all non-hydrogen atoms of the oligopeptide backbone. The isotropic displacement parameter for the hydrogen atoms was fixed at a standard value. In the final stages, a preferred orientation parameter was refined. The final Rietveld refinement (Figure 3) gave $R_{wp} = 0.053$ and $R_p = 0.034$ (data range, $5^\circ \leq 2\theta \leq 60^\circ$; number of reflections: 403; number of profile points: 2857; number of refined variables: 105). The fact that our successful structure determination of Phe-Gly-Gly-Phe was based on the use of experimental data collected on a conventional laboratory powder X-ray diffractometer emphasizes that it is not essential to use synchrotron X-ray powder diffraction data in this field.

The crystal structure of Phe-Gly-Gly-Phe (Figure 4a; see also Table 1 in Supporting Information) comprises ribbons of Phe-Gly-Gly-Phe molecules^[29] extending along the c axis. Adjacent molecules in these ribbons interact through three $\text{N-H}\cdots\text{O}$ hydrogen bonds ($\text{N}\cdots\text{O}$ distances 2.68–3.37 Å) involving the three peptide groups within the oligopeptide backbone (Figure 4b). Importantly, the structure and inter-

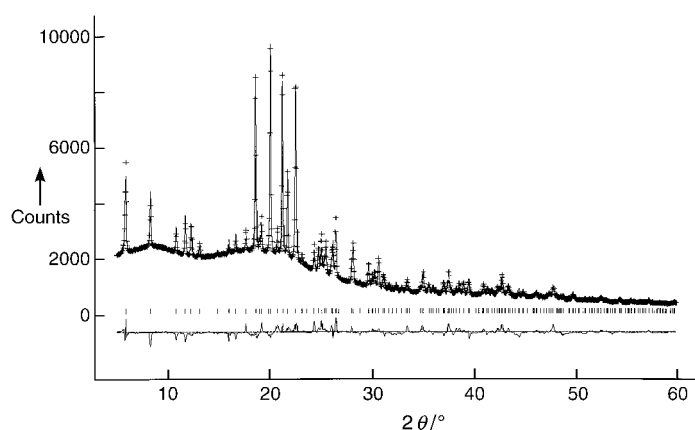


Figure 3. Experimental (+ marks), calculated (solid line), and difference (lower line) powder X-ray diffraction profiles for the Rietveld refinement of Phe-Gly-Gly-Phe. Reflection positions are marked. The calculated powder diffraction profile is for the final refined crystal structure. The refined lattice parameters are: $a = 15.084(1)$, $c = 9.7369(8)$ Å.

actions between molecules in these ribbons are analogous to an antiparallel β -sheet arrangement. In addition to hydrogen bonding, edge-to-face interactions between the phenyl rings of adjacent molecules in these ribbons may also be identified. As shown in Figure 4a, the end-groups of the oligopeptide chains are located around the 4_1 screw axis, and also engage in intermolecular $\text{N-H}\cdots\text{O}$ hydrogen bonding ($\text{N}\cdots\text{O}$ distances 3.05–3.22 Å). Around the 4_1 screw axis in a given unit cell there are four NH_3^+ end-groups (related to each other by the 4_1 screw) and four CO_2^- end-groups (related by the 4_1 screw). The hydrogen bonding pattern comprises two intertwined helical chains (Figure 4c), with $\text{N-H}\cdots\text{O}$ hydrogen bonding interactions existing within each chain but not between the chains. Each individual hydrogen-bonded chain comprises an alternating arrangement of NH_3^+ end-groups and CO_2^- end-groups, and has 2_1 screw symmetry. The overall 4_1 screw symmetry arises when the two individual chains are intertwined.

In conclusion, the successful determination of the crystal structure of the oligopeptide Phe-Gly-Gly-Phe demonstrates the scope and potential of current techniques for the complete determination of molecular crystal structures from powder diffraction data in the case of flexible molecules containing no dominant X-ray scattering atoms, and extends the application of techniques for powder structure solution into the arena of structural biology. The opportunities demonstrated here provide considerable promise for future successful applications of these techniques to solve crystal structures of increasing complexity in this field.

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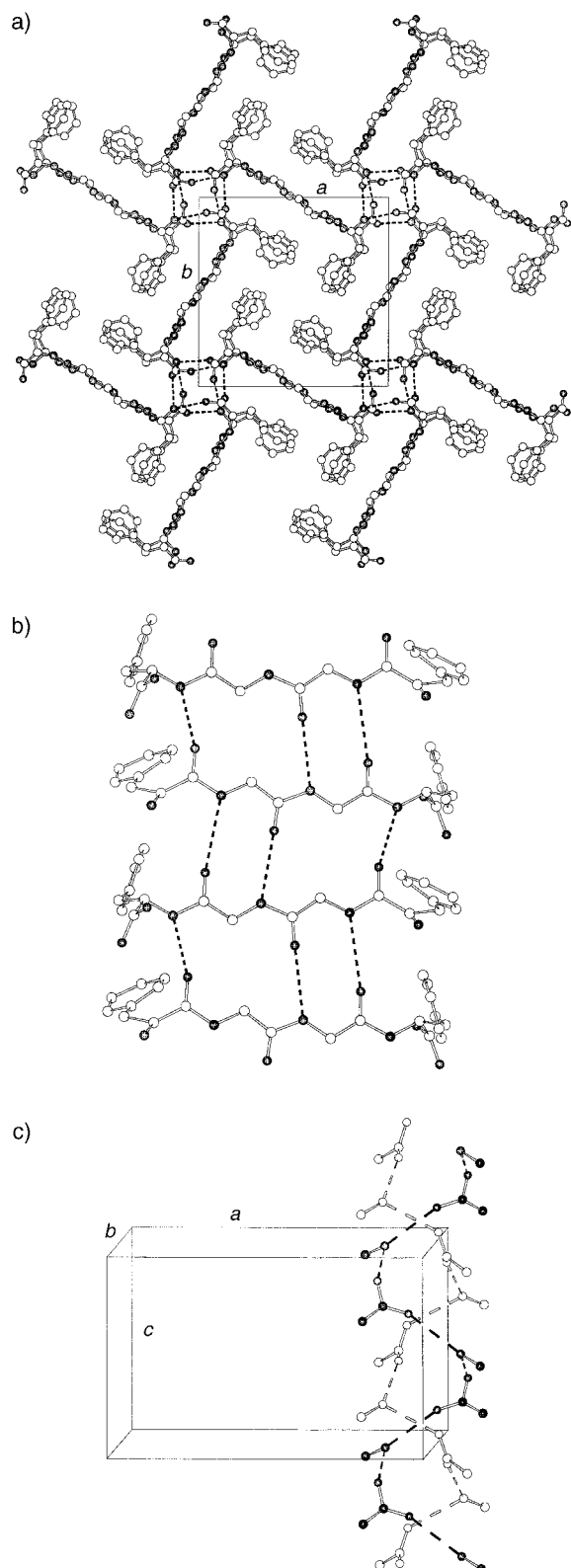


Figure 4. a) Final refined crystal structure of Phe-Gly-Gly-Phe viewed along the c axis. b) Interactions between adjacent molecules in the crystal structure of Phe-Gly-Gly-Phe (viewed perpendicular to the c axis) illustrating the formation of an antiparallel β -sheet arrangement. For clarity, hydrogen atoms are not shown. c) Illustration of the two intertwined helical hydrogen-bonded chains (open circles and filled circles, respectively) running along the c axis (vertical). Fragments comprising four atoms represent $\text{C}-\text{CO}_2^-$ end-groups; fragments comprising two atoms represent the $\text{C}-\text{N}$ component of $\text{C}-\text{NH}_3^+$ end-groups.

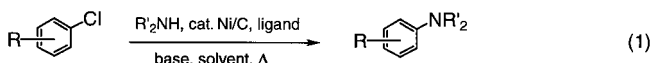
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- [24] A sample of Phe-Gly-Gly-Phe was obtained from Sigma-Aldrich and used directly as received. The powder X-ray diffraction pattern was recorded at 22 °C in transmission mode on a Siemens D5000 diffractometer, using Ge-monochromated $\text{CuK}\alpha_1$ radiation and a linear position-sensitive detector covering 8° in 2θ . The total 2θ range was 5° to 60°, measured in steps of 0.02° over 12 h.
- [25] The powder X-ray diffraction pattern was indexed (using the first 20 observable peaks) by the program TREOR,^[26] giving the unit cell: $a = b = 15.06$, $c = 9.72$ Å, $\alpha = \beta = \gamma = 90^\circ$. Systematic absences are consistent with the tetragonal space group $P4_1$, and density considerations suggest that there is one molecule of Phe-Gly-Gly-Phe in the asymmetric unit. Following unit cell determination and prior to structure solution, parameters describing the experimental lineshape were determined by fitting the powder diffraction profile with arbitrary intensities using the program PowderFit.^[17]
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- [29] The molecular conformation in the crystal structure is specified by the following values of the torsion angles (in standard notation^[30]) defined in Figure 1: $\psi_1 = -103(1)^\circ$; $\chi_1^1 = -175(1)^\circ$; $\chi_1^2 = -33(1)^\circ$; $\phi_2 = +164(1)^\circ$; $\psi_2 = +35(1)^\circ$; $\phi_3 = +176(1)^\circ$; $\psi_3 = +35(1)^\circ$; $\phi_4 = -107(1)^\circ$; $\psi_4 = -70(1)^\circ$; $\chi_4^1 = -50(1)^\circ$; $\chi_4^2 = -67(1)^\circ$.
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Aromatic Aminations by Heterogeneous Ni⁰/C Catalysis**

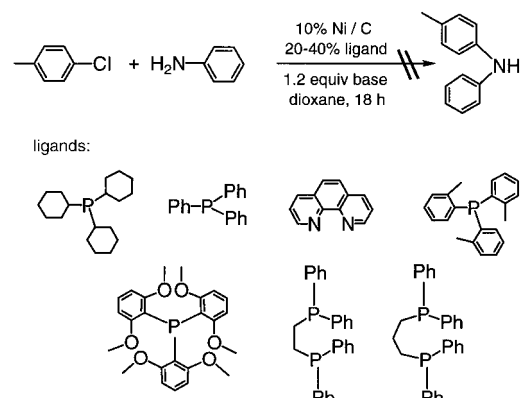
Bruce H. Lipshutz* and Hiroshi Ueda

*Dedicated to Professor Harry H. Wasserman
on the occasion of his 80th birthday*

Replacement of a leaving group on an aryl ring by nitrogen has been the focus of much research of late.^[1] Most successful have been methods which rely on Pd⁰ catalysis,^[2] where a variety of primary and secondary amines can replace a bromide, iodide, or pseudo halide, including in some cases chlorides,^[3] to afford the corresponding aniline compounds. Other approaches, which invoke Ni⁰ catalysis^[4] or titanium–nitrogen fixation,^[5] are uniformly conducted under homogeneous conditions.^[6] Given the importance of aryl amines in a variety of contexts (e.g., pharmaceuticals), a potentially scalable *heterogeneous*^[7] process which utilizes inexpensive precursor chlorides and nickel rather than palladium may provide a welcomed complement to existing protocols. Herein, we describe our initial work leading to an expedient method for effecting aminations of aryl chlorides using the heterogeneous catalyst^[8] nickel-on-charcoal [Ni/C; Eq. (1)].



In our previous work carbon–carbon bond-forming reactions mediated by Ni/C (e.g., Negishi,^[9a] Kumada,^[9b] and Suzuki^[9c] couplings) were usually best achieved in the presence of four equivalents of Ph₃P, relative to the percent loading of Ni(NO₃)₂ onto charcoal (ca. 5–10%). In the presence of Ph₃P, *n*BuLi (two equivalents relative to nickel) was introduced presumably generating the phosphane-ligated active Ni⁰/C. However, this combination was not applicable to displacements by an amine (i.e., conversions were low or no reaction occurred), nor were alternatives based on a screening of several other phosphane ligands (Scheme 1). Eventually it was observed that not only did 1,1'-bis(diphenylphosphanyl)-ferrocene (DPPF) lead to high yields of aminated aryl-ring products, but only one half of an equivalent (relative to nickel) of this bidentate ligand was necessary. In most cases, 5 mole % Ni/C was sufficient, which translated into the use of only 2.5% DPPF. While other ratios of Ni/C:DPPF could be employed (Figure 1), the 2:1 relationship was the best compromise. Either dioxane or toluene at reflux^[10] appears to be suitable as solvent, whereas THF (with a lower boiling point) led to limited levels of educt consumption. Curiously, the base LiOrBu consistently gave statistically significant



Scheme 1. Ligands shown to be unsuitable for displacement by amine.

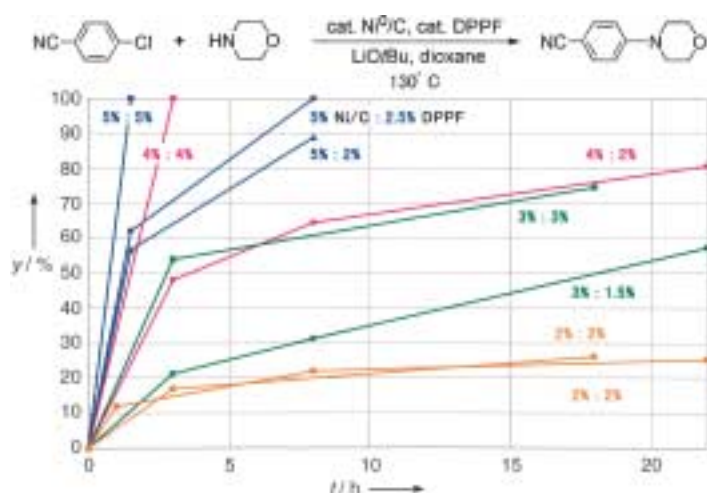
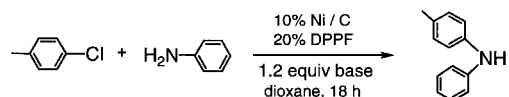
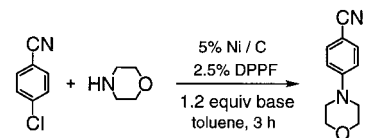


Figure 1. Reactivity of various % ratios of Ni/C:DPPF in aromatic amination reactions. y (%) = % Conversion.

greater rates of coupling and ultimately higher isolated yields than either the sodium^[2a] or potassium analogues (Schemes 2 and 3). Other bases (e.g., Cs₂CO₃) were ineffective. The



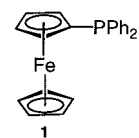
Scheme 2. Base: LiOrBu (93% conversion); NaOrBu (84% conversion); KOtBu (0% conversion).



Scheme 3. Base: LiOrBu (100% conversion); NaOrBu (19% conversion); KOtBu (18% conversion).

monodentate ligand **1**,^[11] examined in place of DPPF,^[12] was ineffective at the 2.5% or 10% level versus substrate (3% and 24% conversion, respectively).

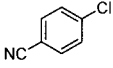
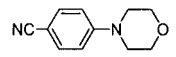
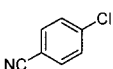
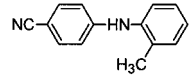
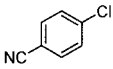
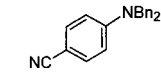
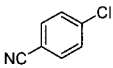
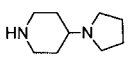
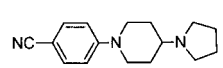
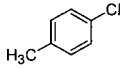
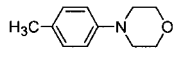
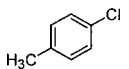
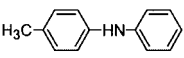
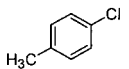
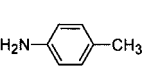
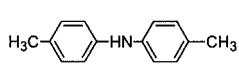
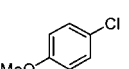
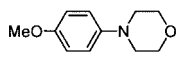
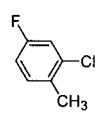
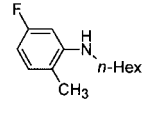
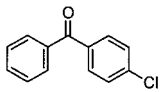
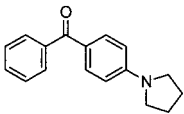
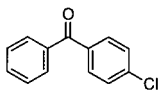
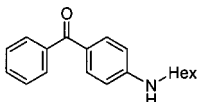
With LiOrBu (1.2 equiv)^[13] and the amine (2 equiv) in the presence of Ni/C, various aryl chlorides could be smoothly converted to their aniline derivatives (Table 1). The aryl chlorides



[*] Prof. B. H. Lipshutz, H. Ueda
Department of Chemistry
University of California
Santa Barbara, CA 93106 (USA)
Fax: (+1) 805-893-8265
E-mail: lipshutz@chem.ucsb.edu

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Table 1. Ni/C-catalyzed aminations of aryl chlorides.

Entry	Halide	Amine	Product ^[a]	Ni/DPPF loading [mol %]	Solvent ^[b]	Time [h]	Yield [%] ^[c]
1				5/10	dioxane	5	91
				5/2.5	dioxane	4.5	87
				5/2.5	toluene	2.5	88
2				10/20	dioxane	6	78
3		Bn ₂ NH		5/2.5	toluene	7	79
4				5/2.5	toluene	3.5	92
5				10/20	dioxane	47	91
6				5/10	dioxane	20	93
7				10/20	dioxane	24	90
8				5/5	toluene	52	70
9		n-HexNH ₂		5/2.5	toluene	46	76
10				5/2.5	toluene	16	87
11		n-HexNH ₂		5/5	toluene	15	69

[a] Fully characterized by IR, NMR, and MS data. [b] Oil bath temperature was maintained at 130 °C. [c] Isolated, chromatographically pure material. Bn = benzyl, Hex = hexyl.

which reacted favorably range from the electron-poor to the electron-rich, and include a test case for steric effects of *ortho* substitution (entry 9). Several anilines could be used as nucleophiles, including an *ortho*-methylated example (entry 2). Pyrrolidyl (entry 10), morpholinyl (entries 1, 5 and 8), and a piperidinyl system (entry 4) all participated, as did primary (entries 9 and 11) and secondary amines (e.g., dibenzylamine, entry 3). No aryl amination reactions were noted with hexamethyldisilazane, imidazole, 13-aza-[15]crown-5,^[14] and the sta base.^[15]

In summary, a novel method for effecting aromatic aminations using aryl chlorides under heterogeneous conditions^[16] is presented. Reliance on the combination of a Ni^{II} salt^[17] supported on activated charcoal may be viewed as an experimentally appealing and economical alternative to existing protocols, in particular to those which utilize Pd⁰ under homogeneous conditions.

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Application of a Spin-Labeled Spin-Trap to the Detection of Nitric Oxide (NO)**

Lucien Marx and André Rassat*

When a dilute monoradical reacts to give diamagnetic products, its EPR signal decays without major changes, while when a diradical is transformed into a monoradical, a new EPR spectrum is generally observed. In the case of a diradical with strong dipolar interaction,^[1] the single broad line is replaced by three narrow lines, and there is a very large increase of the monoradical signal. A narrow-line monoradical in very low concentration and a broad-line diradical in much higher concentration display peaks of similar height,^[2] as shown in Figure 1 where the EPR spectra of synthetic mixtures of tempone (2,2,6,6-tetramethyl-4-oxo-1-oxypiperidine (radical); line width $\Delta H = 0.18$ mT) and of diradical **1**^[3] ($\Delta H = 4.2$ mT) are presented: even when the monoradical constitutes only 0.1% of the total paramagnetic species, its signal can be detected.

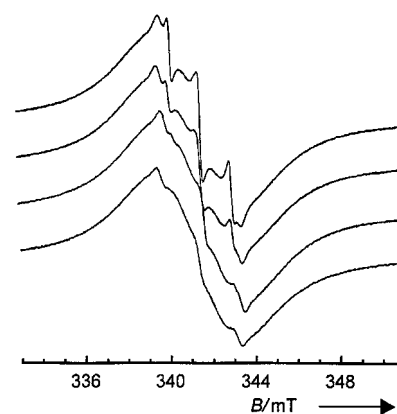


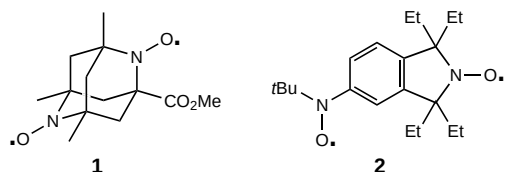
Figure 1. EPR spectra of ethanol solutions of synthetic mixtures containing (from top to bottom) 0, 0.1, 0.5, and 1 mole % of tempone relative to 10^{-3} M diradical **1**.

This suggests a new use of diradicals as *spin-labeled spin-traps*. These reagents would combine a stable radical (R_T), designed to trap selectively the radical to be detected and to yield diamagnetic products, and another radical (R_L), unreactive during this reaction, at a distance such that the diradical EPR spectrum would be a single line.^[4] In this way, as R_T reacts, the signal of the R_L moiety would be detected at the very beginning of the reaction, with both the concentration of spin trap and of spin adduct being monitored on the same spectrum. Furthermore, the rate of the trapping reaction (usually bimolecular) could easily be increased by increasing the biradical concentration.

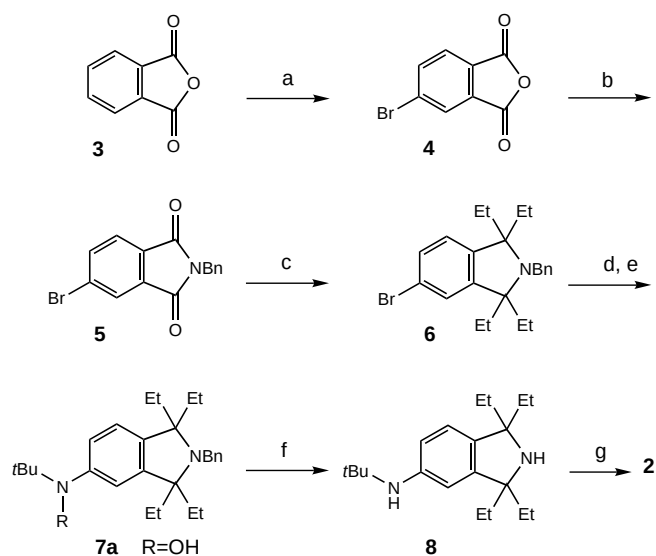
[*] Prof. A. Rassat, Dr. L. Marx
UMR CNRS 8640, Département de Chimie
Ecole Normale Supérieure
24 rue Lhomond, 75231 Paris CEDEX 05 (France)
Fax: (+33) 144323325
E-mail: andre.rassat@ens.fr

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As an example of this method, we have studied the trapping of NO by a new diradical **2**. Aromatic nitroxides like diphenylnitroxide and derivatives^[5] or some dihydroquinoline



nitroxides^[6] are known to react with NO, while the non-aromatic 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-yloxyl (radical) is reported to be unchanged in the presence of NO.^[7] Thus **2** (see Table 1) was designed to combine these two aminoxyl groups on an isoindoline backbone (for the non-aromatic moiety ethyl rather than methyl substituents were chosen to decrease any eventual reactivity)^[8] and was prepared in six steps from phthalic anhydride **3** (Scheme 1).^[9]



Scheme 1. Synthesis of diradical **2**: a) Br₂, 51%; b) BnNH₂, 95%; c) EtMgBr, 25%; d) *t*BuLi, (−78 °C, then 0 °C 30 min); e) *t*BuNO, (−78 °C), 51%; f) H₂, Pd/C, 95%; g) mCPBA, 60%. Bn = benzyl, mCPBA = 3-chloro peroxybenzoic acid.

Figure 2 shows the EPR spectra of **2** in ethanol; in fluid solution (a), the broad line observed ($\Delta H = 2.5$ mT) is in accord with the large dipolar interaction ($D = 400$ MHz, $D/2\mu_B = 14$ mT)^[10] measured on (b). Traces of monoradical (less than 1%) are seen in (a) and are more visible on the central part of (b). After several hours at room temperature, these spectra were unchanged.

When nitric oxide was bubbled into a deoxygenated solution of **2** in ethanol, samples were taken after 10, 30, 60, and 90 minutes and analyzed by thin layer chromatography (tlc) and EPR, a three-line spectrum, not apparent in the spectrum of the sample taken after 10 min, appeared superimposed on the diradical spectrum of the sample taken after 30 min (Figure 3). Four new nitroxides were then detected: they all displayed the same typical isoindolidinyloxyl (radical)

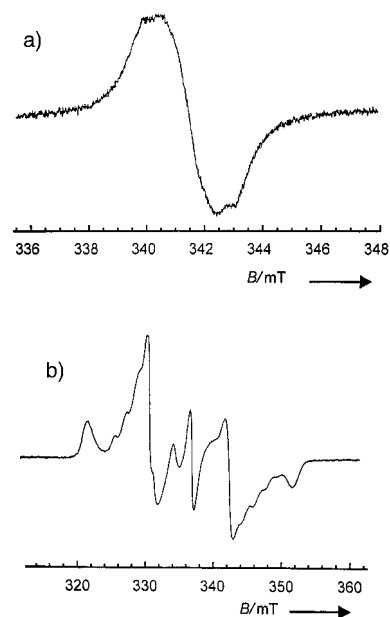


Figure 2. EPR spectrum of the diradical **2** (4×10^{-3} M in EtOH), a) at 20 °C, b) at 114 K.

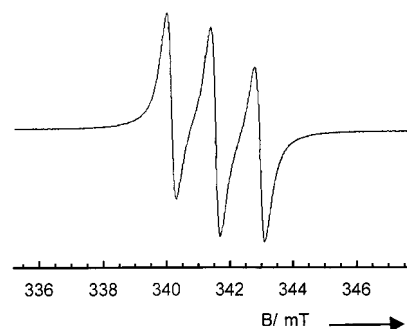
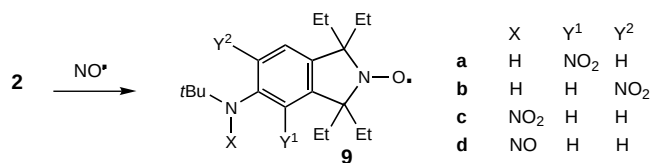


Figure 3. EPR spectrum obtained from a sample taken after 30 minutes of the reaction of diradical **2** with NO.

3-line EPR spectrum (toluene, $a_N = 1.37$ mT), totally different from the 9-line spectrum ($a_N = 1.26$ mT, $a_H = 0.23$ mT, 2H) of **7b** ($R = O^\bullet$, obtained by oxidation of **7a**; Scheme 1). Traces of **2** were no longer observed in the sample taken after 90 min, and after chromatography on alumina, four new compounds **9a–d** (Scheme 2) eluted in this order, were assigned the



Scheme 2. Reaction of diradical **2** with NO.

structures shown on the basis of their EPR, NMR,^[11] and UV spectra^[12] and mass spectrometry (Table 1). These compounds display narrow lines in the EPR spectrum, a characteristic particularly useful in dynamic nuclear polarization (DNP),^[13] and have recently been observed to present a large DNP enhancement factor.^[14]

Table 1. Selected analytical data for **2** and **9a–d**.

2 : red liquid; elemental analysis calcd(%) for $C_{20}H_{34}N_2O_2$: C 72.28, H 9.63, N 8.43; found: C 72.23, H 9.83, N 8.46. 9a : ^1H NMR (400 MHz, $CDCl_3$): δ = 9.2 (s, 1H; CH), 5.1 (s, 1H; CH), 1.5 (s, 9H; CH_3); UV/Vis (EtOH): λ_{max} [nm] (ϵ) = 443 (1800), 246 (16000); high resolution MS (FAB): m/z (%): 364.2589 (21) [$M^+ + 2H$], 363.2536 (40) [$M^+ + H$], 362.2450 (27) [M^+]. 9b : ^1H NMR (400 MHz, $CDCl_3$): δ = 10.4 (s, 1H; CH), 8.5 (s, 1H; CH), 1.6 (s, 9H; CH_3); UV/Vis (EtOH): λ_{max} [nm] (ϵ) = 440 (4600), 285 (shoulder, 4000), 242 (21000); high resolution MS (FAB): m/z (%): 363.2528 (100) [$M^+ + H$], 334.2135 (82.6) [$M^+ - C_2H_4$]. 9c : ^1H NMR (400 MHz, $CDCl_3$): δ = 9.43 (s, 1H; CH), 8.5 (s, 1H; CH), 5.9 (s, 1H; CH), 1.8 (s, 9H; CH_3); high resolution MS (FAB): m/z (%): 364.2594 (63) [$M^+ + 2H$], 363.2518 (60) [$M^+ + H$], 362.2451 (51) [M^+], 348.2657 (73). 9d : ^1H NMR (400 MHz, $CDCl_3$): δ = 9.8 (s, 1H; CH), 9.1 (s, 1H; CH), 5.6 (s, 1H; CH), 1.8 (s, 9H; CH_3); high resolution MS (FAB): m/z (%): 362.2804 (12) [$M^+ + CH_4$], 348.2655 (22) [$M^+ + 2H$], 347.2577 (44) [$M^+ + H$].

The use of this spin-trap for the in vivo detection of NO is more problematic because of the presence of reducing agents: when **2** was treated with ascorbic acid and the reaction monitored by EPR spectroscopy, the typical spectrum of **9e** (Scheme 2 where $X = OH$, $Y^1 = Y^2 = H$) immediately appeared and then decayed, but, as expected, at a much lower rate.^[8] However, it is possible to distinguish between the NO adducts and **9e** by combining different analytical methods such as mass spectrometry, ^1H NMR spectroscopy,^[7] (for **9a** and **9b**) and probably by high-field EPR spectroscopy.^[15]

Analogously other spin-labeled spin-traps may be used to study the reactions of a radical R_T with reactive radicals.^[16]

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Novel NO Biosensor Based on the Surface Derivatization of GaAs by “Hinged” Iron porphyrins**

Deng Guo Wu,* Gonen Ashkenasy,* Dmitry Shvarts, Rachel V. Ussyshkin, Ron Naaman,* Abraham Shanzer,* and David Cahen*

Nitric oxide (NO) plays an important role in biology as a mediator, for example, of the endothelial-derived relaxing factor (EDRF)—an agent responsible for the regulation of blood vessel relaxation and for the maintenance of blood pressure.^[1] Concentrations of 30 to 300 ppm of NO (1 ppm $\approx 3.3 \times 10^{-5}$ M) are sufficient to activate the guanylyl cyclase signaling cascade.^[2] Since NO is a gaseous and highly reactive species, its direct detection at low concentrations is difficult. The methods used to sense NO have generally been based on following changes in the UV/Vis spectra or electrochemical properties of the sensing elements.^[3] Electron paramagnetic resonance (EPR) and NMR image visualization of the distributions of NO free radicals in vivo were also reported, but were found to be limited by spatial resolution and sample size.^[4, 5] We report here a novel approach for the direct detection of low concentrations of NO free radicals in physiological aqueous solution (pH = 7.4).

The Molecular Controlled Semiconductor Resistor (MOCSER; see reference [6]) provides an excellent way to

[*] Prof. Dr. D. Cahen, Dr. D. G. Wu
Dept. of Materials and Interfaces
Weizmann Institute of Science
Rehovot 76100 (Israel)
Fax: (+972) 8-934-4239
E-mail: david.cahen@weizmann.ac.il
Prof. Dr. A. Shanzer, G. Ashkenasy
Dept. of Organic Chemistry
Weizmann Institute of Science
Rehovot 76100 (Israel)
Fax: (+972) 8-9342917
E-mail: coshanzr@wiccmail.weizmann.ac.il
Dr. D. Shvarts
AMOS Ltd., Rehovot 76100 (Israel)
Prof. Dr. R. Naaman, Dr. R. V. Ussyshkin
Dept. Of Chemical Physics
Weizmann Institute of Science
Rehovot 76100 (Israel)
Fax: (+972) 8-9344123
E-mail: ron.naaman@weizmann.ac.il

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detect NO. Recently we showed that the electrical resistivity of this device is controlled by the adsorption of molecules on a GaAs semiconductor surface.^[6, 7] Here we present a modified version of the MOCSEER in which the GaAs surface is derivatized by two-component molecular systems prior to the experiment. Changes in the MOCSEER's current when NO binds to the outer molecular component are examined to follow the binding events in situ.

In these systems iron(III) tetraphenylporphyrin chloride (FeTPPCL) moieties, the NO binders, are not bound directly to the GaAs surface, but rather, they are "hinged" remotely from the surface by bifunctional ligands (Scheme 1). These ligands bind axially, through imidazolyl groups, to the fifth and sixth coordination sites of the iron(III) porphyrin, and simultaneously to the GaAs surface of the MOCSEER through anchoring groups—disulfides or carboxylic acids. A special feature of such a configuration is that the iron porphyrins will, on the average, be oriented perpendicular to the surface. The axial ligation to the metalloporphyrin rings prevents π – π interactions between them—interactions that are expected to decrease the porphyrin activity.^[8] The imidazole rings of the ligands are bound to the Fe^{III} center in a kinetically labile fashion, so that one of them can be replaced by a stronger binding substrate that approaches near the porphyrin.^[9]

Complexes of FeTPPCL with the four bifunctional ligands shown in Scheme 1 were used to probe the molecular features that may affect the sensing event. Ligand **1** has a symmetric bidentate metalloporphyrin binding site made of *N*-alkylimidazoles and a disulfide as the anchoring group; ligand **2** has the same anchor, but with a nonsymmetric metalloporphyrin binding site, in which the NH group of the imidazole ring imposes a weaker binding of the *N*-alkylimidazole group at the opposite face; ligand **3** is symmetric in binding to the iron porphyrin, but it has a malonic acid as an anchor which binds

more strongly to the surface.^[7] Modification in both elements relative to **1** were done in ligand **4**, which contains the nonsymmetric binding site for iron porphyrins and a malonic acid anchor. Devices covered by the iron porphyrin complexes of ligands **2**–**4** were expected to be more sensitive to NO than those covered by the corresponding complexes of the "parent" ligand **1**.

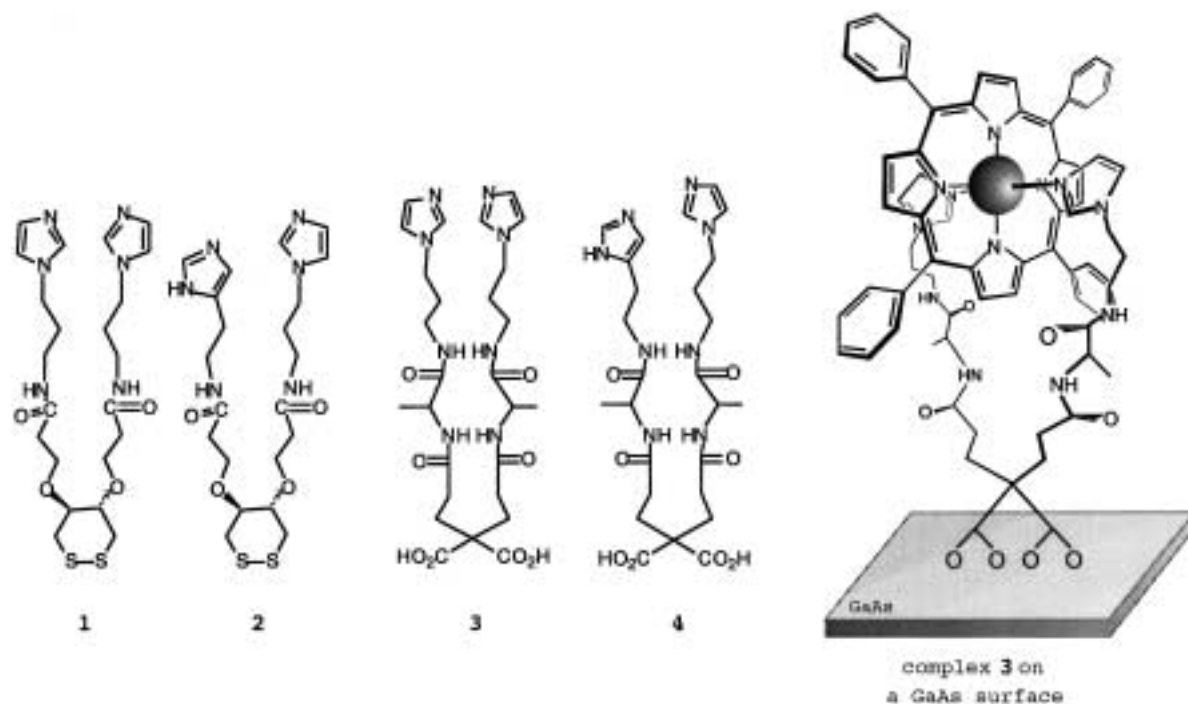
The formation of the expected low-spin ligand–FeTPPCL complexes was evidenced in solution by UV/Vis and ¹H NMR measurements, as described earlier for ligand **1**.^[8] Freshly formed complexes of the four ligands with FeTPPCL were adsorbed onto single crystal GaAs and characterized by FT-IR spectroscopy, using the etched, bare (but oxidized) GaAs as a reference. The main absorbance bands (1700–900 cm^{–1}) are listed in Table 1. For comparison, the corresponding bands of the complexes in KBr are also listed. The typical vibrations

Table 1. Main assignments of the vibrations in the transmission FT-IR spectra (cm^{–1}) for complexes of **1**–**4** on single-crystal GaAs(100) surfaces. The values obtained in KBr are given in parenthesis.

Complex	$\tilde{\nu}_{\text{CONH}}$	$\tilde{\nu}_{\text{COOH}}$	$\tilde{\nu}_{\text{C=C}}$	$\tilde{\nu}_{\text{aromatic}}$	$\tilde{\nu}_{\text{C-O}}$
1	1660 (1653)		1551 (1558)	1440 (1442)	1100 (1096)
2	1651 (1652)		1595 (1597)	1441 (1440)	1093 (1094)
3	1630–1690 ^[a] (1653)	(1715)	1546 (1539)	1439 (1441)	1093 (1094)
4	1626–1683 ^[a] (1670)	(1716)	1552 (1554)	1441 (1440)	1137 (1136)

[a] Broad peak overlapping $\tilde{\nu}_{\text{COO}^-}$ vibration at ≈ 1640 cm^{–1}.

of the carbonyl group of the amide moieties and of the ether bonds (in the complexes formed from ligands **1** and **2**) were observed at approximately 1650 and 1100 cm^{–1}, respectively. Strong and broad bands were observed at 1625–1690 cm^{–1} in the spectra of the complexes formed from **3** and **4** because



Scheme 1.

bands of the carbonyl group of the amide moieties overlapped those of the carboxylate groups. The disappearance of the band corresponding to the carboxylic group in the spectrum (ν_{COOH} (vibration) $1715\text{--}1716\text{ cm}^{-1}$ in KBr) when complexes derived from **3** or **4** were adsorbed onto the GaAs indicates that their carboxylic groups bind to the GaAs surface.^[10] The intensities of the absorbance peaks of ν_{CONH} or ν_{COO^-} were about $0.5\text{--}1.5 \times 10^{-3}$, which indicates that a film of about one monolayer is formed by each of the complexes (from ligands **1–4**) on the GaAs substrate.^[11] The ratios of the intensity of the vibrations corresponding to the ligands to those of the iron porphyrins remain similar to the ratios obtained from the KBr measurements, thus eliminating the possibility that the complexes decomposed during the adsorption process.

Contact potential differences (CPD) were measured under photosaturation conditions, to reveal the surface band bending (BB) of the bare GaAs surface, and of surfaces covered with monolayers of the complexes formed with ligands **1–4**.^[12] Adsorption of the two complexes formed from ligands with disulfide anchors (**1** and **2**) resulted in a small decrease in the BB relative to the bare surfaces. After adsorption of the complexes formed from ligands **3** or **4**, a much more significant decrease in the BB was observed,^[13] which was ascribed to the stronger binding of their carboxylic groups, relative to the disulfide groups, to the surface.

The response of MOCSEs covered by the complexes formed from **1–4** to different concentrations of NO was determined in aqueous buffer solutions (pH = 7.4) at 25 °C. A constant voltage of 100 mV was applied between the ohmic contacts of the MOCSE during the experiment. Figure 1 shows the changes in the current with time for MOCSEs covered by complexes derived from **1**, when four different concentrations of the organic NO precursor, 1-hydroxy-3-methyl-3-(methylaminopropyl)-2-oxo-1-triazene, were released into the solution. At physiological pH this reagent releases quantitatively two equivalents of NO with first order kinetics and $t_{1/2} = 10.1$ minutes at room temperature.^[14–16] As a

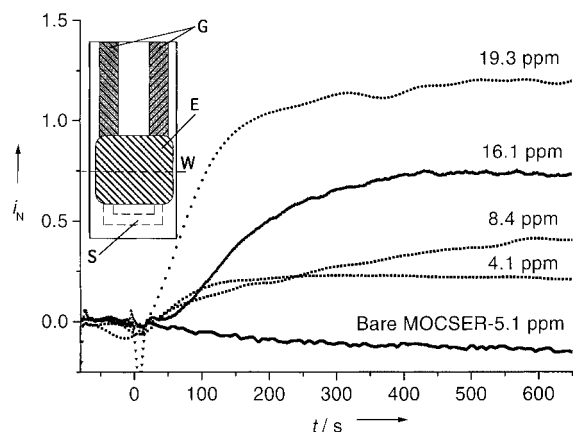


Figure 1. Changes in current over time on exposure of a MOCSE covered by complexes derived from **1** to different concentrations of 1-hydroxy-3-methyl-3-(methylaminopropyl)-2-oxo-1-triazene that releases NO. The lower plot refers to reference measurements with a bare MOCSE. All measurements were done at room temperature, in pH = 7.4 buffer solution, and under N_2 . Inset: schematic drawing of the sensing surface of the MOCSE. S: sensing window (0.3 mm^2), E: epoxy, G: AuGeNi ohmic contacts, W: water level.

consequence of the slight differences in the sensing area and light conditions, the current was normalized by dividing the measured current values by the different absolute responses of the complex-covered MOCSEs to the aqueous medium without NO.^[17, 18] In less than ten minutes following the introduction of NO no further changes in current were observed, and the saturation current increases with an increase of the NO “steady state” concentration.^[19]

The experimental system was kept under anaerobic conditions (N_2) for 30 minutes before and during the experiment to remove practically all the O_2 and to prevent formation of the undesired NO_2 . No changes in current were observed when MOCSEs covered by the complexes were exposed to the unreactive by-product of the proteolysis, that is, *N,N*-dimethyl-1,3-propylamine.^[14] Only a very slight decrease in current was observed when bare MOCSE devices were exposed to various precursor concentrations (0.5–5 ppm). The changes in current with time for the bare MOCSE with 5 ppm of the precursor are included in Figure 1. The less stable current obtained in this measurement shows that adsorption of the complexes stabilizes the device surface.

Figure 2 shows the linear correlation of the saturation current (normalized) of MOCSEs covered by complexes of ligands **2–4** as a function of the NO “steady-state” concentration. To compare the sensitivity of the different MOCSE types toward NO, we followed the (interpolated) current values on exposure of each to 2.0 ppm of NO. This value was below our signal/noise (data not shown) for the MOCSE

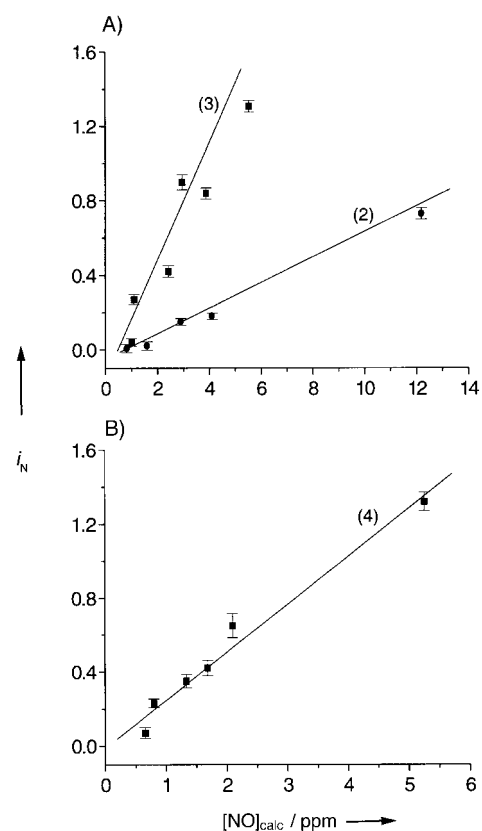


Figure 2. Normalized current of MOCSEs covered by complexes derived from **2** and **3** (A), or **4** (B), as a function of the NO “steady-state” concentration.^[19] Different MOCSEs were used for each measurement.

covered by the complex with **1**, and the normalized current values^[17] for MOCSEs with complexes formed from **2**, **3**, and **4** were 0.07, 0.38, and 0.50, respectively.

Two methods were used to release NO from the saturated sample (complex with **3**, 5.0–10.0 ppm NO) and to regenerate the ability of the MOCSE to detect NO. In the first one, the NO-saturated sample was taken out of the buffer solution, dried in a stream of dry N₂ for less than 15 seconds, and re-immersed in a clean buffer solution. A current typical for the NO-free system could then be measured. In the second method, the NO-containing solution was replaced by a clean buffer solution, and the current of the NO-free system was reached within a few minutes ($t_{90} \approx 120$ s). Subsequent exposure of the samples to the same concentration of NO resulted in the (saturation) current being 70–85 % of the original change, which demonstrates the system is reasonably reversible under these conditions.

The system responds to NO mainly through the binding of NO to the iron(III) porphyrin moieties,^[20] and not by direct binding to the surface. The increase in current when the NO binds to the derivatized surfaces results from a decreased potential drop across the conducting GaAs layer (of the device). Such a decrease is explained by a change in the dipole of the iron porphyrin/ligand complex following the replacement of one coordinated imidazole ring by the NO radical (stronger positive monopole at the binding group).^[21]

Higher sensitivity was obtained for MOCSEs with the nonsymmetric complex formed from **2** than with the complex of **1**, as deduced from the detection values of 2.0 ppm of NO. This result can be understood from the greater ease by which NO replaces the *N*-alkylimidazolyl ring of ligand **2** relative to the imidazolyl ring of ligand **1** as a chelator to the Fe^{III} center. A similar process was observed earlier for replacement of the coordinated *N*-alkylimidazole ring by O₂.^[22] Devices covered by the complexes formed with ligand **3** are also more sensitive to NO than those covered by complexes derived from **1**. Since the Fe^{III} binding sites in the two complexes are the same, this enhancement of the signal with **3** is attributed mainly to a stronger binding of the dicarboxylate group to the surface, relative to the binding of the disulfide group.^[23] The tighter mode of binding is expected to improve the electrical communication between the anchoring site on the GaAs surface and the NO binding site. Such communication can be considered as signaling through the ligand skeleton, via its anchor, to the surface.

Devices covered by the complexes of **4** were found to be the most sensitive to NO. By utilizing the complex formed with **4** we demonstrated simultaneously: a) a better NO binding to FeTPPCL, relative to the complex with **3**, occurs with the nonsymmetric metalloporphyrin binding site, and b) an enhanced signaling to the GaAs surface via the dicarboxylic anchors occurs, relative to that found in devices covered by the complexes formed with **2**. We note that the currents obtained when MOCSEs covered by complexes derived from **4** were exposed to NO are similar to the summation in current obtained in the two separate measurements with devices covered by the complexes derived from **2** or **3** (for example, for 2.0 ppm of NO). This phenomenon suggests that the symmetry of the metalloporphyrin binding site and the

type of anchoring group dictate independently the activity of the entire system.

In recent, preliminary, experiments we observed that dry devices covered by complexes from **1** or **2** are much more sensitive to NO(g) than to O₂(g) or CO(g). This result is reasonable if one considers the weaker binding of O₂(g) and CO(g) to iron(III) porphyrins.^[24] Experiments, designed to sense NO exclusively in a biological environment of competing ligands, for example, other NO_x species, are under way.

Experimental Section

Synthesis: The synthesis of ligands **1** and **2** was described earlier.^[8, 22] and the detailed procedure to make ligands **3** and **4** will be described soon.^[25] **Characterization of 3:** IR (KBr): $\bar{\nu}$ = 1652 (CONH), 1715 cm⁻¹ (COOH); ¹H NMR (CD₃OD): δ = 8.20 (s, 2H), 7.30 (s, 2H), 7.10 (s, 2H), 4.12 (br, 6H), 3.19 (t, *J* = 7.0 Hz, 4H), 2.19 (t, *J* = 6.5 Hz, 4H), 2.12 (t, *J* = 6.5 Hz, 4H), 1.99 (quint, *J* = 7.0 Hz, 4H), 1.30 (d, *J* = 8.0 Hz, 6H); FAB-MS: *m/z*: 604.8 [*M* + H]⁺. **4:** IR (KBr): $\bar{\nu}$ = 1670 (CONH), 1716 cm⁻¹ (COOH); ¹H NMR (CD₃OD): δ = 7.76 (s, 2H), 7.18 (s, 1H), 6.99 (s, 1H), 6.90 (s, 1H), 4.24 (dq, 2H), 4.06 (t, *J* = 6.5 Hz, 2H), 3.35 (t, *J* = 7.0 Hz, 2H), 3.16 (t, *J* = 7.0 Hz, 2H), 2.79 (t, *J* = 7.0 Hz, 2H), 2.21 (br, 4H), 2.14 (br, 4H), 1.98 (quint, *J* = 7.0 Hz, 2H), 1.26–1.34 (dd, 6H); MS (electrospray): *m/z*: 591.4 [*M* + H]⁺.

Complexes of each ligand with FeTPPCL were made by mixing 1/1 solutions (> 15 mm) in DMF (**1** or **2**), CHCl₃/CH₃OH 9/1 (**3**), or CHCl₃/2,2,2-trifluoroethanol (TFE) 8/2 (**4**).

Sample preparation: Etched MOCSEs or GaAs substrates (NH₃/H₂O 1/9) were immersed in solutions of freshly prepared ligand–FeTPPCL complexes overnight. The substrates were then rinsed by CHCl₃/hexane (5 % v/v, **1** or **2**) or by CHCl₃ (**3** or **4**), and dried (N₂). To prevent leakage of the electrical current through the conducting solution, the device was encapsulated using epoxy, leaving a “sensing window” uncovered (inset to Figure 1). The linearity of the *I*-*V* characteristics was checked during the measurements by a Keithley 236 Source Measure Unit.

Sensing NO by the derivatized MOCSEs: The NO-release solution was prepared by dissolving a weighted amount of commercial 1-hydroxy-3-methyl-3-(methylaminopropyl)-2-oxo-1-triazene (Sigma) in a 0.01 M solution of NaOH. These conditions for preparing the NO solution and for NO injection are very similar to those previously described.^[14] At basic pH values the reagent is stable, and at physiological pH it releases 2.0 equivalents of NO with first order kinetics having $t_{1/2}$ = 10.1 min at 22 °C. As shown in ref. [14] NO is the only nitrosyl compound obtained during proteolysis.^[14] After the MOCSE current had been stabilized in the (pH = 7.4) phosphate buffer solution, the precursor was injected, and the saturation current was obtained within 10 min.

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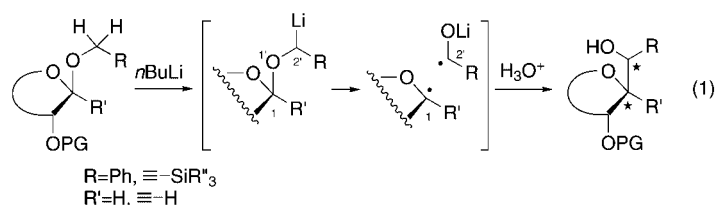
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Stereoselective Synthesis of Highly Functionalized C-Glycosides based on Acetal [1,2] and [1,4] Wittig Rearrangements**

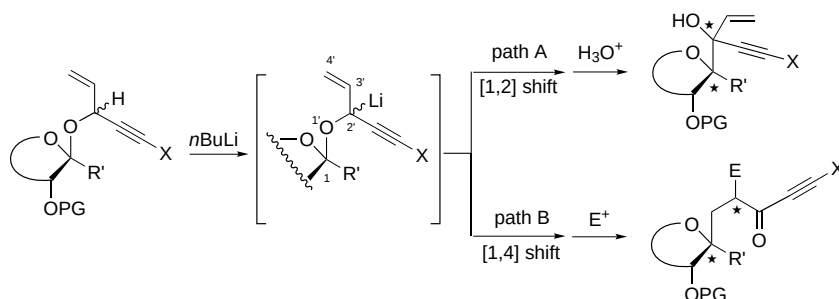
Katsuhiko Tomooka,*
Hiroshi Yamamoto, and Takeshi Nakai

The [1,2] Wittig rearrangement is a classic class of carbanion rearrangements which is now well recognized to proceed by a radical dissociation–recombination mechanism.^[1] De-

spite potentially useful carbon–carbon bond forming reactions, however, synthetic application of this rearrangement still remains severely limited, mainly because of the rather low yields and the restricted range of substrates. To this end, we have recently developed the acetal [1,2] Wittig rearrangement protocol which provides C-glycosides from primary-alcohol-derived O-glycosides in a highly diastereoselective manner [Eq. (1)].^[2, 3]



To extend this protocol we envisioned that the rearrangement of a more functionalized O-glycoside system with a secondary-alcohol-derived migrating terminus might afford the C-glycoside with a tertiary alcohol side chain.^[4] A key feature for the success of the rearrangement is a rational design of a migrating terminus. Thus, we selected the ethynylvinylmethanol system as the migrating terminus with a view that the ethynyl and vinyl substituent not only enhances the [1,2] Wittig reactivity, as a result of the large radical stabilizing effect, but also imparts unique multifunctionality to the products (Scheme 1, path A). Here we report a stereoselective entry to the highly functionalized C-glycosides based on an acetal [1,2] Wittig rearrangement and also a novel



Scheme 1. [1,2] and [1,4] Wittig rearrangement approach to the multi functionalized C-glycosides.

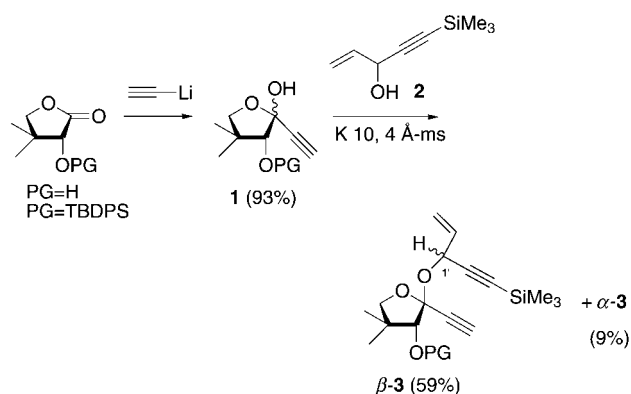
[1,4] Wittig rearrangement of an acetal system (Scheme 1, path B), which was discovered by chance in the [1,2] rearrangement study.

At the outset we studied the rearrangement of acetal β -3 which was prepared from (–)-dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone ((–)-pantolactone) as a 1:1 epimeric mixture at the C1' position in three steps: protection of the hydroxyl groups, addition of lithium acetylide, and a montmorillonite K 10 clay catalyzed acetalization^[5] with the protected ethynylvinylmethanol **2** (racemate) [Eq. (2); TBDPS = *tert*-butyldiphenylsilyl]. Two epimers of β -3 were separable by chromatography on silica gel and their stereochemistries were determined by X-ray crystallography and by chemical conversion.^[6] The reaction of β -(R)-3 with *n*BuLi (3 equiv) in THF at -78°C afforded (< 10 min) the [1,2] re-

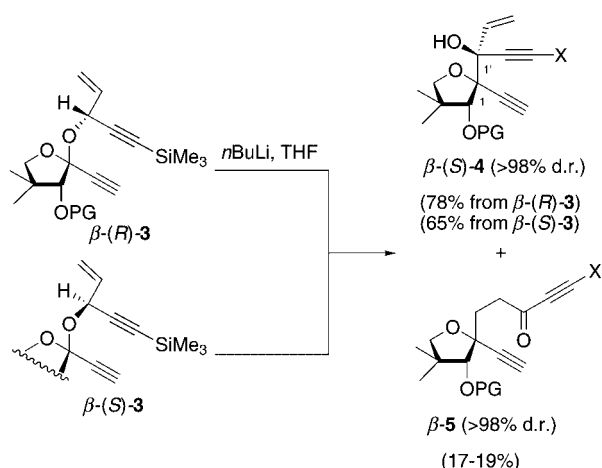
[*] Prof. Dr. K. Tomooka, Dr. H. Yamamoto, Prof. Dr. T. Nakai
Department of Applied Chemistry
Graduate School of Science and Engineering
Tokyo Institute of Technology
Meguro-ku, Tokyo 152–8552 (Japan)
Fax: (+81)3-5734-3931
E-mail: ktomooka@o.cc.titech.ac.jp

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



arrangement product β -(*S*)-4 as a single diastereomer^[6] in 78% yield,^[7] along with a small amount of the [1,4] rearrangement product β -5 (Scheme 2).^[8] Significantly, a similar

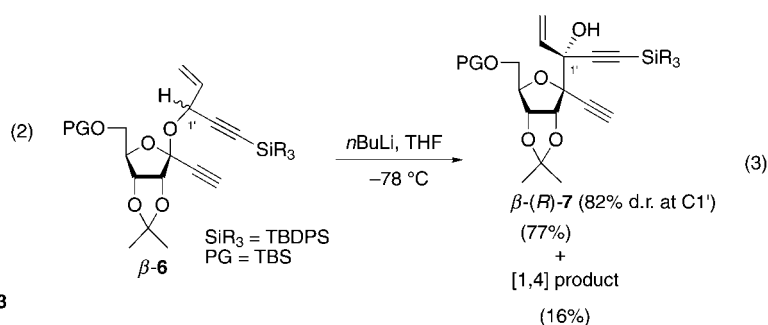


Scheme 2. Wittig rearrangement of *O*-glycosides β -(*R*)-3 and β -(*S*)-3. X = SiMe₃ or H.

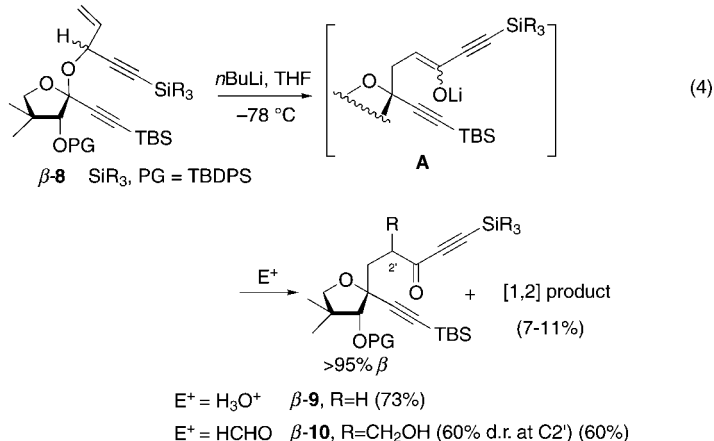
reaction of the β -(*S*)-3 epimer gave the same diastereomer, β -(*S*)-4 (>95% d.r., 65% yield) even though the reaction was slower than with the *R* epimer ($-78 \rightarrow -10^\circ\text{C}$, 50 min). These stereochemical outcomes indicate that acetal 3 undergoes the rearrangement with complete retention at the migrating anomeric center as expected, and in a highly diastereoselective manner (not a stereospecific manner) in regard to the chiral center at the tertiary alcohol in the side chain. This result means that efficient stereocontrol does occur during the rearrangement, and hence no separation of the C1' epimers is required to obtain the single stereoisomer of the rearrangement product.

This protocol is an efficient approach to multifunctionalized *C*-glycosides. In fact, a similar rearrangement of a 1:1 epimeric mixture of *O*-glycoside 6, prepared from the lactone derived from D-ribose, was found to give *C*-glycoside 7 in a highly diastereoselective manner (>95% β , 82% *R* configuration)^[9, 10] in 77% yield [Eq. (3); TBS = *tert*-butyldimethylsilyl].

Next, our interest turned to the previously observed side reaction—the [1,4] rearrangement—because of its potential value as a unique approach to *C*-glycosides. A key issue to



address was the switching of the periselectivity from a [1,2] to a [1,4] fashion. After several attempts, we finally found that the introduction of a *tert*-butyldimethylsilyl group to the ethynyl unit at the C1 position could solve this problem. The reaction of β -8 (1:1 epimeric mixture at C1') with *n*BuLi provided the [1,4] rearrangement product β -9 as the major product [73% yield; Eq. (4)]. This protocol represents the first example of a *C*-glycoside synthesis based on a [1,4] Wittig rearrangement. Moreover, we attempted to intercept the lithium enolate A^[11] with an external electrophile. Thus, β -8 was treated successively with *n*BuLi and formaldehyde to afford the expected adduct 10 (60% d.r.) in good yield.



In summary, we have demonstrated that the [1,2] Wittig rearrangement of the ethynylvinylmethanol-derived *O*-glycoside proceeds in a highly diastereoselective manner to afford the β -*C*-glycoside that has contiguous quarternary chiral centers at the C1 and C1' positions. In addition, we have shown a novel approach to the *C*-glycoside that is based on a [1,4] Wittig rearrangement. The scope and limitation of the present *C*-glycosidation methodologies as well as its applications to natural products synthesis are in progress.

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- [11] The trapping experiment of enolate **A** (SiMe₃Cl, –78 °C) gave an *E*:*Z* mixture (ca. 1:1) of silyl enol ether in 69% yield.

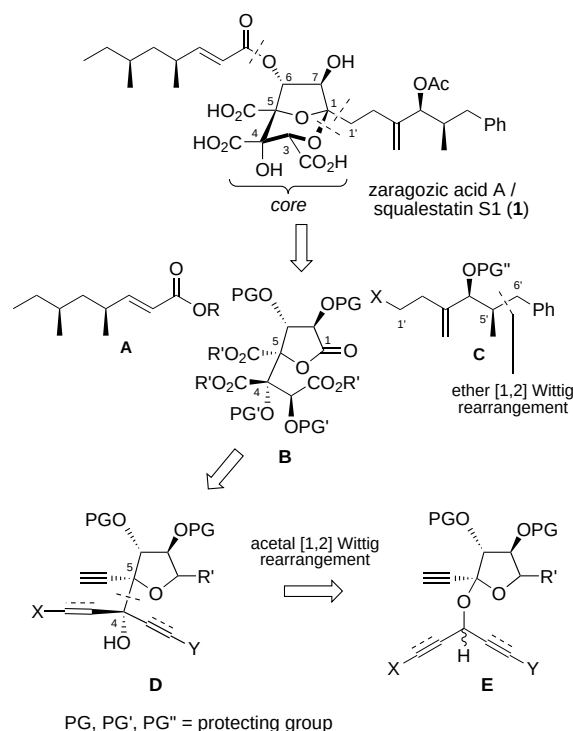
Stereoselective Total Synthesis of Zaragozic Acid A based on an Acetal [1,2] Wittig Rearrangement**

Katsuhiko Tomooka,* Makoto Kikuchi, Kazunobu Igawa, Masaki Suzuki, Ping-Huai Keong, and Takeshi Nakai

The zaragozic acids and squalostatins are a family of naturally occurring fungal metabolites independently isolated and characterized by researchers at Merck^[1] and Glaxo.^[2] These natural products are potent inhibitors of squalene synthase and have potential as therapeutic agents for the treatment of hypercholesterolemia.^[1a, 2a] All the zaragozic acids and squalostatins have a common 2,8-dioxabicy-

clo[3.2.1]octane core with an array of six stereogenic centers including contiguous quarternary carbon atoms, and are different only at the alkyl and acyl side chains at C1 and C6, respectively. Therefore, it is not surprising that these compounds have elicited considerable attention from numerous synthetic chemists.^[3, 4] Zaragozic acid A (squalestatin S1; **1**) is a representative example of this novel class of compounds. Herein, we report the efficient, convergent synthesis of **1** by highlighting the acetal [1,2] Wittig rearrangement^[5] for forming the C4–C5 bond together with the simultaneous creation of the contiguous quaternary carbon atoms as the key step.

Our strategy is shown in Scheme 1. Disconnection of the C6 side chain **A**, unraveling of the C1 acetal, and cleavage of the C1–C1' bond led to lactone **B** and an alkyl anion equivalent



Scheme 1. Retrosynthetic analysis of zaragozic acid A (**1**).

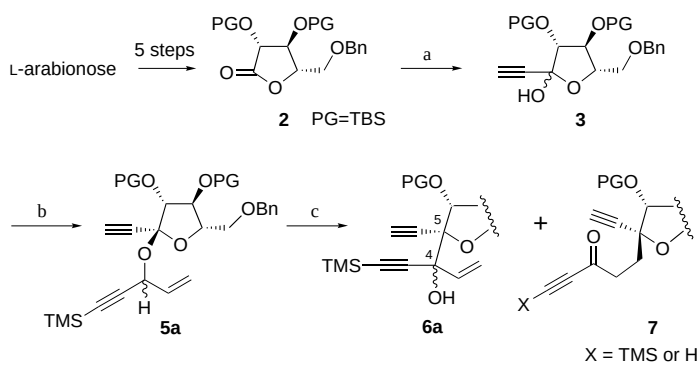
C.^[6] Our interest in the carbanion rearrangement of the sugar derivatives led us to consider a strategy for lactone **B** starting from the C-glycoside **D**, namely an acetal [1,2] Wittig rearrangement product of the O-glycoside **E**. The preceding paper describes the synthesis of this class of highly functionalized C-glycosides based on a rearrangement protocol.^[7] Furthermore, we planned to construct a C5'–C6' bond in segment **C** using the original ether [1,2] Wittig rearrangement.^[8]

Our synthesis begins from the O-glycoside **5a** ($\cong$ **E**), which can be prepared from the hemiketal **3** derived from L-arabino- γ -lactone^[9] and the protected ethynylvinylmethanol **4** (racemate; Scheme 2). Previous work suggested that the carbanion rearrangement of an O-glycoside such as **5a** should produce the [1,2] rearrangement product with high diastereoselectivity and in good yield.^[7, 9] In contrast to this expect-

[*] Prof. Dr. K. Tomooka, Dr. M. Kikuchi, K. Igawa, M. Suzuki, P.-H. Keong, Prof. Dr. T. Nakai
Department of Applied Chemistry
Graduate School of Science and Engineering
Tokyo Institute of Technology
Meguro-ku, Tokyo 152–8552 (Japan)
Fax: (+81)3-5734-3931
E-mail: ktomooka@o.cc.titech.ac.jp

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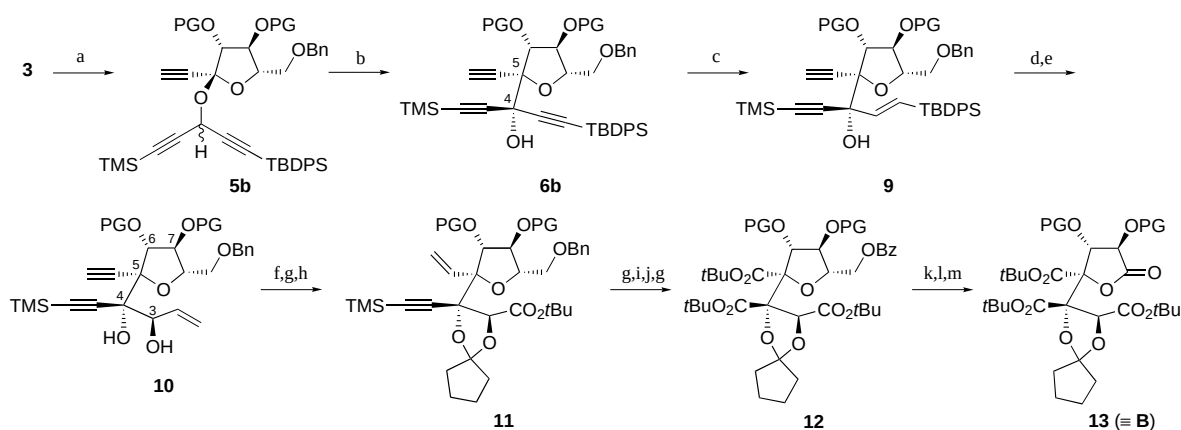


Scheme 2. Synthesis and rearrangement of *O*-glycoside **5a**. a) Lithium acetylide, Et₂O, −78 °C, 75%; b) CH₂=CHCH(OH)C≡C-TMS (**4**), montmorillonite K 10, 4 Å-MS, CH₂Cl₂, RT, 55%; c) *n*BuLi, THF, −78 °C, **6a**: 14%, **7**: 17%, recovered **5a**: 46%. TBS = *tert*-butyldimethylsilyl, Bn = benzyl, TMS = trimethylsilyl.

ation, the reaction of β -**5a** (*n*BuLi, −78 °C, THF) proceeded to form a 1:1 diastereomeric mixture of the desired [1,2] shift product β -**6a** in poor yield, along with a significant amount of the [1,4] rearrangement product **7**. Thus, we tried to redesign the rearrangement system.

Finally, we found that the bis(ethynyl)methanol system satisfied all the requirements as the migrating terminus. The reaction of the *O*-glycoside **5b** ($\cong$ **E**), similarly prepared from ketal **3** and the protected bis(ethynyl)methanol **8** (racemate), exclusively afforded the desired [1,2] Wittig product ($5\beta,4S$)-*C*-glycoside **6b** with high diastereoselectivity (>95% β , 84% d.r. at C4)^[10] in 54% yield. (Scheme 3). Also significant, the anomeric radical, an intermediate in the rearrangement of **5b**, could efficiently discriminate between the enantiotopic faces of the prochiral bis(ethynyl)methanol radical (that is, differentiation of trimethylsilyl and *tert*-butyldimethylsilyl at the γ,γ' positions) during the radical recombination process.

The major diastereomer, separated by column chromatography, was then converted into the key precursor **10**, which has all the stereogenic centers of **B**, by the following sequence.

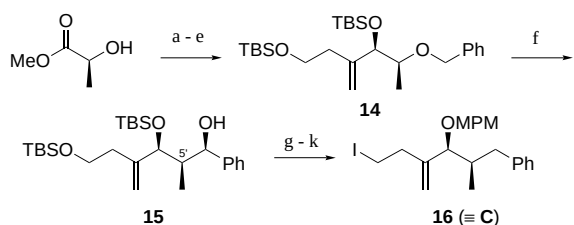


Scheme 3. Synthesis of the lactone **B**. a) TBDPS-C≡CCH(OH)C≡C-TMS (**8**), montmorillonite K 10, 4 Å-MS, CH₂Cl₂, RT, 80%; b) *n*BuLi, THF, −78 °C, 54% 84:16 at C4; c) Red-Al, Et₂O, −15 °C, 83%; d) O₃, MeOH, −78 °C then Me₂S; e) CH₂=CHMgBr, toluene, −78 °C, 49% for two steps; f) cyclopentanone dimethylacetal, *p*TsOH, benzene, RT, 87%; g) O₃, MeOH, −78 °C then Me₂S; NaClO₂, NaH₂PO₄, Me₂C=CHMe, *t*BuOH/H₂O, RT; *N,N'*-diisopropyl-*O*-*tert*-butylisourea, CH₂Cl₂, RT, 73–84% for three steps; h) H₂, cat. Pd/C, MeOH, RT, 88%; i) 0.1N TBAF/THF, THF, 0 °C, 94%; j) H₂, Lindlar cat., MeOH, RT, 93%; k) KOH (aq), MeOH, RT, 78%; l) cat. TPAP, NMO, 4 Å-MS, CH₂Cl₂, RT, 98%; m) O₂, Cu(OAc)₂, 2,2'-bipyridyl, DABCO, DMF, 70 °C, 72%. TBDPS = *tert*-butyldiphenylsilyl, Bz = benzoyl, TBAF = tetrabutylammonium fluoride, TPAP = tetrapropylammonium perruthenate, NMO = *N*-methylmorpholine *N*-oxide, DABCO = 1,4-diazabicyclo[2.2.2]octane.

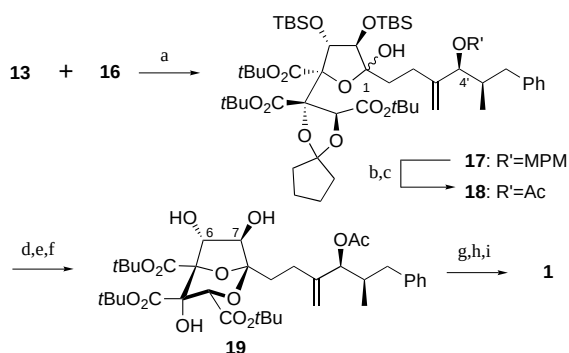
The semireduction of the ethynyl group on **6b** with bis(2-methoxyethoxy)aluminum hydride (Red-Al) in diethyl ether at −15 °C yielded the *tert*-butyldiphenylvinylsilane **9** with high selectivity.^[11] After the ozonolysis of **9**, the addition of vinylmagnesium bromide (toluene, −78 °C) proceeded to give **10** with excellent diastereoselectivity^[12] (>95% d.r.) with the latent C3 carboxy moiety introduced in the form of a vinyl substituent. After protection of the C3 and C4 hydroxyl groups as a cyclopentylidene acetal, the oxidation of the vinyl group at C3 was accomplished through a three-step sequence which involved ozonolysis to the corresponding aldehyde followed by oxidation with buffered NaClO₂ and esterification with *N,N'*-diisopropyl-*O*-*tert*-butylisourea^[13] to give the *tert*-butyl monoester. The tris(*tert*-butyl) ester **12** was obtained in good yield following a similar stepwise oxidation of the ethynyl groups at C4 and C5 to the carboxy moieties. The hydrolysis of the benzoate group at C1^[14] and the two-step oxidation (tetrapropylammonium perruthenate (TPAP)/O₂-Cu(OAc)₂^[15]) of the resulting hydroxymethyl group afforded the lactone **13** ($\cong$ **B**) as a fully elaborated intermediate, to which a nucleophilic side chain equivalent could be added at C1.

Scheme 4 illustrates the synthesis of the C1 side chain equivalent **16** ($\cong$ **C**) using the ether [1,2] Wittig rearrangement as the key step (**14** → **15**). The benzyl ether **15** was synthesized from methyl (*S*)-lactate in five steps. The rearrangement of **14** (*n*BuLi, THF, 0 °C) afforded **15**, which possesses the desired *R* configuration at the C5' position (88% d.r.),^[16] in 32% yield along with 58% of recovered **14**. The eight-step refunctionalization sequence from **15**, which involved removal of the hydroxyl group at C6'^[17] and iodination of the primary alcohol, successfully afforded **16**.

Generation of the alkyl lithium side chain (for C1) derived from iodide **16** followed by the addition of a lactone **13** provided the hemiketal **17** as a mixture of anomers (Scheme 5).^[18] The oxidative cleavage of the MPM ether followed by acetylation of the hydroxyl group at C4' completed the assembly of the hemiketal **18**, the precursor



Scheme 4. Synthesis of the C1 side chain ($\cong$ C). a) $\text{PhCH}_2\text{OC}(\equiv\text{NH})\text{CCl}_3$, cat. $\text{CF}_3\text{SO}_3\text{H}$, cyclohexane, $0^\circ\text{C} \rightarrow \text{RT}$, 90%; b) $i\text{PrMgBr}$, $\text{MeO}(\text{Me})\text{NH} \cdot \text{HCl}$, THF, -10°C , 60%; c) $t\text{BuLi}$, $\text{TBSOCH}_2\text{CH}_2\text{C}(\equiv\text{CH}_2)\text{Br}$, hexane/ Et_2O , -78°C , 92%; d) $\text{Zn}(\text{BH}_4)_2$, Et_2O , $-78 \rightarrow -30^\circ\text{C}$, 93%; e) TBSOTf , 2,6-lutidine, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{RT}$, 95%; f) $n\text{BuLi}$, THF, $-20 \rightarrow 0^\circ\text{C}$, **15**: 32% recovered, **14**: 58%; g) TBAF, THF, RT; acetone dimethylacetal, cat. $p\text{TsOH}$, benzene, RT; 1N HCl (aq), THF, 0°C ; TBSCl , imidazole, CH_2Cl_2 , RT, 62% for four steps; h) Li_0 , liq. NH_3 , $-78 \rightarrow -30^\circ\text{C}$, 85%; i) $\text{MPMOC}(\equiv\text{NH})\text{CCl}_3$, cat. $\text{CF}_3\text{SO}_3\text{H}$, cyclohexane, CH_2Cl_2 , 0°C ; j) TBAF, THF, RT, 81% for two steps; k) I_2 , imidazole, PPh_3 , benzene, RT, 88%. MPM = *p*-methoxyphenylmethyl.



Scheme 5. Total synthesis of zaragozic acid A (**1**). a) 3.2 equiv of **16**, 5.0 equiv of $t\text{BuLi}$, 5:1 hexane:diethyl ether, -40°C , then **13**, -40°C , 96%; b) DDQ, CH_2Cl_2 , RT, 96%; c) Ac_2O , DMAP, CH_2Cl_2 , RT, 95%; d) 10.20:1 TFA: CH_2Cl_2 : H_2O , RT; e) N,N' -diisopropyl-*O*-*tert*-butylisourea, CH_2Cl_2 , RT; f) 1N TBAF, THF, RT, 51% for three steps; g) $(t\text{BuOCO})_2\text{O}$, 4-pyrrrolidinopyridine, CH_2Cl_2 , 0°C , 81%; h) **20** ($\cong$ A, R = H), DCC, DMAP, CH_2Cl_2 , RT, 82%; i) TFA, CH_2Cl_2 , RT, 73%. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DMAP = 4-dimethylaminopyridine, TFA = trifluoroacetic acid, DCC = 1,3-dicyclohexylcarbodiimide.

to the bicyclic core and associated C1 side chain. Hydrolysis/ketalization of the hemiketal **18** with trifluoroacetic acid (TFA), followed by the esterification and TBAF treatment provided the desired bicyclic ketal **19**.^[19] Selective protection of the hydroxyl group at C7 with a *tert*-butoxycarbonyl (Boc) group^[20] followed by acylation with the C6 side chain **20** ($\cong$ A, R = H),^[21] then acid treatment afforded zaragozic acid A (**1**), whose spectroscopic data were in excellent agreement to those of a natural sample (^1H and ^{13}C NMR, IR, HPLC, and $[\alpha]_D$).

In conclusion, we have described the total synthesis of zaragozic acid A, which is highlighted by developing the acetal [1,2] Wittig rearrangement for the construction of the C4–C5 contiguous quaternary chiral centers. This synthetic strategy could be applied to the synthesis of a variety of naturally occurring zaragozic acids as well as designed synthetic analogues.

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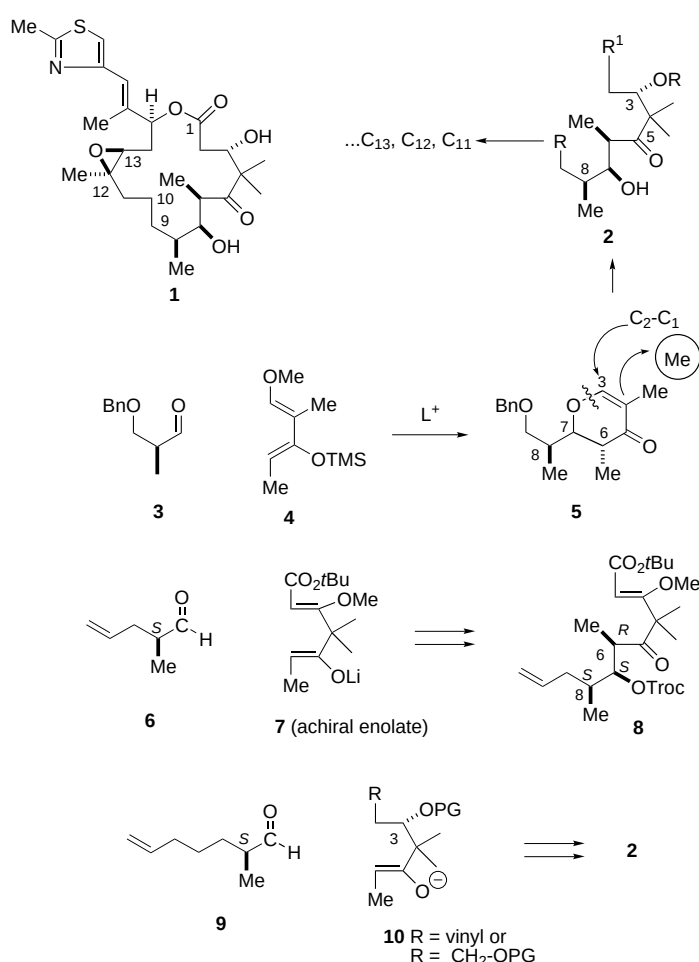
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Subtle Variations in the Long-Range Transmission of Stereochemical Information: Matched and Mismatched Aldol Reactions**

Zhicai Wu, Fei Zhang, and Samuel J. Danishefsky*

In this paper we describe findings with respect to an important question in stereospecific synthesis, that is, how might remote stereogenic loci influence the diastereofacial sense in which covalent bond formation occurs. To address this type of issue in concrete rather than abstract terms, we focused on the very promising antitumor agents, the epothilones (such as epothilone B (**1**), shown in Scheme 1). These compounds have stimulated a great deal of research from the point of view of total synthesis.^[1–6] In this paper we do not directly address the total synthesis problem per se. Rather, we focus on the construction of the C6–C7 bond in the acyl sector en route to the natural products, as a paradigm for accomplishing high orders of selection by long-range induction.

By way of background we note that, originally,^[1a, 1c] the stereochemical information for building the “acyl” sector of the epothilones (see **2** in Scheme 1) was stored in dihydropyrone **5** which itself arose from a cyclocondensation reaction of enantiomerically pure **3** and diene **4**. In our second-generation synthesis,^[7] the enantiomerically homogenous **6** reacted with



Scheme 1. Structure of epothilone B (**1**) and several methods for the construction of the C6–C7 bond in the acyl sector (**2**) of the epothilones. Bn = benzyl, TMS = trimethylsilyl, Troc = trichloroethoxycarbonyl, PG = protecting group.

achiral enolate **7**, giving rise selectively (approximate ratio = 5.5:1) to **8**, with the required *R*, *S*, and *S* configurations at C6, C7, and C8, respectively. The configuration at C3 was subsequently induced by catalytic, reagent-based, asymmetric reduction. System **2** can now be prepared in a highly convergent fashion on a multigram scale by this synthesis.^[6]

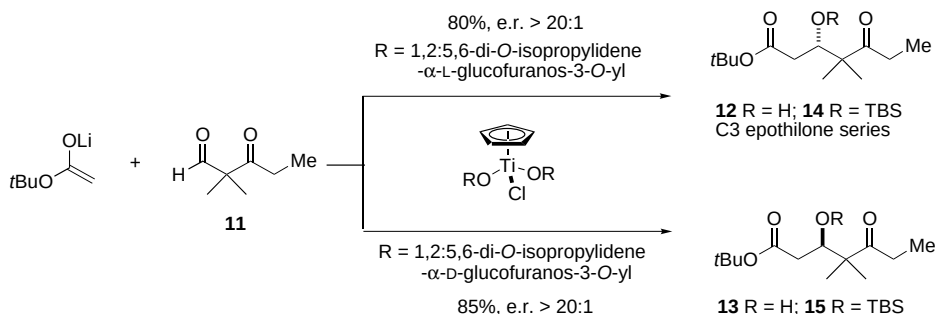
The background result which was central to the program described below was reported by Schinzer and co-workers,^[3a] and was subsequently used by others.^[4e, 8] The essence of Schinzer's claim^[9] was that aldol condensation of the enantiomerically defined lithio enolate system (see **10** in Scheme 1) with the enantiomerically defined aldehyde **9** occurs with high stereoselection in the required sense at the emerging C6–C7 bond, leading, eventually, to a product of type **2**. In essence, in the approach of Schinzer et al., the chirality at C3 had induced the required C6(*R*) and C7(*S*) configurations. This is in contrast to our second generation approach where the stereogenicity was induced solely by the *S* aldehyde **6**.

We undertook to revisit this type of aldol condensation for several reasons. The role of the pre-C8 *S* methyl group in **9** in delivering the high diastereofacial selectivity at C6 and C7

[*] Prof. S. J. Danishefsky, Dr. Z. Wu, F. Zhang
Department of Chemistry
Columbia University
Havemeyer Hall, 3000 Broadway, New York, NY 10027 (USA)
E-mail: s-danishefsky@ski.mskcc.org
Prof. S. J. Danishefsky
Laboratory for Bioorganic Chemistry
Sloan–Kettering Institute for Cancer Research
1275 York Avenue, New York, NY 10021 (USA)
Fax: (+1) 212-772-8691

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was not established. The β -hydroxy *tert*-butyl ester **12** was prepared with high enantioselectivity by the reaction of aldehyde **11** with lithio *tert*-butyl acetate following precedents and protocols described by Duthaler and co-workers in other contexts (Scheme 2).^[10] With the expectation of probing the basis of Schinzer et al.'s findings in greater detail, we also



Scheme 2. Asymmetric synthesis of stereogenic center C3. e.r. = enantiomeric ratio, TBS = *tert*-butyldimethylsilyl.

prepared the *ent* β -hydroxyester **13** and converted the enantiomeric C3 alcohols into their TBS derivatives **14** and **15**. However, we were unable to effect aldol condensation between **6** and the lithio enolates derived from **12** or **13** owing to vulnerabilities in the β -siloxyester network.

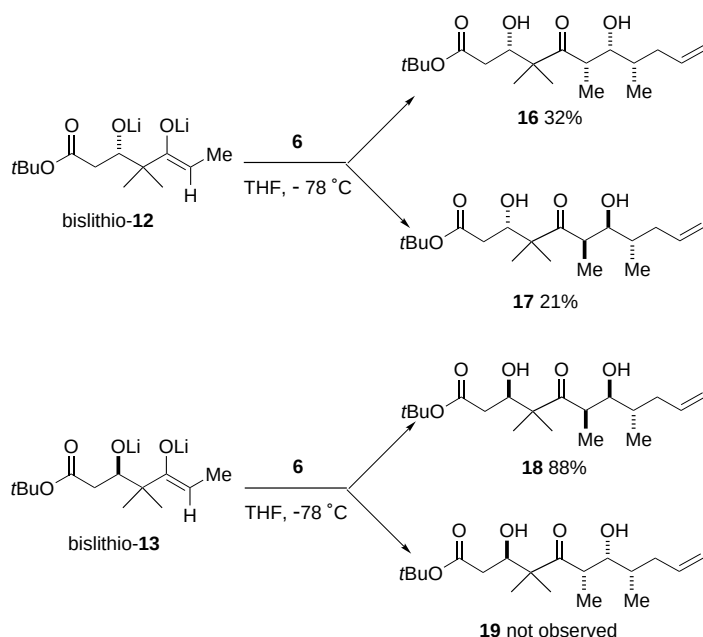
Given our inability to couple **14** and **15** as their lithium enolates, we examined the feasibility of using the enantiomeric β -hydroxy esters **12** and **13** in the form of their lithio enolate alkoxides, in the coupling reactions with **6** (Scheme 3).^[11] In these cases, β -elimination of the C3 oxygen was not a problem and aldol condensation proceeded smoothly. The results were most interesting. When the C3(*S*) enolate corresponding to **12** was treated with enantio-

merically pure aldehyde **6**, there was generated a 3:2 ratio of two products. Subsequently, it was shown by correlation (see below) that the major product, **16**, corresponds to a C6/C7 *bis-epi* version of **2** (that is, C6(*S*) and C7(*R*)), while the latter **17**, has the required epothilone configurations, C6(*R*) and C7(*S*) (in addition to being *S* at C3).

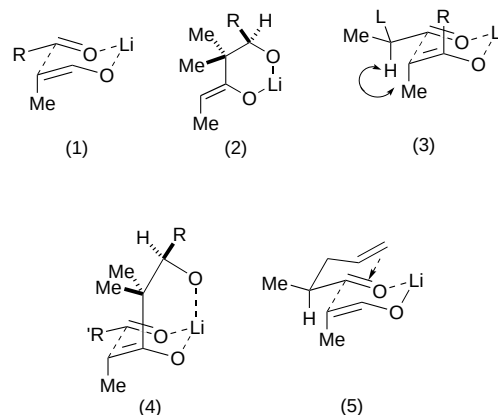
We then examined the corresponding reaction employing the enolate alkoxides derived from the *ent* precursor **13**. Remarkably, this reaction gave rise to a single aldol product (Scheme 3). Correlation with other intermediates in the program (see below) established that the C6 and C7 configurations in **18** correspond to those required to reach an intermediate such as **2**. Of course, the configuration at C3 in **18** is *R* rather than the required *S*. Hence, the use of compound **18** in a total synthesis of a naturally configured epothilone would require inversion at C3.

Nonetheless, these results are quite informative. They teach that the transition state of the aldol reaction of the C3-unprotected enolate system with **6** is very different from that of the protected enolate **10** with **9**.^[3, 8] Thus, in the C3-protected series (in the case of Schinzer et al.) *S* chirality at C3 is needed to deliver the required C6(*R*),C7(*S*) configurations in the aldol product, following condensation with enantiomerically pure (*S*)-**9**. By contrast, in the C3-unprotected dilithio series (see **13**), it is the C3(*R*) enantiomer which delivers the C6(*R*),C7(*S*) configurations with the corresponding very similar aldehyde **6**.

In addition to accounting for the sharp difference in outcomes as a function of the state of protection at C3, we sought to explain why the **12**+**6** case gave rise to a virtually 1:1 mixture of **16** and **17**, while the **13**+**6** combination is matched, leading cleanly to a single product **18**. We propose that there are five desiderata for such an aldol condensation (Scheme 4): 1) The usual chair transition state in the C–C bond formation,^[12] 2) chelation of the lithium counterion (or counterions) by the alkoxido and enolate oxygens,^[13] 3) a *syn* relationship between the C2 proton of **6** and the C6 methyl group of the



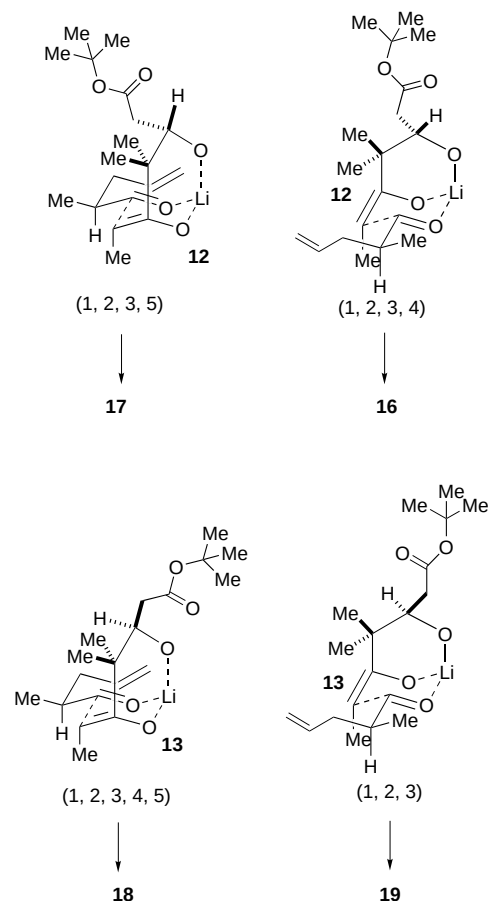
Scheme 3. Aldol reactions of the enantiomeric β -hydroxy esters **12** and **13** with **6**.



Scheme 4. Five desiderata for the aldol condensation.

enolate,^[14] 4) attack by the aldehyde *anti* to the large resident R group of the enolate, and 5) attack by the enolate on the face of the aldehyde which is *anti* to the resident allyl or pentenyl group. In starting with electrophile **6**, the special γ,δ -unsaturated aldehyde effect^[7b] will tend to orient the double bond of the allyl function for a favorable interaction (perhaps by π -stacking) with the aldehyde group.^[7]

While the relative weightings of these factors are not known, we note that in the reaction of *R* enolate **13** with **6** to give **18**, the transition state of the aldol step shown incorporates each of the energy-lowering features (1)–(5) (Scheme 5). Thus, the two enantiotopicities of **13** and **6** are

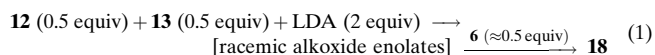


Scheme 5. Proposed transition states in the aldol reactions of **12** and **13** with **6**.

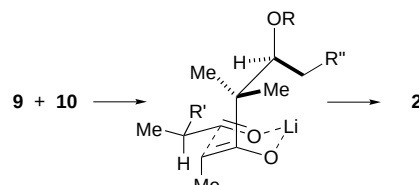
matched to produce **18**.^[15] The transition state alignment that would be necessary to produce the alternative, not observed, C6,C7-*erythro* product **19**, given the incorporation of features (1)–(3), would be lacking favorable elements (4) and (5). In a similar vein, in combining the *S* enolate **12** with **6**, with inclusion of features (1)–(3) as given, each transition state is seriously unoptimal. To reach **17**, attack of aldehyde **6** must occur *syn* to the large R group projecting from the chelate. Conversely, in reaching **16**, the effect from overlap of the terminal vinyl group with the aldehyde is forfeited.

To explore these concepts further, we conducted a critical experiment to test the postulate implied in Scheme 2. We reasoned that if ensembles pre-**16** and pre-**17** correspond to

mismatches, while **18** is matched, there could be measurable kinetic consequences. We probed this matter by using a racemic mixture of enolates **12** and **13** with a limiting concentration of enantiopure aldehyde **6**. In practice this experiment turned out to be most revealing. Reaction of racemic **12**:**13** (one equivalent) with lithium diisopropylamide (two equivalents) to achieve double deprotonation was followed by addition of approximately half an equivalent of optically pure (*S*)-**6**. Remarkably, this reaction produced **18** (70% yield) virtually free of **16** or **17** (or **19**) [Eq. (1)]. In short, the matched/mismatched hypothesis has been corroborated at the kinetic level in a remarkable instance of molecular recognition in covalent bond formation through an aldol condensation.



In addition, our data strongly suggest that in the C3-protected series (such as **10**), the chirality induction does not involve chelation between C3 and C5.^[16] Indeed, the protecting groups which have been used at C3^[3, 8] tend to be large and thus disfavor a chelate-directed conformational organization. By contrast the transition state in Scheme 6 nicely accounts

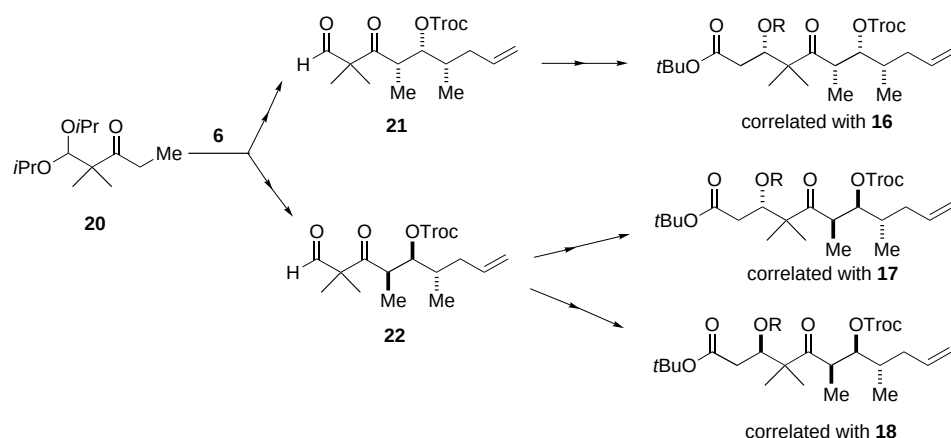


Scheme 6. Proposed transition state for C3-protected series.

for the sense of the stereochemical transmission in the C3-protected enolate series. We propose that the C3 “OR” group is positioned *anti* to the C4–C5 bond. In the C3(*S*) enolate **10**, attack of aldehyde **9** *syn* to the C3 hydrogen leads to **2**, with C6(*R*),C7(*S*) configuration (as required for the epothilones). This combination can be termed as matched. The formation of the C6(*S*),C7(*R*) product from the ensemble of **9** and **10** as proposed would require attack to occur *syn* to the CH₂R’ face of the enolate on the R’ face of the aldehyde.

The structures of **16**–**18** were established by methods which demonstrate the capacity to control the configuration at C3 by the “titano” *tert*-butyl acetate method (Scheme 7).^[10] Correlations started with condensation of lithio-**20** with **6** to afford the C6(*R*),C7(*S*) and C6(*S*),C7(*R*) aldol products in a 4:1 ratio. Protection of each compound as the Troc derivative and deprotection of the aldehyde function afforded compounds **21** and **22**. Following diastereospecific two-carbon homologation of these compounds, correlation with **16**–**18** was readily accomplished.

In summary, the strikingly different senses of long-range transmission (over five bonds) of stereochemical information in seemingly related aldol condensations have been rationalized by comparing the effects of resident silyl ether and “lithio alkoxy” functions. In the case of the lithio alkoxy enolates, a huge kinetic advantage favoring the matched series has been



Scheme 7. Independent synthesis of compounds for correlation with 16–18.

demonstrated. Extensions of these findings to other instances of long-range transmission of stereochemical bias are a continuing area of interest in our laboratory.

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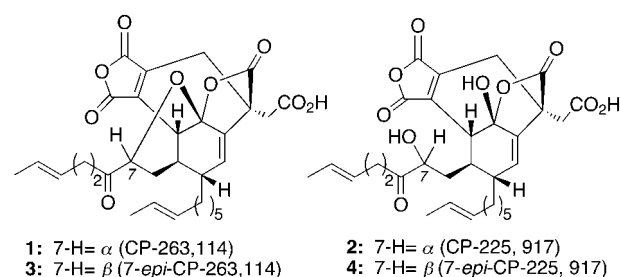
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The Synthesis of CP-263,114 and CP-225,917: Striking Long-Range Stereocontrol in the Fashioning of C7**

Qiang Tan and Samuel J. Danishefsky*

During a screening exercise which was designed to target inhibitors of Ras farnesyl transferase and squalene synthase, Pfizer scientists reported the isolation of two natural products CP-263,114 (**1**) and CP-225,917 (**2**) as fungal metabolites extracted from juniper twigs in Texas.^[1] These compounds have fostered a great deal of creative research from synthetic organic chemists. The interest accrues from the novel and challenging structures of the CP metabolites rather than from compelling biological imperatives. Three total syntheses of **1** and **2** have been described.^[2] The first of these was reported by Nicolaou and co-workers.^[2a-c] When appropriately modified, their synthesis revealed the configuration of **1** and **2**. These compounds are now known to correspond to the absolute stereostructures shown. This finding was independently confirmed by the groups of Shair^[2d] and Fukuyama.^[2e]

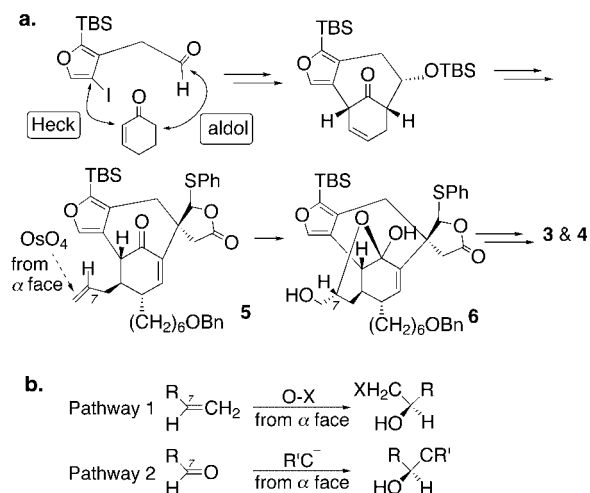


In our earlier report^[3] we disclosed the total synthesis of the 7-*epi* series of the CP compounds (**3** and **4**). We are now confident that these 7-*epi* compounds are themselves less abundant natural products, found in the fermentation broth.^[4] Nonetheless, since the goal structures of our synthetic venture were compounds **1** and **2**, we undertook the challenge of reaching these natural products. In the course of this study, we

encountered some remarkable instances of stereochemical guidance by remote functional groups arising from the novel architectures of the pre-CP intermediates. These findings, as well as the attainment of our synthesis goals in reaching **1** and **2**, are described herein.

On casual inspection it would appear that the synthetic problem could readily be solved by equilibration of **3** or **4**, or their precursors, at C7. However, as was detailed in our total synthesis report,^[3d] epimerization at C7 under apparent thermodynamic control only proceeds in the direction of the 7-*epi* diastereomer. Hoped for solutions based on kinetic quenching of enolates derived from deprotonation at C7 under irreversible conditions were unsuccessful. Complicating both of these strategies for inverting the C7 configuration was the general instability of the CP systems to several intended deprotonation protocols. Hence it was necessary to retreat to earlier stages of the synthesis to accomplish our objective of reaching **1** and **2** through our total synthesis.

The reaction that established the eventual 7-*epi* stereochemistry arose from the action of osmium tetroxide on the side-chain allyl group of compound **5** (Scheme 1a). This oxidation resulted in a hemiacetal bearing a hydroxymethyl



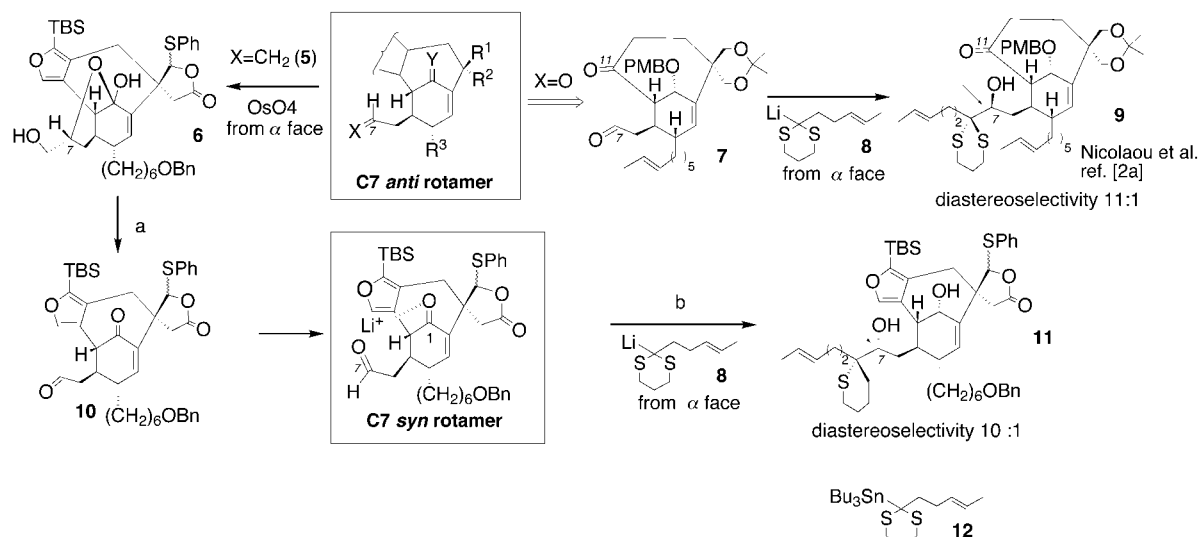
Scheme 1. TBS = *tert*-butyldimethylsilyl, Bn = benzyl.

group at C7. The dihydroxylation was essentially stereo-specific. The hemiacetal was shown to have the stereochemistry of **6** by its eventual conversion into **3** and **4**. In retrospect this result can be explained by α -face attack of the oxidant upon an “extended *anti*” conformation as proposed for **5** (Scheme 1a). While this was a disappointing result at the time, a seemingly workable solution virtually suggested itself. The thought was to gain access to the required C7 side-chain stereochemistry by inverting the order of element linkage to the achiral C7 sp^2 precursor (Scheme 1b). Thus, in the dihydroxylation reaction (Pathway 1) an oxygen atom had been added to the C7 methane carbon of a terminal methylene group. Now we hoped to reverse the stereochemical outcome by adding a carbanion equivalent to a C=O linkage (Pathway 2).

Specifically we sought to add lithio dithiane **8** to aldehyde **10**. Of course the successful realization of the scheme

[*] Prof. S. J. Danishefsky, Q. Tan
 Laboratory for Bioorganic Chemistry
 Sloan–Kettering Institute for Cancer Research
 1275 York Avenue, New York, NY 10021 (USA)
 Fax: (+1) 212-772-8691
 E-mail: s-danishefsky@ski.mskcc.org
 Prof. S. J. Danishefsky
 Department of Chemistry
 Columbia University
 Havemeyer Hall, 3000 Broadway, New York, NY 10027 (USA)

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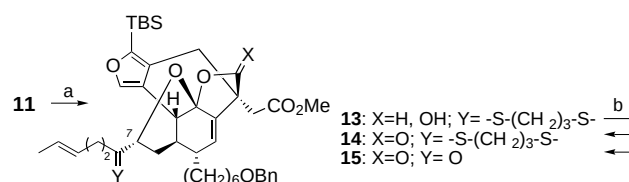


Scheme 2. a) $\text{Pb}(\text{OAc})_4$, PhMe, 0°C , 20–45 min, 90%; b) **12**, MeLi/LiBr, THF, -78°C , then add aldehyde at -200°C , warm to -78°C , 15 min, 70% total. PMB = *para*-methoxybenzyl.

presumed that the conformation of the reacting formyl group of **10** would also be “extended *anti*” and that the nucleophile would again (as with the osmium tetroxide reaction) attack from the α -face. Indeed as we were preparing for this very experiment, Nicolaou and co-workers reported that the addition of lithio dithiane **8** to aldehyde **7** produced **9** in an approximately 11:1 ratio relative to the C7 epimer (Scheme 2).^[2a] The result from the Nicolaou group was consistent with our dihydroxylation result in that opposite diastereomers had been produced at C7 following the opposite linkage orders.

Notwithstanding its dominantly hemiacetal character, **6** reacted with lead tetraacetate to afford **10** (Scheme 2). The latter reacted smoothly with **8** (generated in situ from its *n*-butylstannyl derivative **12**) to afford substantially a single carbinol in a 10:1 ratio with the C7 diastereomer. Our satisfaction with the result was short-lived when it was learned that the carbinol was **11**. This realization followed a two step conversion of **11** into **13** followed by oxidation of the γ -lactol and the deprotection of the dithio-ketal (Scheme 3). The resultant **15** had been previously encountered in our synthesis of the 7-*epi* systems **3** and **4**.

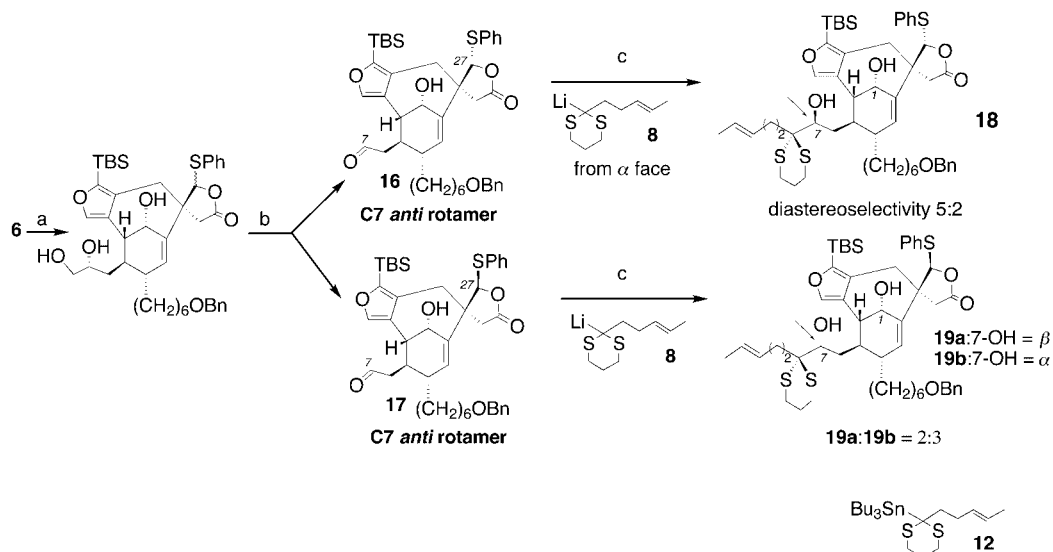
It seemed that a factor to explain the massive difference (11:1 versus 1:10) between our case and that of Nicolaou and co-workers was the absence or existence of the C1 ketone. Perhaps the presence of this ketone in our substrate **10** occasioned a shift in either



Scheme 3. a) 1. LiOH, THF, 36 h; 2. CH_2N_2 , 60%; b) (COCl)₂, DMSO, CH_2Cl_2 , Et₃N, -78°C , 75%, **14**:**15** = 1:1.

the nature of the reactive aldehyde rotamer or the sense of attack on the corresponding rotamer. For instance, formation of a local “lithio channel”, by the C1 ketone and the C7 aldehyde, would favor an otherwise unfavorable *syn*-like rotamer as shown in Scheme 2.

This argument was evaluated following conversion of **6** into **16** and **17** as shown in Scheme 4. While not productive with respect to our goal, these studies provided striking instances

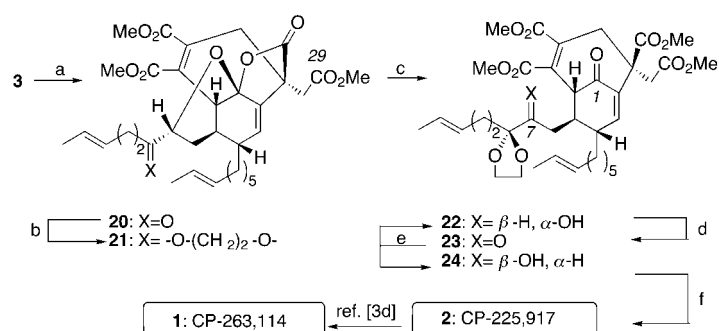


Scheme 4. a) NaBH_4 , PhMe/*i*PrOH, 6.5 h, 70%; b) $\text{Pb}(\text{OAc})_4$, PhMe, 0°C , 20–45 min, 90%; c) **12**, MeLi/LiBr, THF, -78°C , then add aldehyde at -200°C , warm to -78°C , 15 min, 70% total.

of long-range effects. Indeed, even the two C27 epimeric thiophenyl compounds reacted quite differently with **8**. In the case of **16**, the ratio of **18** and its C7 epimer was 5:2, whereas with **17**, the ratio of **19a:19b** was 2:3.^[5]

Qualitatively at least, removal of the C1 ketone did markedly shift the sense of addition of anion **8** in the predicted sense. Failure to achieve the very high selectivity described by the Nicolaou and co-workers^[2a] may be a consequence of the presence of the C11 ketone in their substrate **7**. This ketone could well provide additional guidance for α -face attack by the nucleophile on the rotamer shown.

While **18** and **19a** are potentially valuable precursors toward the CP systems **1** and **2**, a more rapid progress was registered. This involved the reduction of a C7 ketone to reach our goal (Scheme 5). Here too neighboring group influences



Scheme 5. a) TMSCHN₂, 15 min, >90%; b) (TMSOCH₂)₂, TMSOTf, CH₂Cl₂, 5 h, 90%;^[6] c) 1. LiOH, THF/H₂O, 1.2 h; 2. TMSCHN₂, 0.5 h, 70% overall; d) Dess–Martin periodinane, NaHCO₃, CH₂Cl₂, 4 h, 90%; e) LiAlH(OtBu)₃, toluene, –10°C, 5 min, 70%, **22:24** = 1:1; f) 1. LiOH, THF–H₂O, 24 h; 2. TFA/CH₂Cl₂/H₂O (54/4/1), 30 min, 70% overall.

are pivotal. The sequence started with the interesting reaction of trimethylsilyldiazomethane with **3** to afford **20** and thence **21**. The latter reacted with lithium hydroxide and then trimethylsilyldiazomethane to give **22**, which following oxidation yielded **23**. Treatment of this compound with lithium tri(*tert*-butoxide) hydride provided **22** and **24**. We note again^[3d] that, in the case of **21**, a “cascade”^[2b] driven by carboxylate participation can not be invoked, since the lactone saponification occurs with the C29 methylester intact.

It is also likely that the regiospecific reduction of the C7 ketone relative to that at C1 is orchestrated by the proximal dioxolane protecting group. For instance, the corresponding ketodithiane analogue undergoes more rapid reduction at the C1 ketone relative to that at C7. The reduction of **23** as shown affords **24** as well as the separable and recyclable **22**. The four methyl esters are cleaved through long-term treatment of **24** with lithium hydroxide and reconstruction of the system was accomplished through the action of TFA (see structure **2**). The conversion of **2** into **1** has been reported.^[3d]

The contrathermodynamic conversion of the **3**, **4** minor series of metabolites into the more prevalent **1**, **2** family has been accomplished, which thus completes our file on all the known components of the fermentation mixture. Clearly the densely functionalized architectures of these four compounds

and their synthetic precursors give rise to quite striking intramolecular signaling which invites further experimentation and elucidation.

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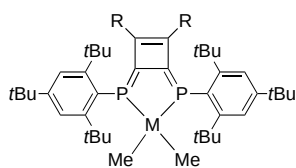
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- [4] These compounds have now been identified directly in the fermentation mixture as will be described in detail.
- [5] The ratios were determined by oxidizing the C1 alcohol of the dithiane adducts (PDC, PhMe, approximately 80%), and comparing the NMR spectra of the resultant ketones with those of **11**. PDC = pyridinium dichromate.
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Synthesis and Reactions of Palladium and Platinum Complexes Bearing Diphosphinidenecyclobutene Ligands: A Thermally Stable Catalyst for Ethylene Polymerization**

Shintaro Ikeda, Fumie Ohhata, Masaki Miyoshi, Rika Tanaka, Tatsuya Minami, Fumiyuki Ozawa,* and Masaaki Yoshifuji*

Diphosphaalkenes are phosphorus analogues of diimines and form stable chelate rings with transition metal species.^[1–3] The coordination structures resemble those of diimine complexes. However, owing to the unique electronic nature of P=C bonds, diphosphaalkene complexes are expected to possess rather intriguing chemical properties, significantly different from the diimine analogues. Thus, we have been interested in the reaction chemistry of diphosphaalkene complexes, particularly in catalytic systems; data for such systems is extremely limited.^[2]

Herein we examine the synthesis, structures, and catalytic properties of some methylpalladium and platinum complexes (**1–4**) coordinated with 3,4-bis[(2,4,6-tri-*tert*-butylphenyl)-phosphinidene]cyclobutene derivatives.^[4] A cationic monomethylpalladium complex derived from **1** exhibits good catalytic activity for ethylene polymerization.



- 1:** M = Pd, R = Ph **3:** M = Pd, R = H
2: M = Pd, R = SiMe₃ **4:** M = Pt, R = Ph

The diphosphaalkene ligands in **1–4** were chosen with reference to findings reported by Brookhart et al. on nickel- and palladium-based catalysts for ethylene polymerization.^[5, 6] There the key to high catalytic activity was the use of α -diimine ligands having bulky aryl groups at the nitrogen atoms. The diphosphinidenecyclobutene ligands possess similar structural features.

[*] Prof. Dr. F. Ozawa, S. Ikeda, F. Ohhata, M. Miyoshi, R. Tanaka, Dr. T. Minami
 Department of Applied Chemistry, Faculty of Engineering
 Osaka City University
 Sumiyoshi-ku, Osaka 558-8585 (Japan)
 Fax: (+81) 6-6605-2978
 E-mail: ozawa@chem.eng.osaka-cu.ac.jp

Prof. Dr. M. Yoshifuji
 Department of Chemistry, Graduate School of Science
 Tohoku University
 Aoba, Sendai 980-8578 (Japan)
 Fax: (+81) 22-217-6562
 E-mail: yoshifuji@mail.cc.tohoku.ac.jp

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Complexes **1** and **2** were prepared by ligand displacement of [PdMe₂(tmeda)] (tmeda = tetramethylethylenediamine) with phenyl- and trimethylsilyl-substituted ligands in Et₂O and isolated as orange crystals in 94% and 63% yields, respectively. Complex **3** was not obtained by this method but prepared in 60% yield by using [PdMe₂(cod)] (cod = cyclooctadiene) in place of [PdMe₂(tmeda)]. The platinum complex **4** was synthesized from [Pt₂Me₄(SMe₂)₂] in 89% yield. All complexes were characterized by NMR spectroscopy and elemental analysis (see Supporting information).

The X-ray structure of **4**,^[7] which clearly shows chelate coordination of the diphosphinidenecyclobutene ligand (Figure 1). The two aryl rings on the phosphorus atoms are almost

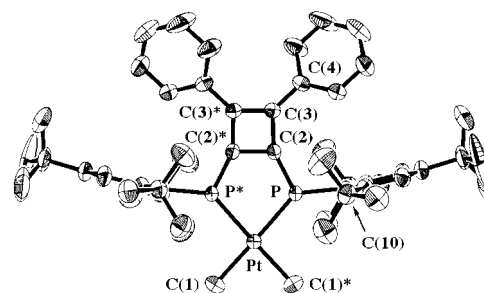


Figure 1. Molecular structure of **4**. Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Pt–C(1) 2.093(3), Pt–P 2.2909(8), P–C(2) 1.669(3), P–C(10) 1.829(3), C(2)–C(2)* 1.498(5), C(2)–C(3) 1.480(4), C(3)–C(3)* 1.410(6), C(3)–C(4) 1.449(4); P–Pt–P* 82.85(4), C(1)–Pt–C(1)* 85.1(2), P–Pt–C(1) 178.5(1), P–Pt–C(1)* 96.1(1), Pt–P–C(2) 111.2(1), Pt–P–C(10) 136.9(1), C(2)–P–C(10) 111.8(1), P–C(2)–C(2)* 117.3(1), P–C(2)–C(3) 154.4(2), C(2)*–C(2)–C(3) 88.3(2), C(2)–C(3)–C(3)* 91.7(2), C(2)–C(3)–C(4) 131.5(3), C(3)*–C(3)–C(4) 136.7(2).

perpendicular to the square planar coordination plane, making a dihedral angle of 92.52(8)°. The distance between platinum and the methyl carbon (Pt–C(1) = 2.093(3) Å) is slightly longer than that of the diimine analogues (2.05–2.079 Å),^[8] showing a *trans*-influence for the diphosphaalkene ligand that is slightly higher than that of the diimine ligands.

Table 1 lists the results of the ethylene polymerization catalyzed by the palladium complexes. Similar to the diimine analogues, dimethylpalladium complexes **1–3** were inactive, while their monomethyl derivatives (**5–7**), generated in situ by the treatment of **1–3** with H(OEt)₂BAR₄ (Ar = 3,5-(CF₃)₂C₆H₃),^[9] catalyzed the polymerization.

Table 1. Ethylene polymerization catalyzed by diphosphinidenecyclobutene complexes of palladium.

Entry ^[a]	Precursor complex	Temperature [°C]	Pressure [kgfcm ^{–2}]	Activity [kg h ^{–1} (mol cat) ^{–1}]	M _w (M _w /M _n) ^[b] [kg mol ^{–1}]
1	1	60	10	86.3	13.9 (10.5)
2	1	70	5	54.3	21.2 (9.2)
3	1	70	10	128	18.7 (14.8)
4	1	70	30	121	4.2 (6.6)
5	1	80	10	123	28.9 (13.9)
6	1	100	10	90.7	28.6 (12.4)
7	2	70	10	4.1	33.1 (5.2)
8	3	70	10	2.6	11.8 (6.8)

[a] Reactions were performed in chlorobenzene (20 mL) for 1 h using 10 μmol of catalysts generated from the precursor complexes and H(OEt)₂BAR₄ (Ar = 3,5-(CF₃)₂C₆H₃). [b] Determined by GPC based on polyethylene standards.

A typical procedure for polymerization (entry 3) is as follows. An orange solution of **5**, which was prepared by the reaction of **1** (9.30 mg, 10 μ mol) with H(OEt)₂BAR₄ (10.7 mg, 10 μ mol) in chlorobenzene (5 mL) at room temperature, was transferred by cannula into a 150 mL pressure bottle and diluted with chlorobenzene (15 mL). Ethylene gas was charged, and the mixture was mechanically stirred at 70 °C for 1 h under a constant pressure (10 kgf cm⁻²; 1 kgf = 9.81 N). At this stage, the reaction system was a clear orange solution containing a small quantity of white precipitates. The mixture was poured into MeOH (80 mL), and the resulting precipitates were collected by filtration and dried under vacuum to give a white solid of polyethylene (1.34 g). The reaction could be carried out in 1,2-dichloroethane in place of chlorobenzene at almost the same catalytic activity (124 kg h⁻¹ (mol cat)⁻¹). In this case, however, precipitation of polymer from the reaction solution was considerable and the molecular weight was lowered ($M_w = 12.9$ kg mol⁻¹, $M_w/M_n = 7.1$).

As seen from entries 3, 7, and 8 in Table 1, the catalytic activity was highly sensitive to the R groups of the diphosphinidenecyclobutene ligands; the phenyl-substituted catalyst **5** derived from **1** exhibited much higher activity than the others. The activity thus observed is similar to the level of the diimine-based palladium catalysts.^[6] Higher pressure tends to improve the catalytic activity but causes a drop in the molecular weight (entries 2–4). The activity reached the maximum at around 70 °C (entry 3).

Noteworthy is that the present catalysts bearing diphosphinidenecyclobutene ligands possess extremely high thermal stability in the reaction solutions; no sign of decomposition was observed even at 100 °C (entry 6). This property is remarkable when compared with the diimine analogues^[10] and may be attributed to good coordination ability of the phosphorus-based ligands with a soft palladium center.

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- [9] The formation of **5** in solution was supported by NMR spectroscopy. The monomethyl structure of **5** was further confirmed by using its acetonitrile adduct which was isolated. ¹H NMR (300 MHz, CD₂Cl₂): $\delta = 1.39$ (dd, $J_{\text{PH}} = 9.3, 3.3$ Hz, 3 H; PdMe), 1.44, 1.45, 1.59, 1.60, 1.60, 1.61 (each s, each 9 H; *t*Bu), 2.30 (s, 3 H; MeCN), 6.78 (d, $J_{\text{HH}} = 7.8$ Hz, 2 H; *o*-Ph), 6.84 (d, $J_{\text{HH}} = 8.1$ Hz, 2 H; *o*-Ph), 6.97 (t, $J_{\text{HH}} = 8.1$ Hz, 2 H; *m*-Ph), 6.98 (t, $J_{\text{HH}} = 7.8$ Hz, 2 H; *m*-Ph), 7.24 (t, $J_{\text{HH}} = 7.8$ Hz, 2 H; *p*-Ph), 7.57 (s, 4 H; BAr), 7.61 (d, $J_{\text{PH}} = 2.7$ Hz, 2 H; PAr), 7.71 (d, $J_{\text{PH}} = 4.2$ Hz, 2 H; PAr), 7.73 (s, 8 H; BAr); ¹³C{¹H} NMR (75 MHz, CD₂Cl₂): $\delta = 3.4$ (s, MeCN), 14.5 (dd, $J_{\text{CP}} = 94, 3$ Hz, PdMe), 31.3, 31.5, 33.8, 33.8, 34.1, 34.1 (each s, CMe₃), 35.8, 36.0, 38.7, 38.7, 39.8, 39.9 (each s, CMe₃), 122.7 (s, NCMe); ³¹P{¹H} NMR (121 MHz, CD₂Cl₂): $\delta = 133.6, 159.9$ (each d, $J_{\text{PP}} = 30$ Hz); elementary analysis calcd (%) for C₈₇H₈₆BF₂₄NP₂Pd: C 58.68, H 4.87, N 0.79, found: C 58.25, H 4.75, N 0.73.
- [10] It was found that the diimine complex [PdMe{Ar'N=C(Me)-C(Me)=NAr'}]⁺[BAR₄]⁻ (Ar' = 2,6-(*i*Pr)₂C₆H₃)^[5a] under the reaction conditions of entry 3 in Table 1 immediately starts to decompose giving metallic palladium and loses all catalytic activity within 15 min, 1.10 g of a sludgy, dark polymer was obtained using 10 μ mol of the catalyst.

Highly Selective Ring-Opening/Cross-Metathesis Reactions of Norbornene Derivatives Using Selenocarbene Complexes as Catalysts*

Hiroyuki Katayama, Hideto Urushima, Tsuneo Nishioka, Chikaya Wada, Masato Nagao, and Fumiyuki Ozawa*

With the advent of well-defined alkylidene catalysts of molybdenum^[1] and ruthenium,^[2] olefin-metathesis has been invested with a great deal of utility in synthetic organic

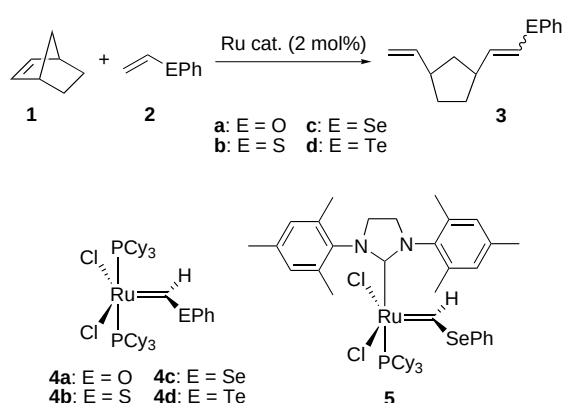
[*] Prof. Dr. F. Ozawa, Dr. H. Katayama, H. Urushima, T. Nishioka, C. Wada, M. Nagao
Department of Applied Chemistry, Faculty of Engineering
Osaka City University
Sumiyoshi-ku, Osaka 558-8585 (Japan)
Fax: (+81) 6-6605-2978
E-mail: ozawa@chem.eng.osaka-cu.ac.jp

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chemistry.^[3] This transformation includes several reaction patterns which are dependent on the olefinic substrates employed. The most extensively investigated is the ring-opening metathesis polymerization (ROMP) of cyclic olefins. Ring-closing metathesis (RCM) of dienes or enynes has also found a number of applications in organic synthesis. Very recently, a highly efficient catalyst for inter- and intramolecular cross-metathesis (CM) of acyclic olefins has been developed.^[4]

While the intermolecular coupling between cyclic olefins and acyclic olefins by a sequence of ring-opening/cross-metathesis reactions (ROCM) should be another useful variation of olefin metathesis, selective systems for this tandem protocol are rare.^[5] A major problem is the concurrent self-metathesis of cyclic olefins, giving the ROMP products. The polymer formation is particularly pronounced when highly reactive cyclic olefins such as norbornene and 7-oxanorbornene are employed. Consequently, ROCM is usually conducted in a dilute solution using an excess of acyclic olefin,^[5] and, on occasion, it is necessary to reduce the reactivity of cyclic olefin by steric protection.^[5a] In contrast, we found in this study that a highly selective ROCM of norbornene (**1**) could be performed without such devices, by using phenyl vinyl selenide **2c** as an acyclic olefin and selenocarbene ruthenium **4c** as a catalyst (Scheme 1).^[6]



Scheme 1. Catalytic ROCM of norbornene with vinyl chalcogenides.

A dichloromethane solution of **1** and **2c** (each 0.25 M) was treated with a catalytic amount of **4c** (2 mol %) at room temperature for 2 h. GLC analysis revealed selective formation of the ROCM product **3c** ($E/Z = 65/35$), which was isolated in 92 % yield by column chromatography. Complex **4c** was the sole ruthenium species observed by $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopy throughout the reaction and recovered almost quantitatively from the system.

The ROCM reaction could be conducted using $[\text{Ru}(\text{=CHPh})\text{Cl}_2(\text{PCy}_3)_2]$ (**6**)^[2a] or $[\text{Ru}(\text{=C=CHtBu})\text{Cl}_2(\text{PCy}_3)_2]$ (**7**)^[8] in place of **4c**. The reaction with **6** (2 mol %), which is the precursor of **4c**,^[6] proceeded at a comparable rate, giving **3c** ($E/Z = 64/36$) in 89 % yield. In this case, treatment of **6** with **2c** prior to mixing with **1** was essential for the high product yield; otherwise a considerable amount of poly(**1**) was formed. While complex **7** was less reactive than **4c**

and **6**, the ROCM product **3c** was still obtained in good yield (83 %; $E/Z = 62/38$) at elevated temperature (60 °C, 4 h).

The reactions of **1** with a series of phenyl vinyl chalcogenides are summarized in Table 1. The vinyl selenide **2c** (entry 4) is much more effective in ROCM than the others. Thus the reactions with vinyl ether **2a** (entries 1 and 2) and sulfide **2b** (entry 3) were accompanied by considerable ROMP of **1**.^[9] The reaction with telluride **2d** was sufficiently selective but incomplete because of decomposition of the ruthenium catalyst (entry 5).

Table 1. Ruthenium-catalyzed ROCM of norbornene (**1**) with the vinyl chalcogenides **2**.

Entry ^[a]	$\text{H}_2\text{C=CHPh}$	Catalyst	Reaction time [h]	Yield of 3 ^[b] [%]	E/Z ^[c]
1	2a (E = O)	4a	1	0 ^[d]	–
2	2a (E = O) ^[e]	4a	5	17	15/85
3	2b (E = S)	4b	6	63	63/37
4	2c (E = Se)	4c	2	92	65/35
5	2d (E = Te)	4d	18	34 (98) ^[f]	46/54

[a] Initial concentration: $[\textbf{1}] = [\textbf{2}] = 0.25 \text{ M}$, $[\text{catalyst}] = 5.0 \text{ mM}$. Conditions: in CH_2Cl_2 at room temperature. [b] Isolated yield. [c] Determined by ^1H NMR spectroscopy. [d] See ref [9]. [e] 1.3 M of **2a** was used. [f] Conversion in parentheses.

Although the ROCM of **1** was further examined with several α -olefins, no selective systems were found out. For example, the reaction with styrene or 1-hexene in the presence of **6** (2 mol %) at room temperature rapidly afforded a quantitative yield of poly(**1**) which was isolated by precipitation from MeOH. The reaction with allyltrimethylsilane, which has been reported as a good cross-metathesis reagent,^[5] also gave the polymer in 85 % yield. In addition, several types of cross-metathesis products as well as a homodimer of allyltrimethylsilane were formed (see the GCMS chart in Supporting Information).

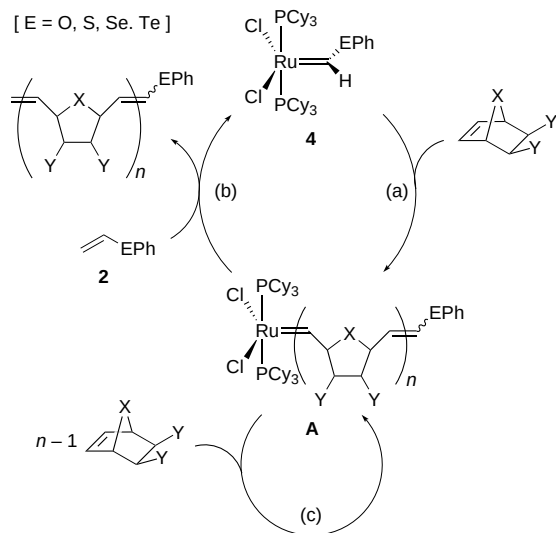
The catalytic ROCM system using vinyl selenide **2c** could be applied to several norbornene derivatives. The representative results are given in Table 2. 7-Oxanorbornene derivatives (**8** and **10**) reacted at room temperature to give the corresponding ROCM products (**9** and **11**, respectively) in high yields (entries 1 and 2). The reactions of less reactive *endo*-5,6-disubstituted norbornenes (**12** and **14**) were successfully conducted at 40 °C using catalyst **5** bearing a 1,3-dimesityl-4,5-dihydroimidazol-2-ylidene ligand (see Scheme 1).^[7]

The variation in the product-selectivity depending on the sort of chalcogen atoms (Table 1) may be rationalized by the catalytic mechanism depicted in Scheme 2, where the Fischer-type carbene complex **4** is proposed as a key intermediate. Although Fischer-type complexes are considered to have little reactivity toward olefin metathesis,^[10] complexes **4a–4d** based on the Grubbs' catalyst **6** were so reactive as to readily catalyze the ROMP of norbornene (**1**) and *exo*-5,6-bis(methoxycarbonyl)-7-oxanorbornene (**8**) at room temperature.^[11] Process (a) giving the alkylidene intermediate **A** ($n = 1$) is the common initiation step for both ROCM and ROMP, which subsequently invoke processes (b) and (c), respectively. Therefore, it is possible that **A** further reacts with norbornene

Table 2. Ruthenium-catalyzed ROCM of norbornene derivatives with **2c**.

Entry ^[a]	Substrate	Product	Yield ^[b] [%]	E/Z ^[c]
1			99	84/16
2			82	72/28
3			96	55/45
4			91	58/42

[a] All reactions were run in CH_2Cl_2 for 20–48 h. Initial concentration: [substrate] = **2c** = 0.25 M, [catalyst] = 5.0 mM. Catalyst: **4c** (entries 1, 2), **5** (entries 3, 4). Reaction temperature: room temperature (entries 1, 2), 40 °C (entries 3, 4). [b] Isolated yield. [c] Determined by ^1H NMR spectroscopy. TBDMS = SiMe_2tBu .



Scheme 2. Proposed mechanism for catalytic ROCM reactions, a), b), and c) are explained in the text.

derivatives according to process (c) to form the ROMP products. However, when vinyl chalcogenide **2** is sufficiently more reactive than the norbornene derivatives, intermediate **A** selectively undergoes process (b) to afford the ROCM products. Thus the catalytic reactions using phenyl vinyl selenide **2c** produces no notable amounts of polymers and the ROCM products are obtained in high yields.

There is another condition for the selective ROCM: the cross-metathesis between **A** ($n = 1$) with **2** must be a highly regioselective process, regenerating **4** exclusively. Thus the greater thermodynamic stability of the Fischer-type complex **4** compared with the corresponding Schrock-type analogue $[\text{Ru}(=\text{CH}_2)\text{Cl}_2(\text{PCy}_3)_2]$ should provide this high regioselectivity, leading to the well-controlled ROCM reactions.^[12]

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Crystal Structure of $[(\text{Ph}(\text{Me})\text{C}:\text{N}:\text{C}(\text{H})\text{Ph})\text{K} \cdot (\text{tBuOK})_2 \cdot (\text{thf})_2]_{\infty}$: A Unimetallic Mixed Anion Model for a “Superbase”?

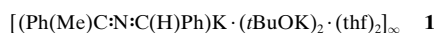
Philip C. Andrews,* Gary D. Fallon, Melissa Maguire, and Anna C. Peatt

In memory of Ron Snaitth

Mixed-anion “superbase” complexes of the type $[\text{RM} \cdot \text{M}'\text{OR}]_n$ ($\text{M} = \text{Li}$, $\text{M}' = \text{Na}$, K) are of great interest since they can exhibit differing selectivity and greater deprotonating ability in synthetic organic procedures than the alkylmetal complex alone.^[1] This behavior has also been described for other alkali metal species such as amides, $[\text{R}_2\text{NM} \cdot \text{MOR}]_n$, and enolates derived from ketones and esters.^[2] The reason for the exceptional reactivity of this family of complexes has been attributed to the formation of mixed-anion aggregates in solution; however, in attempts to isolate these aggregates, the heavier alkali metal species and the alkoxide are, more often than not, deposited separately or isolated only as a microcrystalline solid.^[3] This explains why there is still limited solid-state structural information on mixed-anion systems, though several examples have now been described in each class.^[4] Of most importance and relevance here are the structurally characterized amide/alkoxide complexes; $[[\text{iPr}_2\text{NLi}][(\text{Me}_2\text{NCH}_2)_2\text{CHOLi}]]_2$,^[5] which is unimetallic, and the mixed-metal complexes, $[[[\text{PhN}(\text{H})]_2(\text{tBuO})\text{LiNaK} \cdot (\text{tmen})_2]_2$ ^[6] ($\text{tmen} = N,N,N',N'$ -tetramethylethylenediamine) and $[[(\text{MeOPhN}(\text{H}))_2(\text{tBuO})\text{NaLi}]_2]$.^[7]

Recently we reported that the chiral secondary amine (S)- α -(methylbenzyl)benzylamine, $[(\text{Ph}(\text{Me})\text{CH})(\text{PhCH}_2)\text{NH}]$, readily undergoes transformation to an aza-allylic anion on reaction with $n\text{BuM}$ ($\text{M} = \text{Li}$, Na , K) in the presence of the tridentate N-donor ligand pmdta (N,N',N'',N''' -pentamethyldiethylenetriamine) and other ligand systems which reduce the aggregation state of the complexes to a monomer in solution.^[8] This is consistent with the detailed structural and theoretical studies that have been described for the formation of the 1,3-diphenyl-2-aza-allyl anion from Group 1 complexes of dibenzylamine.^[9] The Na and K complexes of the chiral amine are generally obtained from the reaction with either $n\text{BuNa}$ or $n\text{BuK}$ in the presence of various Lewis donors. $n\text{BuM}$ ($\text{M} = \text{Na}$, K) are synthesized by the reaction of $n\text{BuLi}$ with the appropriate *tert*-butoxide, $t\text{BuOM}$, and isolated and stored as solvent-free solids.

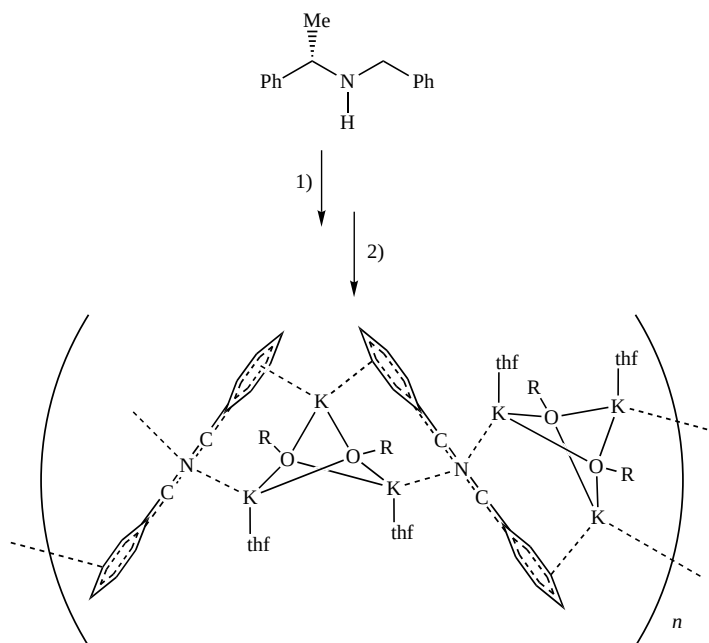
Herein we report on the solid-state structure of the potassium mixed-anion complex **1** and its reproducible,



moderately high-yielding synthesis. While investigating the conversion of (S)- α -($\text{Ph}(\text{Me})\text{CH})(\text{PhCH}_2)\text{NH}$ to the aza-

allylic anion on its reaction with $n\text{BuK}$ in the presence of tmen and THF, we obtained a small quantity of pale red needlelike crystals from the reaction mixture. Analysis by ^1H NMR spectroscopy showed signals relating to both the aza-allylic and $t\text{BuO}^-$ ions in a 1:2 ratio. There were also signals corresponding to THF but, surprisingly, not to tmen. It became clear that a small but significant amount of $t\text{BuOK}$ remained in the $n\text{BuK}$, either from the nonstoichiometric addition of $n\text{BuLi}$ or from inefficient washing of the solid. Repeated NMR experiments on several samples of the crystals indicated that the anion ratios were consistent and the product homogeneous.

In a more rational approach to the synthesis of **1**, the amine was added to a suspension of three molar equivalents of $t\text{BuOK}$ in hexane at -78°C followed by addition of one equivalent of $n\text{BuLi}$ (1.6 M in hexane; Scheme 1). Replacement of hexane by THF, followed by two equivalents of tmen and cooling, led to a moderate yield of red needlelike crystals; thus, the presence of tmen appears crucial.^[10]



Scheme 1. Synthesis of **1**; 1) 3 $t\text{BuOK}$, $n\text{BuLi}$, hexane, -78°C ; 2) 2 tmen, THF ($-\text{hexane}$). $\text{R} = t\text{Bu}$.

The red crystals proved to be air- and moisture-sensitive, decomposing to a yellow solid, and have a melting range of between 131 – 132°C . Low-temperature single-crystal X-ray diffraction studies revealed the novel polymeric structure of the mixed-anion complex **1** (Figure 1). The addition of tmen, in conjunction with the THF already present, provides the driving force for the elimination of H_2 from the amido unit. This results in an almost planar aza-allylic anion, which in the polymer is interspersed by K_3O_2 clusters comprising three K^+ ions and two $t\text{BuO}^-$ ions. The cluster core itself has approximate threefold rotation symmetry with the axis bisecting the two O atoms and structurally is, as far as we are able to ascertain, without precedent. All three K^+ ions are involved in bonding to the aza-allylic units in such a way that

[*] Dr. P. C. Andrews, Dr. G. D. Fallon, M. Maguire, A. C. Peatt
Department of Chemistry, Monash University
Clayton, Melbourne, Victoria 3800 (Australia)
Fax: (+61) 3-9905-4597
E-mail: p.andrews@sci.monash.edu.au

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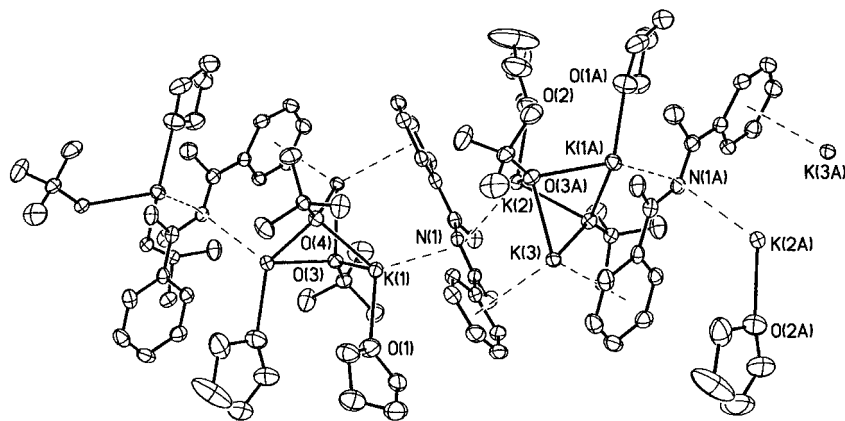


Figure 1. Molecular structure of **1** (30% probability ellipsoids). All hydrogen atoms are removed for clarity. Important bond lengths [Å]; K(1)–O(1, 3, 4) 2.661(3), 2.595(2), 2.572(2), K(1)–N(1) 2.827(3), K(1)–C(1, 8) 3.206(3), 3.294(3), K(2)–O(2, 3, 4) 2.686(3), 2.569(2), 2.600(2), K(2)–N(1) 2.837(3), K(1)–C(1, 8) 3.409(3), 3.256(3), K(3)–O(3, 4), 2.605(2), 2.594(2), K(3)–C(2) 3.263(3), K(3)–C(3) 3.275(3), K(3)–C(4) 3.302(4), K(3)–C(5) 3.333(4), K(3)–C(6) 3.288(3), K(3)–C(7) 3.242(3), K(3)–C(9) 3.273(3), K(3)–C(10) 3.280(4), K(3)–C(11) 3.324(4), K(3)–C(12) 3.359(4), K(3)–C(13) 3.327(3), K(3)–C(14) 3.263(3).

each aza-allyl anion actually accommodates four K^+ ions in total; two on each side. Thus, two of the K centers in the cluster, K(1) and K(2), which are also coordinated by single THF molecules, bond to the same aza-allyl anion at the delocalized C:N:C unit, while the third K^+ center K(3) is sandwiched between phenyl groups from neighboring anions. These phenyl groups tilt towards the K center to allow maximum interaction of the metal with the π -electron density; this leads to a range of bond lengths of between 3.242(3) and 3.359(4) Å for K(3)–C(7) and K(3)–C(12), respectively. These distances are comparable with those observed in $[PhCH_2K \cdot 0.5toluene]_\infty$ which also shows a similar polymeric zigzag arrangement.^[11] The aza-allyl anions therefore sit over the two four-membered butterfly-type rings described by K(1A), O(3), O(4), K(3) and K(2), O(3), O(4), K(3) with the two THF molecules occupying the vacant site defined by the third four-membered butterfly ring, K(1), O(4), K(2), O(3) (Figure 2). Closer inspection of the K_3O_2

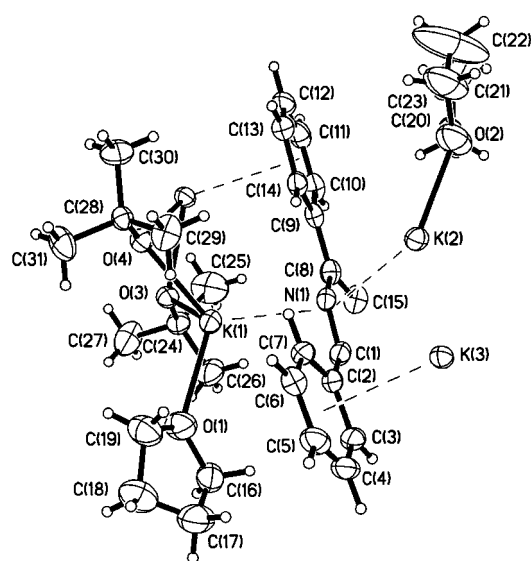
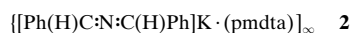


Figure 2. Bonding environment of the aza-allyl anion in **1**.

core reveals that the O atoms in each butoxo anion bridge the three K centers in a μ_3 manner; all the bonds are approximately equal in length (range between 2.569(2) and 2.605(2) Å for K(2)–O(3) and K(3)–O(3), respectively). The symmetry within the core is highlighted by the angles subtended at the metal cations by O(3) and O(4); K(1) 74.65(7)°, K(2) 74.60(7)°, and K(3) 74.11(7)°.

The dative THF to K bonds are slightly longer (K(2)–O(2) 2.686(3), K(1)–O(1) 2.661(3)). K(1) and K(2) bond with the delocalized π -electron density in the C:N:C units; the K(1)–N–K(2) angle is 146.3(1)°. An

interesting comparison can be made here with **2**,^[12] which is also polymeric and contains K ions bridging aza-allylic anions,



although in this case there are two types of cation–anion attachment; one with the aza-allyl unit and the other with an allylic unit with the *ipso*-carbon atom of the phenyl ring at its center and including the benzylic carbon. This type of arrangement is not evident in **1** though there is a degree of asymmetry in the bonding of the K centers to the aza-allylic moieties. The K(1)–N(1) and K(2)–N(1A) distances in **1** of 2.827(3) Å and 2.837(3) Å, respectively, are shorter than those in **2** (2.996(2) Å), though the metal-to-carbon distances are significantly longer; K(1)–C(1, 8) 3.206(3), 3.294(3) Å compared with 2.930(6) Å in **2**, though they are shorter than the K– α C distance of 3.366(7) Å in the allylic group in **2**.

^1H and ^{13}C NMR studies were conducted in $[D_8]THF$ and showed all the expected chemical shifts and splitting patterns associated with the two anions and the THF molecules. We reported previously that it appears possible that there is a low-energy pathway for the elimination of MeH in these systems which can lead to the formation of the 1,3-diphenyl-2-aza-allyl anion. However, there was no indication in the NMR studies that this was occurring in this case and we are attempting now to reproduce the above results with dibenzylamine.

The stability of **1** can most likely be traced back to several factors; 1) the aza-allyl anion is fully delocalized and thus allows a great deal of flexibility in the way the K^+ ions can bond with it, 2) the essentially planar nature of this anion means that as well as offering a large surface area of electron density it has very little three-dimensional bulk, a property which has been described as being important for the stability of mixed-anion systems, and 3) the geometry of the K_3O_2 cluster core allows exposed K^+ vertices, leading to the metal cations being capable of maximizing their bonding interactions and coordination environments.

This provides useful information as to why the addition of tmen may be important in the formation of **1**. Crystal

structures of the Group 1 complexes of (S)-[(Ph(Me)-CH)(PhCH₂)NH] reveal the amido ligand adopts a “butterfly in flight” configuration in which the phenyl groups are almost perpendicular to each other,^[8] and as such can be viewed as having significant steric bulk with the charge concentrated on the N[−] ion. This is less likely to give stable mixed-anion complexes, and can be contrasted to [[PhN(H)]₂(tBuO)Li-NaK·(tmen)₂]₂ and [(MeOPh)N(H)]₂(tBuO)NaLi₂, both of which are derived from primary amines.

Experimental Section

1: (S)- α -(Ph(Me)CH)(PhCH₂)NH (5 mmol, 1.05 g) was added dropwise to a chilled (−78 °C) suspension of *t*BuOK (15 mmol, 1.55 g) in hexane (20 mL). *n*BuLi (5.5 mmol, 3.2 mL, 1.6 M in hexane) was added dropwise resulting in the formation of a bright red suspension. The reaction mixture was stirred and allowed to warm to room temperature over 2 h during which time the suspension darkened to a deeper red. Hexane was removed in vacuo and THF added (ca. 20 mL) which with gentle warming allowed complete dissolution. The ligand tmen (10 mmol, 1.51 mL) was added, then the solvent was reduced in vacuo by approximately a third and the mixture was placed in the freezer at −20 °C overnight. This resulted in a crop of red needlelike crystals which were isolated and then washed with hexane. Yield 57% (1.7 g). M.p. 131–132 °C; elemental analysis C₃₁H₄₈NK₃O₄ (%); calcd: C 60.5, H 7.8, N 2.3; found: C 61.1, H 7.5, N 2.1; ¹H NMR ([D₈]THF, 25 °C, 300 MHz): δ = 7.21 (d, *J* = 7.9 Hz, 2H; *o*-H), 6.90 (brs, 2H; *o*-H), 6.81 (t, *J* = 7.7 Hz, 2H; *m*-H), 6.72 (t, *J* = 7.6 Hz, 2H; *m*-H), 6.19 (m, 2H; *p*; PhCH), 6.06 (t, *J* = 7.1 Hz, 1H; *p*-H), 3.57 (m, 4H; THF), 1.89 (s, 3H; Me), 1.73 (m, 4H; THF), 0.96 (s, 18H; *t*Bu); ¹³C NMR ([D₈]THF, 25 °C, 75.5 MHz): δ = 146.33 (*q*C), 145.78 (*q*C), 129.14 (*o*C), 129.09 (*o*C), 129.00 (*o*C), 128.70 (*m*C), 127.35 (*m*C), 127.32 (*m*C), 119.16 (PhCH), 115.81 (*p*C), 114.41 (PhCMe), 105.75 (*p*C), 37.5 (*t*Bu), 12.35 (Me).

Crystallographic data for **1**: Data collected on an Enraf Nonius Kappa CCD at 123 K. Crystal mounted under oil. C₃₁H₄₈NK₃O₄, *M*_r = 615.01, monoclinic, space group *P*₂₁/*c* (no. 14), red acicular crystal measuring 0.30 × 0.24 × 0.10 mm, *a* = 10.7096(5), *b* = 16.4210(8), *c* = 19.8427(9) Å, β = 91.875(2)°, *V* = 3487.7(2) Å³, *Z* = 4, ρ_{calcd} = 1.171 g cm^{−3}, $\lambda(\text{MoK}\alpha)$ = 0.71073 Å, $\mu(\text{MoK}\alpha)$ = 4.23 cm^{−1}, 42 594 reflections measured, 8661 unique, 3936 were observed (*I* > 3.00σ(*I*)), *R* = 0.043, *R*_w = 0.051, GoF 1.30. The structure was solved by direct methods with all H atoms placed in idealized positions (*r* = 0.95 Å). The aza-allylic anion is disordered in the crystal, as indicated by the occupancy of Me on C1 and C8 of 50:50. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-147738. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Catalytic Asymmetric Aziridination with Arylborate Catalysts Derived from VAPOL and VANOL Ligands**

Jon C. Antilla and William D. Wulff*

The ring opening of chiral aziridines is a potent method for the synthesis of optically active amines.^[1] The driving force of this process derives from the ring strain of the aziridine and this can be used to extend the ring opening to a variety of nucleophiles to produce difunctional amines with vicinal chiral centers. Since no general method for catalytic asymmetric aziridination exists,^[2] chiral aziridines have been largely made from materials in which the chiral centers already exist.^[1d] Given the synthetic and strategic advantages of asymmetric catalysis we began a program to find a chiral catalyst for asymmetric aziridination, and recently reported

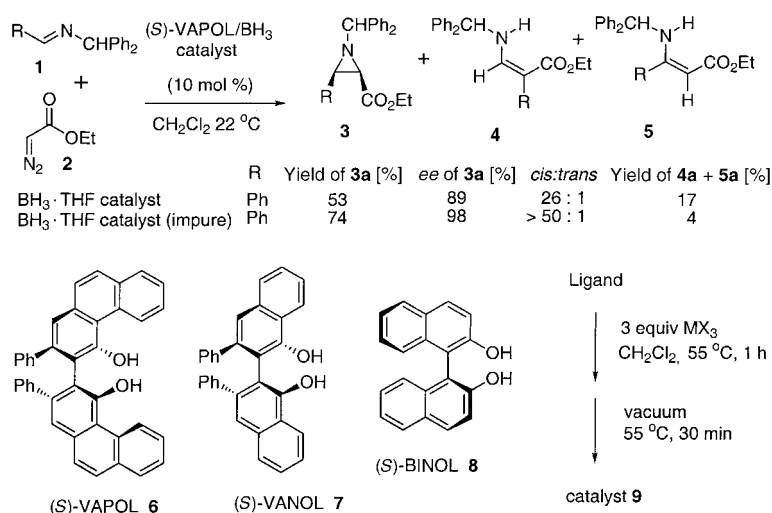
[*] Prof. W. D. Wulff, J. C. Antilla
Department of Chemistry
Michigan State University
East Lansing, MI 48824
Fax: (+1) 517-353-1793
E-mail: wulff@cem.msu.edu

[**] This work was supported by the National Institutes of Health and was performed at the University of Chicago. VAPOL = 2,2'-diphenyl-[3,3'-biphenanthrene]-4,4'-diol, VANOL = 3,3'-diphenyl-[2,2'-binaphthalene]-1,1'-diol.

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the first catalyst that is generally applicable for a range of substrates.^[3] This methodology involves the addition of ethyl diazoacetate (EDA) to imines mediated by a catalyst prepared from the VAPOL ligand and the $\text{BH}_3 \cdot \text{THF}$ complex.^[4] We have since found that the success of the reaction is dependent on the purity of the $\text{BH}_3 \cdot \text{THF}$ complex used to generate the catalyst. This observation led to the present report of the development of a catalyst that is not only more reliable, but which can be extended to previously unreactive substrates, gives increased chemical yields, and greatly increased diastereoselectivity while maintaining high asymmetric inductions. It was also found that the vaulted biaryl ligand VANOL^[4c, 5] is equally effective for this process whereas the 1,1'-binaphth-2-ol (BINOL) ligand fails to give significant asymmetric induction.

A catalyst prepared from VAPOL and a sample of $\text{BH}_3 \cdot \text{THF}$ that is greater than 98 % pure gives the aziridine **3a** in 89 % *ee* from the addition of ethyl diazoacetate to the imine **1a**. However, batches of $\text{BH}_3 \cdot \text{THF}$ that are not so pure can lead to a catalyst with a much improved profile: up to 98 % *ee*, d.r. $\geq 50:1$, and improved yields as well as a significantly reduced level of side products (Scheme 1). One of the impurities that we identified in the commercially available samples of $\text{BH}_3 \cdot \text{THF}$, especially in aged ones, was tributylborate. This observation prompted a study of catalysts prepared from the VAPOL ligand and a variety of borate esters, and the results are given in Table 1. Indeed, it was found that a catalyst prepared from tributylborate gave results nearly identical with the best observed with impure $\text{BH}_3 \cdot \text{THF}$. Lower asymmetric induction was seen with trimethyl- and triethylborate while higher induction was seen with triisopropylborate, although the rate was much slower with the latter. The performance of aluminum and gallium ethoxides were far inferior to any of the borate esters, and in the case of aluminum this may be attributed to poor solubility. The optimal catalyst was that prepared from



Scheme 1. Synthesis of aziridine **3a** and the catalyst **9**. MX₃ = triphenylborate.

triphenylborate, which gave an 81 % yield of **3a** in 95 % *ee* and with a d.r. $\geq 50:1$ in favor of the *cis* isomer.

A comparison of catalysts prepared from triphenylborate and the biaryl ligands BINOL, VANOL, and VAPOL was undertaken. The BINOL-derived catalyst gave very low induction (20 % *ee*) for the addition of ethyl diazoacetate to the imine **1a** (Table 1, entry 11) as well as significantly reduced *cis/trans* selection and an increased proportion of the noncyclized products **4** and **5**. In contrast, the catalysts prepared from the VANOL and VAPOL ligands and triphenylborate were found to be essentially equally effective with remarkably small variation between the two catalysts for all of the substrates (Table 2). In all other reactions that we have previously examined the VANOL and VAPOL ligands have always led to disparate levels of induction.^[4] In the present case, the asymmetric inductions track nearly identically with each substrate and only small differences are seen. For some reactions slightly higher inductions were observed with the VANOL catalyst while for others the higher induction was

Table 1. Asymmetric aziridination of imine **1a** (R = Ph) with catalysts **9**.^[a]

Entry	Metal complex	Catalyst	<i>t</i> [h]	Conversion ^[b]	Yield of <i>cis</i> - 3a [%] ^[c]	<i>ee</i> of <i>cis</i> 3a [%] ^[d]	<i>cis:trans</i> of 3a ^[e]	Yield of 4a [%] ^[f]	Yield of 5a [%] ^[f]
1	BH ₃ · THF	9a ^[g]	20	100	53	89	26:1	11.1	6.3
2	BH ₃ · THF	9a ^[g, h]	3	100	58	91	21:1	15	5.3
3	B(OnBu) ₃	9b ^[i]	10	100	72	96	44:1	3	2.4
4	B(OnBu) ₃	9b ^[i, j]	2	100	71	96	> 50:1	4.8	5.0
5	B(OiPr) ₃	9c ^[k]	92	41	20	97	> 50:1	1.3	1.0
6	B(OMe) ₃	9d	20	92	74	91	35:1	3.6	2.5
7	B(OEt) ₃	9e	20	67	51	92	30:1	3.2	2.3
8	Al(OEt) ₃	9f	20	16	—	—	5:1	6.0	—
9	Ga(OEt) ₃	9g	20	84	29	30	7:1	16.2	12.3
10	B(OPh) ₃	9h	1	100	81	95	> 50:1	4.3	1.4
11	B(OPh) ₃	9h ^[l]	3	100	61	20	17:1	12.6	9.0

[a] Unless otherwise specified, all reactions were run in dichloromethane (0.5 M in the imine) with 0.1 mmol of (S)-VAPOL at 22 °C with 1.1 equiv of ethyl diazoacetate with respect to **1a** and 10 mol % of the catalyst. [b] Determined by ¹H NMR spectroscopy. [c] Isolated after purification by chromatography on silica gel. [d] Determined by HPLC on a Chiralcel OD-H column. [e] Ratio calculated by integration of the methine protons from the *cis*- and *trans*-aziridines. [f] Determined by ¹H NMR spectroscopy of the crude reaction mixture by integration of the signals relative to those of *cis*-**3a**. [g] BH₃ · THF contained less than 2 % B(OnBu)₃. [h] Catalyst prepared at 0 °C for 24 h. [i] 5 mol % of the catalyst. [j] Syringe pump addition of imine (in 1 mL of CH₂Cl₂) to a solution of the catalyst and ethyl diazoacetate over 1 h and with stirring for an additional 1 h. [k] Catalyst prepared from 1 equiv of borate and 1 equiv of (S)-VAPOL. [l] (S)-BINOL was used as the ligand to give **3a** with the same configuration as (S)-VAPOL.

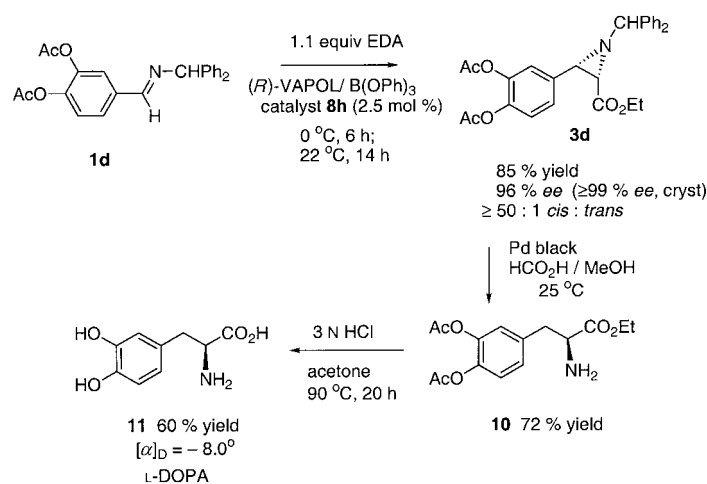
Table 2. Asymmetric aziridination with VAPOL and VANOL catalysts prepared from B(OPh)₃.^[a]

Entry	Imine	R	Ligand	<i>t</i> [h]	Yield of <i>cis</i> - 3 [%] ^[b]	<i>ee</i> of <i>cis</i> - 3 [%] ^[c]	<i>cis:trans</i> - 3 ^[d]	Yield of 4 [%] ^[e]	Yield of 5 [%] ^[e]
1	1a	Ph	(<i>S</i>)-VAPOL	48	77 ^[f]	95	> 50:1	4.4	1.7
2			(<i>S</i>)-VANOL	0.5	85	96	> 50:1	3.3	1.0
3	1b	<i>p</i> -BrC ₆ H ₄	(<i>S</i>)-VAPOL	5	91 ^[g]	98	> 50:1	3.4	2.2
4			(<i>S</i>)-VANOL	1	85	98	> 50:1	8	3
5	1c	<i>o</i> -MeC ₆ H ₄	(<i>S</i>)-VAPOL	14	69	94	40:1	11.1	7.1
6			(<i>S</i>)-VANOL	16	65	91	> 50:1	6	4
7	1d	3,4-(OAc) ₂ C ₆ H ₃	(<i>R</i>)-VAPOL	20	85 ^[f, h]	96	> 50:1	< 1	< 1
8			(<i>S</i>)-VANOL	5	83	97	> 50:1	< 1	< 1
9	1e	2-furyl	(<i>R</i>)-VANOL	16	55 ^[i]	93	> 50:1	< 1	< 1
10	1f	1-naphthyl	(<i>S</i>)-VAPOL	12	87	92	> 50:1	3.8	0.3
11	1g	<i>n</i> -propyl	(<i>S</i>)-VAPOL	20	54 ^[i]	91	> 50:1	8.3	9.2
12			(<i>S</i>)-VANOL	5	60	90	> 50:1	6.7	9.2
13	1h	<i>tert</i> -butyl	(<i>S</i>)-VAPOL	12	78	91	40:1	< 1	< 1
14			(<i>S</i>)-VANOL	5	77 ^[k]	97	> 50:1	< 1	< 1
15	1i	<i>c</i> -C ₆ H ₁₁	(<i>S</i>)-VAPOL	8	74	94	38:1	< 1	< 1

[a] Unless otherwise specified, all reactions were run in dichloromethane containing 0.5 M imine at 22 °C with 1.1 equiv of ethyl diazoacetate with respect to imine **1a** and 10 mol % of the catalyst. [b] Isolated after purification by chromatography on silica gel. [c] Determined by HPLC on a Chiralcel OD-H column. [d] Ratio calculated by integration of the methine protons from *cis*- and *trans*-aziridines. [e] Determined by ¹H NMR spectroscopy of the crude reaction mixture by integration of the signals relative to those of *cis*-**3**. [f] Reaction with 2 mol % of the catalyst (entry 1) or 2.5 mol % (entry 7) and 1.0 M of the imine. [g] Reaction in toluene:dichloromethane (1:1). [h] Reaction was run at 0 °C for 4 h and then 16 h at 22 °C. [i] Reaction conditions: toluene, 0 °C for 16 h. [j] Reaction conditions: toluene, 0 °C for 4 h, then 22 °C for 16 h. [k] Reaction conditions: toluene, 0 °C for 4 h, then 22 °C for 1 h.

observed with the VAPOL catalyst. What is remarkable is that for both ligands and for all substrates the asymmetric induction for all combinations falls in the range of 90–98 % *ee*. The chemical yields of **3** for each catalyst are similar and both are generally higher than the yields observed with the BH₃·THF complex. The reaction times indicated in Table 2 are not indicative of rate since the times were not optimized and in some cases different catalyst loadings were employed. The diastereoselectivities are very high for both catalysts but the VANOL catalyst gave a selectivity of greater than 50:1 for every substrate. The diastereoselectivity is one of the greatest differences between the catalysts generated from triphenylborate and the BH₃·THF complex. For example, the *cis:trans* selectivity for the imine **1c** derived from *ortho*-methylbenzaldehyde was 3:1 for the catalyst prepared from VAPOL and the BH₃·THF complex,^[3] while it was 40:1 with the corresponding catalyst derived from the triphenylborate catalyst. Another dramatic difference is that the imines prepared from pivaldehyde and 1-naphthylaldehyde are unreactive with the catalyst prepared from VAPOL and BH₃·THF, whereas these substrates react with similar rates using the triphenylborate catalyst to give asymmetric inductions and chemical yields similar to those observed for the other substrates. Finally, the rates of the reaction could not be increased by the slow addition of the imine with the VAPOL–borate catalyst as was found to be the case with the VAPOL–borane catalyst.^[3]

An application of the VAPOL–triphenylborate catalyst in the synthesis of L-3,4-dihydroxyphenylalanine (L-DOPA) is illustrated in Scheme 2. The reaction of imine **1d** with ethyl diazoacetate in the presence of 2.5 mol % of the catalyst derived from (*R*)-VAPOL and triphenylborate led to the formation of the aziridine **3d** in 85 % yield and in 96 % *ee* on a multigram scale. The optical purity could be improved to greater than 99 % *ee* with a single crystallization of the product from hexane:dichloromethane (10:1; the first crop gave 85 % recovery).^[6] Hydrogenation of the nitrogen–



Scheme 2. Synthesis of L-DOPA.

benzylic bond occurred with cleavage of the benzhydryl group to give the amino ester **10** in 72 % yield. After hydrolysis of the three ester linkages L-DOPA was obtained in 60 % yield with an optical rotation of [α]_D = -8.0°. This rotation corresponds to 98 % *ee*, and thus very little if any epimerization occurred during the hydrolysis.^[7]

Further studies on the mechanism, scope, and synthetic applications of this asymmetric catalytic aziridination reaction will be reported in due course.

Experimental Section

3d: (*R*)-VAPOL (174 mg, 0.323 mmol) was added to a flame-dried flask cooled under argon and then dissolved in CH₂Cl₂ (6 mL). After the addition of triphenylborate (281 mg, 0.969 mmol) the mixture was heated to 55 °C for 1 h and then a vacuum (0.5 mm Hg) was applied for 30 min with the temperature maintained at 55 °C. The catalyst was dissolved in CH₂Cl₂ (3.0 mL) and transferred by syringe to a 50-mL flame-dried flask which was previously charged with a stirring bar and filled with argon. A solution of

imine **3d** (5.00 g, 12.9 mmol) in CH_2Cl_2 (10 mL) was added by syringe to the catalyst solution at 0 °C. Stirring the mixture for 10 min gave an orange solution to which was rapidly added ethyl diazoacetate (1.484 mL, 14.2 mmol) by syringe. Some bubbling was noted after the addition. The reaction was allowed to proceed for 6 h at 0 °C and then for 14 h at room temperature (22 °C). The reaction mixture was transferred to a 500-mL flask, diluted with hexanes (250 mL), and then the volatiles were removed under vacuum to give the crude aziridine **3d** as an off-white solid. The ^1H NMR spectrum of this material revealed **3d** with *cis:trans* $\geq 50:1$ and indicated that $<1\%$ of **4d** and **5d** were formed. Purification of **3d** by column chromatography on silica gel with a mixture of ethyl acetate:hexanes (3:7) gave aziridine **3d** (5.20 g, 11 mmol) as a white solid in 85% yield. The optical purity of this material was determined to be 96% *ee* by HPLC analysis (OD-H column). Crystallization from hexanes: CH_2Cl_2 (10:1, 300 mL) gave **3d** (4.43 g) with 99% *ee*. A second crop was taken but found to be only 90% *ee*. Spectral data for **3d**: m.p. 141–143 °C (hexanes: CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): δ = 0.99 (t, J = 7 Hz, 3H), 2.24 (s, 3H), 2.25 (s, 3H), 2.68 (d, J = 7 Hz, 1H), 3.18 (d, J = 7 Hz, 1H), 3.95 (s, 1H), 3.95 (m, 2H), 7.07 (d, J = 9 Hz, 1H), 7.19 (m, 1H), 7.28 (m, 7H), 7.45 (d, J = 7 Hz, 2H), 7.81 (d, J = 7 Hz, 2H). ^{13}C NMR (100.6 MHz, CDCl_3): δ = 13.84, 20.64, 46.57, 47.03, 60.89, 77.49, 122.75, 122.78, 126.05, 127.18, 127.30, 127.45, 127.61, 128.55, 128.65, 133.97, 141.35, 141.57, 142.21, 167.45, 168.07, 168.24; IR (thin film) 3030 (w), 2980 (w), 1770 (s), 1731 (s), 1600 cm^{-1} (m); MS (EI): m/z (relative intensity): 474 (21) [$M+1$], 306 (12), 195 (10), 167 (100); m/z calcd for $\text{C}_{28}\text{H}_{27}\text{NO}_6$: 474.1903, found 474.1903. Elemental analysis calcd for $\text{C}_{28}\text{H}_{27}\text{NO}_6$: C 71.02, H 5.75, N 2.96; found: C 71.23, H 5.88, N 2.94. $[\alpha]_{\text{D}}^{25} = -17.3^\circ$ (C = 1 from CH_2Cl_2) taken on 99% *ee* material (HPLC). Further experimental details can be found in the Supporting Information.

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- [5] The VANOL ligand was prepared according to the same procedure that has been reported for the VAPOL ligand.^[4c]
- [6] A second crop was taken but had only approximately 90% *ee*.
- [7] The optical rotation of an authentic sample of L-DOPA was found to be $[\alpha]_{\text{D}} = -8.2^\circ$.

Stereoselective Synthesis of R_p - and S_p -Dithymidine Phosphorothioates via Chiral Indolooxazaphosphorine Intermediates Derived from Tryptophan**

Yixin Lu and George Just*

The use of phosphorothioates as DNA analogues useful in antisense-based therapy is well established^[1] and led to the development of Vitravene as the first antisense drug.^[2] Several other phosphorothioate oligonucleotides (PS-Oligos) are in clinical trials. Although Stec et al. described an elegant oxathiaphospholane-based approach^[3] for preparing diastereomerically pure phosphorothioates, it has not been used for large-scale production of PS-Oligos, which are still used as a mixture of about 10^6 diastereomers. The use of cyclic *N*-acylphosphoramidites as promising monomers for the stereocontrolled synthesis of phosphorothioates was recently reported by Beaucage et al.^[4]

Previously, we developed cyclic phosphoramidites^[5] such as sugar-derived oxazaphosphorinanes,^[5b,c] indolooxazaphosphorines (**a**),^[5e,f] and indoleimidazoles^[5i] for the stereoselective synthesis of PS-Oligos. Here we report on the use of promising indolooxazaphosphorine precursors derived from tryptophan which do not require a difficult chromatographic separation and may form the basis of a practical process.

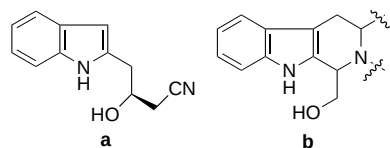
As demonstrated in the indolooxazaphosphorine approach,^[5f] chiral auxiliary **a** led to the stereoselective synthesis of phosphorothioate in solution. When it was applied to solid-phase synthesis, a β -elimination caused rearrangement to give

[*] Prof. Dr. G. Just, Dr. Y. Lu
Department of Chemistry
McGill University
Montreal, PQ, H3A 2K6 (Canada)
Fax: (+1) 514-398-3797
E-mail: just@chemistry.mcgill.ca

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a cyanoethyl phosphonate.^[5g] We therefore decided to synthesize a chiral auxiliary of type **b**, which can be derived from tryptophan. Removal of the chiral auxiliary could then be

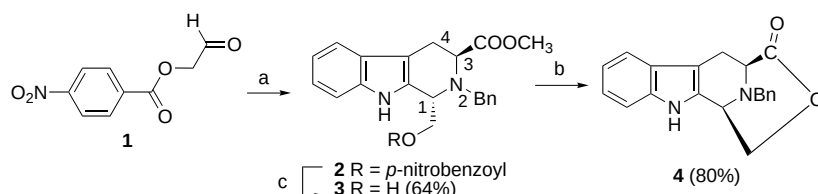


effected by direct displacement of the primary alcohol group or by neighboring-group participation of an appropriately positioned functionality. When benzyl serves as a protecting group for N_b of tryptophan, a Pictet–Spengler reaction tends to yield a single diastereomer.^[6] This could well solve the chirality problem of the new chiral auxiliary. Furthermore, the chiral auxiliary can be derived from natural chiral tryptophan, making the method very attractive and inexpensive.

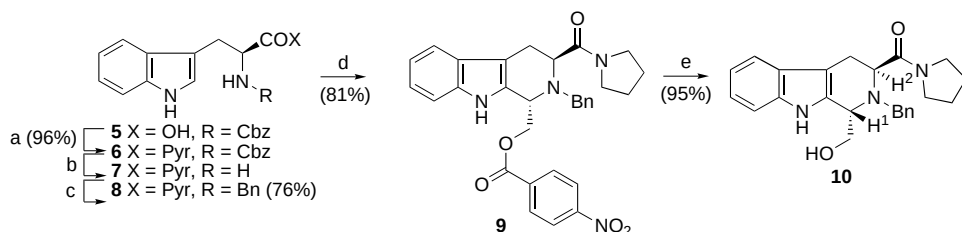
The synthesis of the chiral auxiliary is illustrated in Scheme 1. Dimethylallyl alcohol was transformed into its *p*-nitrobenzoate, ozonolysis of which then gave aldehyde **1**. Pictet–Spengler reaction between **1** and N_b -benzyl tryptophan methyl ester^[6] in toluene without acid catalyst afforded the desired condensed product **2**. Ester **2** was then methanolized by reaction with sodium methoxide in methanol. To our surprise, lactone **4** or its enantiomer was obtained as the product.

Cook et al. showed that Pictet–Spengler reactions between N_b -benzyl tryptophan methyl ester and bulky aldehydes led to stereospecific formation of the *trans* diastereomer.^[6a] Since our product was a single isomer, it follows that epimerization occurs readily at C1 or C3. These facile epimerizations are preceded.^[6b] Participation of the neighboring amide group has been reported.^[5d, 7] We therefore considered synthesizing a chiral auxiliary with an amide group. Removal of the chiral auxiliary by neighboring-group participation can be expected at the end of the synthesis should facile epimerization occur during removal.

Chiral auxiliary **10** with a neighboring pyrrolidine amide (Pyr) group was easily prepared (Scheme 2). Commercially available N_b -Cbz-L-tryptophan (**5**) was coupled with pyrrolidine to yield amide **6**. The Cbz protecting group was then removed to give **7**, which upon reductive amination with benzaldehyde was transformed into **8**. Pictet–Spengler condensation of **8** with aldehyde **1** afforded **9** as a single diastereomer, which was then hydrolyzed to furnish **10** as a single isomer. The absence of cross-peaks



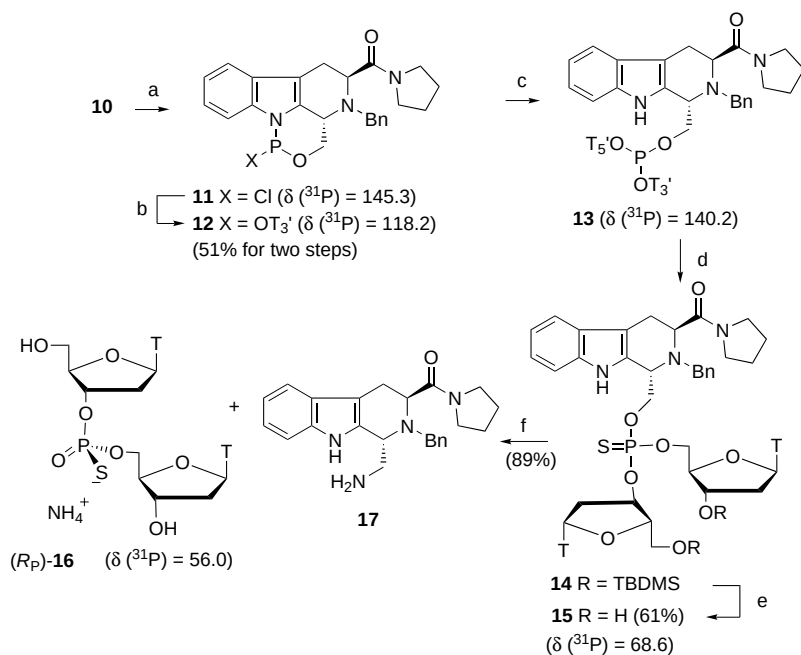
Scheme 1. a) N_b -Benzyl tryptophan methyl ester, toluene, reflux, Dean–Stark trap; b) NaOMe, MeOH; c) *i*PrNH₂, 80 °C.



Scheme 2. a) Pyrrolidine, Et₃N, HBTU, CH₃CN, RT; b) H₂, Pd/C, ethyl acetate/H₂O/AcOH; c) PhCHO, NaCNBH₃, MeOH, pH 6; d) **1**, toluene, reflux, Dean–Stark trap; e) NaOH, H₂O/THF, 55 °C. Cbz = carboxybenzyl, HBTU = *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, Pyr = 1-pyrrolidyl.

between the H¹ and H² protons in the NOESY spectra of **10** means the expected *trans* configuration is very likely.^[6a]

The application of chiral auxiliary **10** to the stereoselective synthesis of phosphorothioates is outlined in Scheme 3. A solution of **10** and triethylamine in dichloromethane was added slowly to phosphorus trichloride at 0 °C. Initially, several ³¹P NMR signals were observed. After heating at 50 °C for one day to equilibrate, a single peak at around $\delta = 145$ was observed, corresponding to **11**. When intermediate **11** was further treated with 5'-*O*-TBDMS-thymidine (T₃OH) in the



Scheme 3. a) PCl₃, Et₃N, CH₂Cl₂; b) T₃'OH, Et₃N, CH₂Cl₂; c) T₃'OH, DBU, THF; d) Beaucage's reagent; e) TBAF; f) conc. ammonia/EtOH, 55 °C. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, T = thymine residue, TBAF = tetrabutylammonium fluoride, TBDMS = *tert*-butyldimethylsilyl, T₃OH = 5'-*O*-TBDMS-thymidine, T₃'OH = 3'-*O*-TBDMS-thymidine.

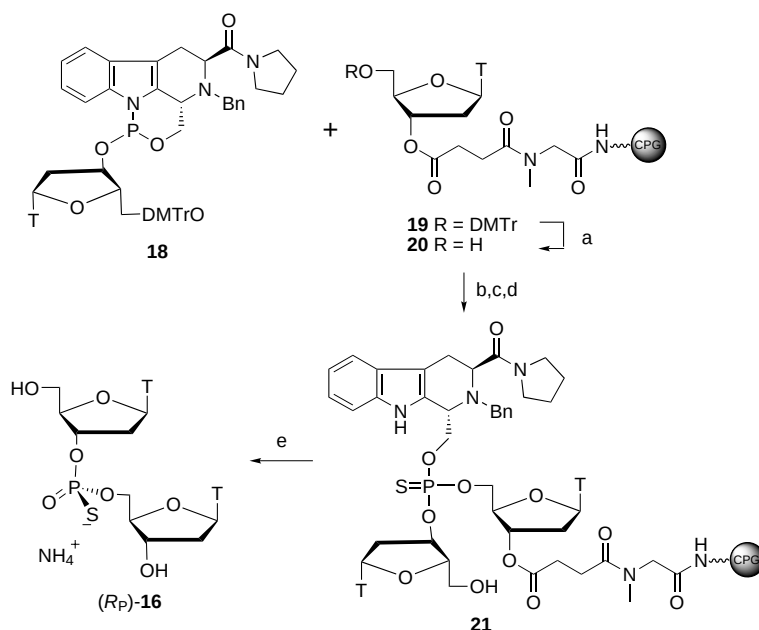
presence of triethylamine in dichloromethane, the reaction went to completion in 10 min to yield a single isomer of phosphoramidite **12** with a ^{31}P NMR signal at $\delta = 118.2$. Cyclic phosphoramidite **12** was stable enough to be easily purified by column chromatography on silica gel. When purified **12** was treated with 3'-*O*-TBDMS-thymidine (T_5OH) in the presence of five equivalents of DBU, the displacement was completed within 20 min at room temperature, as established by the formation of a characteristic major ^{31}P NMR peak at $\delta = 140$ corresponding to phosphite triester **13**. The DBU was removed by filtration through a short column, and sulfurization with Beaucage's reagent^[8] afforded phosphorothioate **14** with a major ^{31}P NMR signal at around $\delta = 68$. Removal of silyl protecting groups gave dithymidine phosphorothioate **15**.

We next attempted to remove the chiral auxiliary. The solution of **15** in water/THF was first heated at 55°C . No reaction had occurred after one day. We then treated **15** with concentrated ammonia/ethanol (3/1) at 55°C for 16 h. To our delight, phosphorothioates **16** were obtained, as indicated by the presence of two peaks at $\delta = 56.1$ and 56.0 in a ratio of 1 to 40 in the ^{31}P NMR spectrum. The configurations of minor and major isomers were assigned as S_{P} and R_{P} , respectively, on the basis of their ^{31}P NMR chemical shifts.^[5b] The chiral auxiliary we recovered had structure **17**, and this means direct attack by ammonia was responsible for its removal. Should the chiral auxiliary epimerize to give a *cis* configuration, neighboring-group participation would be expected to yield lactone **4** or its hydrolyzed product after deprotection.

The methyl ester chiral auxiliary **3** was used for the stereoselective synthesis of phosphorothioates in a similar manner, and the same results were obtained.

To demonstrate that our methodology is compatible with solid-phase synthesis of oligonucleotides, we synthesized R_{P} - and S_{P} -dithymidine phosphorothioates on a solid phase. The solid-phase syntheses of dithymidine phosphorothioates were performed manually (Scheme 4). 5'-*O*-DMTr-thymidine (**19**), obtained by immobilizing thymidine on controlled pore glass (CPG) with a DBU-resistant sarcosinyl-succinoyl linker,^[9] was detritylated and treated with excess DBU followed by the addition of L-tryptophan-derived monomer **18**. After 20 min at room temperature, the solid support was washed with acetonitrile and sulfurized with Beaucage's reagent, followed by detritylation to furnish phosphorothioate **21**. The solid support was heated in concentrated ammonia at 55°C for 16 h to cleave the dimer and remove the chiral auxiliary to furnish (R_{P})-dithymidine phosphorothioate **16**.

The configuration at the phosphorus center of **16** was established by comparison of reverse-phase HPLC (RP-HPLC) chromatograms of **16** and thymidine dimers synthesized by a nonstereoselective method. It is documented in the literature^[10] that the R_{P} isomer of the dinucleotide eluted faster than S_{P} isomer in RP-HPLC. The configuration of **16** was accordingly assigned as R_{P} . The identity of **16** was also proved by HPLC-MS, which showed the peak of the



Scheme 4. a) Cl_3CCOOH ; b) DBU, then **18**; c) Beaucage's reagent; d) Cl_3CCOOH ; e) conc. ammonia/EtOH, 55°C . DMTr = 4,4'-dimethoxytrityl.

dithymidine phosphorothioate anion. Similarly, the monomer derived from D-tryptophan led to the formation of the S_{P} dimer, which was also confirmed by HPLC comparison and HPLC-MS analysis.

In summary, chiral indolooxazaphosphorine auxiliaries can be easily prepared from L- and D-tryptophans. When applied in solution and solid-phase syntheses of dithymidine phosphorothioates, the L-tryptophan-derived chiral auxiliary led to the formation of the R_{P} isomer, and the D-tryptophan-derived precursor to the S_{P} isomer. The adaptation of the methodology to routine solid-phase synthesis of oligonucleotides is underway.

Experimental Section

10: Amide **8** (0.35 g, 1 mmol) and aldehyde **1** (0.23 g, 1.1 mmol) in toluene were heated to reflux with removal of water. After 4 h, evaporation to a small volume provided a light yellow crystalline product **9** (0.32 g, 60%). The mother liquid was purified by chromatography to give more **9** (0.11 g, 21%; HRMS (FAB): calcd for $\text{C}_{31}\text{H}_{31}\text{N}_4\text{O}_5$: 539.2294; found: 539.2293). A solution of **9** (110 mg, 0.2 mmol) in 2N aqueous NaOH (3 mL) and THF (3 mL) was warmed to 55°C for 1 h. Extraction with ethyl acetate provided **13** as a white solid (74 mg, 95%). ^1H NMR (500 MHz, CDCl_3): $\delta = 8.05$ (br, 1H), 7.56 (d, 1H, $J = 7.5$ Hz), 7.10–7.30 (m, 8H), 4.10 (m, 2H), 4.01–4.03 (m, 2H), 3.90–3.96 (m, 1H), 3.72–3.80 (m, 2H), 3.40–3.58 (m, 4H), 2.72–2.76 (dd, 1H, $J = 4.0, 16.5$ Hz), 1.87–2.03 (m, 4H); ^{13}C NMR (125.7 MHz, CD_3COCD_3): $\delta = 169.79, 139.32, 136.17, 130.35, 128.58, 128.42, 127.12, 126.83, 121.83, 119.40, 118.27, 110.73, 108.66, 63.71, 57.75, 56.86, 52.75, 46.17, 26.30, 23.99, 19.04, 14.02$; HRMS (EI) calcd for $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_2$: 389.2103; found: 389.2099.

12: A solution of **10** (39 mg, 0.1 mmol) in CH_2Cl_2 containing Et_3N (30.7 μL , 0.22 mmol) was added slowly to a solution of PCl_3 (8.7 μL , 0.1 mmol) in CH_2Cl_2 (0.25 mL) at 0°C . The NMR tube was then sealed. After heating at 50°C for 24 h, a solution of 3'-*O*-TBDMS-thymidine (39 mg, 0.11 mmol) and Et_3N (15.4 μL , 0.11 mmol) was added to the solution of **11**, and the NMR tube was resealed. When ^{31}P NMR spectroscopy showed the disappearance of the signal at $\delta = 144$ and the formation of new signal at around $\delta = 118$, the solvent was removed in vacuo. Purification by flash chromatography afforded **12** as a white solid (39 mg, 51%). ^{31}P NMR

(202.3 MHz, CDCl₃): δ = 116.96; ¹H NMR (500 MHz, CDCl₃): δ = 8.48 (br, 1 H), 7.54 (d, 1 H, $J_{4,5}$ = 7.5 Hz), 7.51 (d, 1 H, $J_{6,7}$ = 7.5 Hz), 7.45 (s, 1 H), 7.19–7.35 (m, 7 H), 6.12 (m, 1 H), 4.90 (m, 1 H), 4.36 (m, 1 H), 3.45–4.00 (m, 8 H), 3.50 (m, 3 H), 3.26 (m, 1 H), 3.13 (m, 1 H), 2.96 (m, 1 H), 2.23 (m, 1 H), 2.01–2.06 (m, 1 H), 1.82–1.95 (m, 7 H), 0.91 (s, 9 H), 0.08 (2s); HRMS (FAB): calcd for C₄₀H₅₃N₅O₇PSi: 774.3452; found: 774.3455.

15: DBU (37 μ L, 0.25 mmol) was added to a stirred solution of **12** (38.6 mg, 0.05 mmol) and 3'-O-TBDMS-thymidine (35.6 mg, 0.1 mmol) in dry THF (2 mL) at room temperature. After 20 min, the ³¹P NMR spectrum of the reaction mixture showed a single signal at around δ = 140. The mixture was then loaded onto a short column to filter off DBU. After evaporation of the solvent, the residue was dissolved in dry CH₂Cl₂, and Beaucage's reagent (10 mg, 0.05 mmol) was added at room temperature. After stirring for 5 min, ³¹P NMR spectroscopy revealed a single peak at around δ = 68. The solvent was removed, the residue was dissolved in THF, and TBAF was added. After 2 h, the solvent was removed in vacuo. CH₂Cl₂ was added to the residue, and the extract was washed with water and brine and dried over magnesium sulfate. Purification by column chromatography afforded **15** as a white solid (28 mg, 61 %).

16 and 17: Phosphorothioate **15** (28 mg, 0.026 mmol) was dissolved in concentrated ammonia/ethanol (3/1, 5 mL). The mixture was heated at 55 °C for 16 h. The ³¹P NMR signal shifted quantitatively from δ = 68 to 56. The reaction mixture was extracted with chloroform several times. The combined organic extracts were washed with brine and dried over magnesium sulfate. The solvent was removed to provide **17** (9.2 mg, 91 %) as a light yellow solid. The aqueous crude product was purified by flash chromatography to give **16** (15 mg, 89 %).

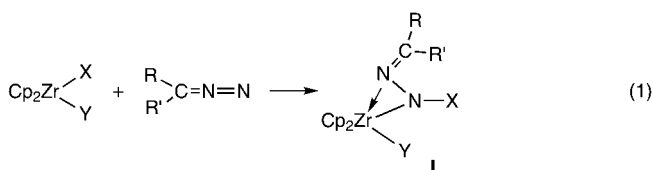
17: ¹H NMR (500 MHz, CDCl₃): δ = 7.41 (d, 1 H, J = 8.0 Hz), 7.23–7.35 (m, 6 H), 7.13 (m, 1 H), 7.02 (m, 1 H), 4.71 (br, 1 H), 4.04–4.14 (m, 2 H), 3.94 (m, 2 H), 3.68–3.86 (m, 2 H), 3.11–3.23 (m, 4 H), 2.97 (m, 1 H), 2.82 (m, 1 H), 1.55–1.82 (m, 5 H); HRMS (FAB): calcd for C₂₄H₂₉N₄O: 389.2341 [(M+1)⁺]; found: 389.2343.

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Early Transition Metal α -Diazoalkane Complexes**

Victorio Cadierno, Maria Zablocka, Bruno Donnadiu, Alain Igau, and Jean-Pierre Majoral*

The reaction of diazoalkanes with transition metals results either in coordination through the nitrogen atoms or in formation of a carbon–metal bond. Many N-bonded substituted transition metal diazoalkanes have been studied,^[1] but only four series of C-bonded late transition metal compounds have been isolated.^[2] α -Metalated diazoalkanes L_nM–C(N₂)R containing an early transition metal have not yet been reported. Treatment of zirconocene Zr^{IV} complexes with diazoalkanes results in insertion into Zr–C,^[3] Zr–H,^[3c] Zr–P,^[4] and zirconium–metal bonds^[5] to give the corresponding hydrazone ligands **I** [Eq. (1)].



We have shown that 1-aza-zirconacyclopentene complexes **1a** and **1b**^[6] (see Scheme 1) contain a strongly electrophilic metal center (mostly because of the inductive electron-withdrawing properties of the σ -bonded nitrogen atom) and a rather nucleophilic imido group that does not share its lone-pair electron density with the adjacent metal center because of the strain in the metallacycle.^[7] Indeed phosphanes **1a** and **1b** activate C–H bonds in relatively acidic carbonic acids, such as acetylenes and methylene compounds.^[8] Although electrophilic, metal-mediated, aliphatic C–H bond cleavage is preceded,^[9] its application to the functionalization of complex substrates is very rare. The present report deals with a novel electrophilic sp²–C–H bond activation and its application for the preparation of α -diazomethylzirconium complexes, which are the first C-metalated diazoalkanes L_nMC(N₂)R with an early transition metal.

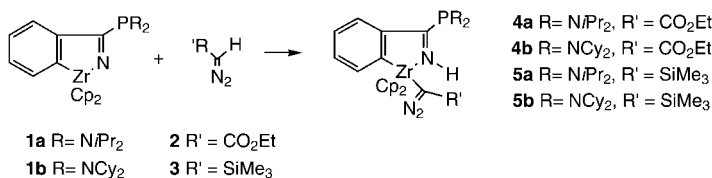
Treatment of **1a** and **1b** with ethyl diazoacetate (**2**) in THF at room temperature gave the light yellow, thermally stable solids **4a** and **4b** (Scheme 1) in excellent yields (>88 %). The ³¹P NMR spectra of **4a** and **4b** display a singlet at δ = 49.4 and 58.5, respectively. The total disappearance of the sp²–CH

[*] Dr. J.-P. Majoral, Dr. V. Cadierno, Dr. B. Donnadiu, Dr. A. Igau
Laboratoire de Chimie de Coordination, CNRS
205 route de Narbonne, 31077 Toulouse Cedex (France)
Fax: (+33) 05-61-55-30-03
E-mail: majoral@lcc.toul.lcc-toulouse.fr
Dr. M. Zablocka
Polish Academy of Sciences
Sienkiewicza 112, 90-363 Lodz (Poland)

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

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Scheme 1. Synthesis of α -diazomethyl zirconium complexes **4a, b** and **5a, b**.

chemical shift of the starting reagent **2** was monitored by ^1H NMR spectroscopy, which showed the presence of a broad, low-field signal for the NH proton at $\delta = 9.55$ and 9.74 in the final products **4a** and **4b**, respectively. In addition to the chemical shifts corresponding to the aza-zirconocene metallacycle skeleton (the resonances for the Cp groups appear as one singlet), the ^1H and ^{13}C NMR spectra exhibited signals in the region expected for the CO_2Et ester group bonded to the diazo functionality. The most striking data of the C-coordinated $\text{C}(\text{N}_2)$ diazo group in **4a** and **4b** are the ^{13}C NMR chemical shifts at $\delta = 48.1$ and 47.0 ,^[2, 10] respectively, and the intense band at 2029 cm^{-1} in the IR spectra for $\text{C}=\text{N}=\text{N}$, which is shifted to lower frequency in comparison with that of the starting diazoalkanes.^[2] All these data are fully in accordance with the structure proposed for **4a** and **4b**.

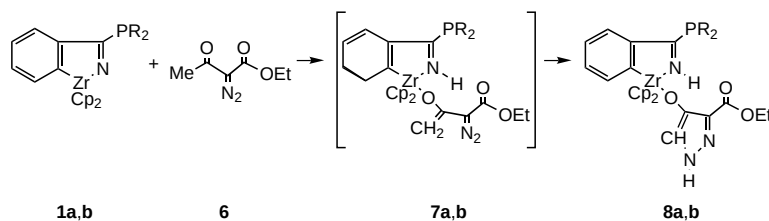
An identical experimental procedure was conducted with trimethylsilyldiazomethane (**3**) and **1a, b** to prepare the corresponding C- α -metalated diazoalkane complexes **5a** and **5b**, which were isolated in good yield (Scheme 1). The ^1H and ^{13}C NMR spectra exhibited the expected chemical shifts for the SiMe_3 fragment and showed signals at $\delta = 30.5$ (**5a**) and 36.7 (**5b**) for the $\text{Zr}-\text{C}(\text{N}_2)$ carbon atom.^[2, 10] The IR spectra displayed a characteristic band at 2069 (**5a**) and 2049 cm^{-1} (**5b**) for the diazo $\text{C}=\text{N}=\text{N}$ group. Thus spectroscopic and analytical data^[11] are consistent with the formulation of **4a, b** and **5a, b** as the first α -C-zirconated diazoalkanes.

Interestingly when **1a** and **1b** were allowed to react with the “nonterminal” diazo compound **6**, the pyrazoles **8a** and **8b** were obtained in good yield (Scheme 2). These new compounds were unambiguously characterized by mass spectrometry as well as by IR and NMR spectroscopy (see the Experimental Section). Formation of the metalated heterocycles **8a** and **8b** could be rationalized by O–H bond activation of the enol tautomer of substrate **6**, leading to the zirconocene diazo intermediates **7a** and **7b**, which rearrange by an intramolecular [3+2] cycloaddition to give the isolated products **8a** and **8b**.

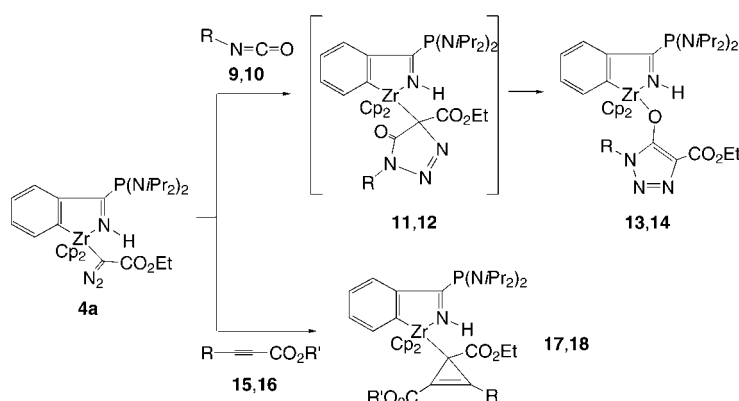
In a first approach, the reactivity of the C- α -metalated diazoalkane complex **4a** was tested by addition of phenyl isocyanate (**9**) to afford the 1,2,3-triazolato zirconocene enolate complex **13** (Scheme 3). As evidenced from ^{31}P , ^1H , and ^{13}C NMR spectroscopies, the structure of the aza-zirconocene metallacycle skeleton remained unchanged. In particular, the ^1H NMR signal of the NH group appeared as expected at $\delta = 9.59$ and the ^{13}C NMR shift for $\text{P}-\text{C}=\text{N}$ was observed at $\delta = 201.3$ with $J_{\text{C,P}} = 38.5\text{ Hz}$ (**4a**: $\delta(^{13}\text{C}) = 203.9$,

$J_{\text{C,P}} = 39.3\text{ Hz}$). No IR absorption corresponding to a diazo group was identified in **13**. In the ^{13}C NMR spectrum the signals corresponding to the triazole skeleton at $\delta = 119$ and 162 are in the range usually observed for sp^2 carbon atoms bonded to ester and ether groups, respectively.

An identical reaction was conducted with naphthyl isocyanate (**10**) and **4a** (Scheme 3). The isolated triazole **14**^[11]



Scheme 2. Reactions of the enolizable diazo compound **6** with **1a, b**: **a**: R = NiPr_2 , **b**: R = NCy_2 .



Scheme 3. Cycloaddition reactions of the α -diazomethyl zirconium complex **4a**: **9, 11, 13**: R = Ph; **10, 12, 14**: R = α -Naph; **15, 17**: R = CO_2Et , R' = Et; **16, 18**: R = H, R' = Me.

exhibited the same spectroscopic data as **13**. However, to characterize unambiguously the cycloaddition products **13** and **14**, a single-crystal X-ray analysis of **14** was undertaken (Figure 1, see the Experimental Section). The features at the

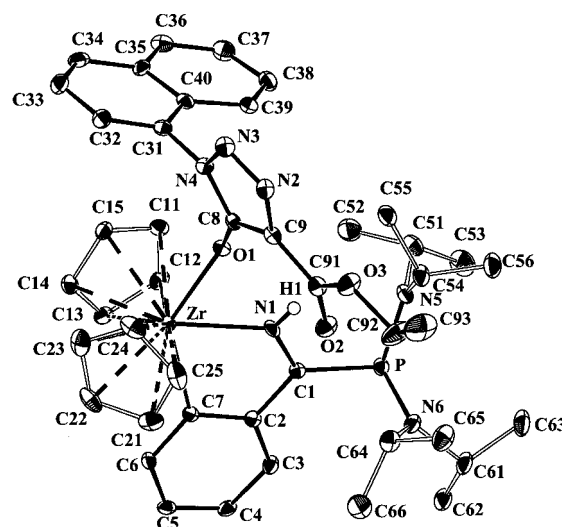
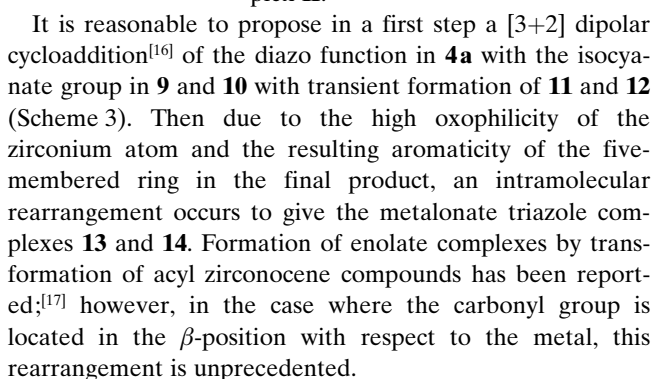


Figure 1. ZORTEP drawing of the molecular structure of **14**. Selected bond lengths [Å] and angles [°]: Zr–O1 2.220(4), Zr–N1 2.258(4), Zr–C7 2.390(4), N1–C1 1.281(5), C1–P 1.861(4); O1–Zr–N1 70.89(12), N1–Zr–C7 67.71(13), Zr–O1–C8 146.1(2), Zr–N1–C1 126.1(3), Zr–C7–C2 116.3(3).

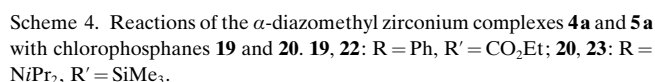


Diazo compounds are classical precursors of carbenes. It is well known that transition metal complexes catalyze the decomposition of diazoacetates in the presence of acetylenes.^[18] Addition of **4a** to the acetylene dicarboxylate **15** resulted in loss of nitrogen and formation of the stable C-metalated cyclopropene compound **17**,^[11] which was isolated in 96 % yield (Scheme 3). The intense $\nu(\text{C}(\text{N}_2))$ absorption at 2029 cm^{-1} for **4a** totally disappeared, while characteristic bands for **17** at 1566 cm^{-1} ($\text{C}=\text{N}$), 1725 cm^{-1} (CO), and 3335 cm^{-1} (NH) were observed. The ^1H and ^{13}C chemical shifts of the aza-zirconocene metallacycle skeleton in **17** appeared in the expected range (^1H NMR: $\delta(\text{NH})=10.64$; ^{13}C NMR: $\delta(\text{P}-\text{C}=\text{N})=202.0$, $J_{\text{C,P}}=38.5\text{ Hz}$). The cyclopropene ring in **17** exhibited, beside the chemical shift of the corresponding CO_2Et ester groups, two singlets at $\delta=118.7\text{ ppm}$ ($\text{sp}^2\text{-C}$) and 146.1 ppm ($\text{Zr}-\text{C}$).

The terminal acetylenic compound **16** reacted with **4a** to give the corresponding cyclopropene complex **18** as the unique product (Scheme 3); no C/H insertion was observed.^[18] In the ¹H NMR spectrum the resonance for the imino=NH group was identified at $\delta = 11.22$, and the signal at $\delta = 7.79$ was unambiguously assigned to the =CH group of the three-membered ring.^[19] The ¹³C{¹H} NMR spectrum showed three singlets at $\delta = 117.3$ (=CH), 144.7, and 145.3 (ZrC and sp²-C) for the cyclopropene ring.^[19] We can propose, for the first time, that the loss of nitrogen from **4a** in the formation of the cyclopropene rings **17** and **18** is initiated by the presence of the zirconium metal fragment.

Organometallocene Group 4 complexes are useful intermediates in organic synthesis since various procedures for the cleavage of carbon–zirconium bonds with electrophiles have been reported.^[20] Addition of chlorodiphenylphosphane (**19**)

to **4a** resulted in transfer of the diazo C(N₂)R' moiety to the phosphorus atom to give, as the unique products, **21** and the α -diazophosphane **22** (³¹P NMR: $\delta = -21$; Scheme 4). However **22** decomposed by intermolecular reactions of the diazo moiety with the lone pair on phosphorus.^[21] Then **5a** was treated with **20** to provide the stable diazo compound **23**^[22] along with the metal complex **21**.



In summary we have prepared by an unprecedented sp²-C–H bond activation the first stable α -C-zirconated diazoalkanes **4a,b** and **5a,b**. The reactions of **4a** with isocyanates and acetylenic derivatives showed the large versatility of these species to give metallacycle compounds (**13/14**, **17/18**) without or with the loss of nitrogen. In future work the thermal and photochemical behavior of the C-metalated zirconocene diazo complexes will be examined.

Synthetic procedures and spectroscopic data for compounds **13**, **14**, and **21** can be found in the Supporting Information.

4a, b: Ethyl diazoacetate (**2**; 0.048 mL, 0.465 mmol for **4a**; 0.063 mL, 0.599 mmol for **4b**) was added at room temperature to a solution of **1a** (0.258 g, 0.465 mmol) or **1b** (0.428 g, 0.599 mmol) in toluene (5 mL). The mixture was stirred at room temperature for 10 min (30 min for **4b**) and then evaporated to dryness. The resulting solid residue was extracted with pentane (20 mL) and filtered. The volatile components were removed from the solution to give the product.

4a: Yellow solid, 0.283 g (91 % yield). IR (KBr): $\bar{\nu}$ = 1571 $\text{v}(\text{C}=\text{N})$, 1661 $\text{v}(\text{C}=\text{O})$, 2029 $\text{v}(\text{C}=\text{N}=\text{N})$, 3375 cm^{-1} $\text{v}(\text{N}-\text{H})$; $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): δ = 49.4 (s); ^1H NMR (C_6D_6): δ = 1.09 (d, $J_{\text{H,H}}$ = 6.5 Hz, 12H; CH_3), 1.16 (t, $J_{\text{H,H}}$ = 7.0 Hz, 3H; OCH_2CH_3), 1.19 (d, $J_{\text{H,H}}$ = 5.9 Hz, 12H; CH_3), 3.46 (m, 4H; NCH), 4.14 (q, $J_{\text{H,H}}$ = 7.0 Hz, 2H; CH_2), 5.75 (s, 10H; CH_{Cp}), 7.00–7.18 (m, 2H; CH_{arom}), 7.47 (d, $J_{\text{H,H}}$ = 7.3 Hz, 1H; CH_{arom}), 8.30 (m, 1H; CH_{arom}), 9.55 (d, $J_{\text{H,P}}$ = 5.3 Hz, 1H; NH); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): δ = 15.5 (s; OCH_2CH_3), 24.4 (d, $J_{\text{C,P}}$ = 6.1 Hz; CH_3), 24.8 (d, $J_{\text{C,P}}$ = 5.5 Hz; CH_3), 48.1 (s; $\text{C}=\text{N}=\text{N}$), 49.2 (d, $J_{\text{C,P}}$ = 11.6 Hz; NCH), 59.8 (s; CH_2), 109.6 (s; CH_{Cp}), 123.3, 129.5, 142.3 (s; CH_{arom}), 129.7 (d, $J_{\text{C,P}}$ = 21.0 Hz; CH_{arom}), 146.3 (d, $J_{\text{C,P}}$ = 27.6 Hz; ZrCC), 174.7 (s; $\text{C}=\text{O}$), 196.9 (d, $J_{\text{C,P}}$ = 6.9 Hz; ZrC), 203.9 (d, $J_{\text{C,P}}$ = 39.3 Hz; PC=N); elemental analysis calcd for $\text{C}_{33}\text{H}_{48}\text{N}_5\text{O}_2\text{P}_3\text{Zr}$ (668.96) (%): C 59.25, H 7.23, N 10.47; found: C 59.23, H 7.29, N 10.51.

4b: Yellow solid, 0.437 g (88 % yield). IR (KBr): $\bar{\nu}$ = 1571 ν (C=N), 1661 ν (C=O), 2029 ν (C=N=N), 3355 cm^{-1} ν (N-H); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): δ = 58.5 (s); ^1H NMR (C_6D_6): δ = 0.83–1.89 (m, 40H; CH_2), 1.21 (t, $J_{\text{H,H}}$ = 7.1 Hz, 3H; OCH_2CH_3), 3.13 (m, 4H; NCH), 4.23 (q, $J_{\text{H,H}}$ = 7.1 Hz, 2H; OCH_2), 5.85 (s, 10H; CH_eq), 7.08–7.20 (m, 2H; CH_arom), 7.52 (d, $J_{\text{H,H}}$ = 8.0 Hz, 1H; CH_arom), 8.50 (m, 1H; CH_arom), 9.74 (d, $J_{\text{H,P}}$ = 5.5 Hz, 1H; NH); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): δ = 15.4 (s; OCH_2CH_3), 26.3, 27.1 (s; CH_2), 35.5 (d, $J_{\text{C,P}}$ = 6.1 Hz; CH_2), 36.1 (d, $J_{\text{C,P}}$ = 4.2 Hz; CH_2), 47.0 (s; C=N=N), 58.8 (d, $J_{\text{C,P}}$ = 9.8 Hz; NCH), 59.9 (s; OCH_2), 109.6 (s; CH_eq), 123.4, 129.3, 142.1 (s; CH_arom), 130.1 (d, $J_{\text{C,P}}$ = 19.3 Hz; CH_arom), 146.7 (d, $J_{\text{C,P}}$ = 28.2 Hz; ZrCC), 174.5 (s; C=O), 196.9 (d, $J_{\text{C,P}}$ = 6.5 Hz; ZrC), 204.0 (d, $J_{\text{C,P}}$ = 43.6 Hz; PC=N); elemental analysis calcd for $\text{C}_{45}\text{H}_{64}\text{N}_5\text{O}_2\text{PZr}$ (829.23) (%): C 65.18, H 7.77, N 8.44; found: C 65.12, H 7.81, N 8.42.

5a,b: (Trimethylsilyl)diazomethane (**3**, 2.0 M in hexanes; 0.302 mL, 0.604 mmol for **5a**; 0.489 mL, 0.979 mmol for **5b**) was added at room temperature to a solution of **1a** (0.335 g, 0.604 mmol) or **1b** (0.700 g, 0.979 mmol) in toluene (8 mL). The mixture was heated under reflux for 20 min and then evaporated to dryness. The resulting solid residue was extracted with pentane (20 mL) and filtered. The volatile components were removed from the solution to give the product.

5a: Yellow solid, 0.287 g (71 % yield). IR (KBr): $\tilde{\nu}$ = 1571 ν (C=N), 2069 ν (C=N=N), 3325 cm^{-1} ν (N-H); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): δ = 57.2 (s); ^1H NMR (C_6D_6): δ = 0.35 (s, 9H; SiMe₃), 1.11 (d, $J_{\text{H,H}}$ = 6.6 Hz, 12H; CH₃), 1.18 (d, $J_{\text{H,H}}$ = 6.6 Hz, 12H; CH₃), 3.25 (m, 4H; NCH), 5.90 (s, 10H; CH_{CP}), 6.98–7.40 (m, 3H; CH_{arom}), 8.18 (d, $J_{\text{H,H}}$ = 7.9 Hz, 1H; CH_{arom}), 9.62 (d, $J_{\text{H,P}}$ = 5.6 Hz, 1H; NH); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): δ = 2.5 (s; SiMe₃), 24.5 (d, $J_{\text{C,P}}$ = 7.1 Hz; CH₃), 24.6 (d, $J_{\text{C,P}}$ = 6.2 Hz, CH₃), 30.5 (s, C=N=N), 49.6 (d, $J_{\text{C,P}}$ = 10.9 Hz, NCH), 110.2 (s; CH_{CP}), 122.7, 129.5, 141.2 (s; CH_{arom}), 129.7 (d, $J_{\text{C,P}}$ = 12.7 Hz; CH_{arom}), 146.9 (d, $J_{\text{C,P}}$ = 23.9 Hz; ZrCC), 198.1 (d, $J_{\text{C,P}}$ = 6.4 Hz; ZrC), 202.2 (d, $J_{\text{C,P}}$ = 37.4 Hz; PC=N); elemental analysis calcd for C₃₃H₅₂N₃PSiZr (669.08) (%): C 59.24, H 7.83, N 10.46; found: C 59.19, H 7.79, N 10.51.

5b: Yellow solid, 0.600 g (74 % yield); IR (KBr): $\tilde{\nu}$ = 1571 ν (C=N), 2049 ν (C=N=N), 3315 cm^{-1} ν (N-H); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): δ = 63.7 (s); ^1H NMR (C_6D_6): δ = 0.35 (s, 9H; SiMe₃), 0.83–2.05 (m, 40H; CH₂), 2.91 (m, 4H; NCH), 5.93 (s, 10H; CH_{CP}), 6.98–7.40 (m, 3H; CH_{arom}), 8.28 (d, $J_{\text{H,H}}$ = 7.6 Hz, 1H; CH_{arom}), 9.70 (d, $J_{\text{H,P}}$ = 5.7 Hz, 1H; NH); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): δ = 2.5 (s; SiMe₃), 26.3, 27.5 (s; CH₃), 35.6 (d, $J_{\text{C,P}}$ = 5.6 Hz; CH₂), 35.9 (br; CH₂), 36.7 (s; C=N=N), 59.7 (br; NCH), 110.2 (s; CH_{CP}), 122.7, 129.4, 141.1 (s; CH_{arom}), 129.8 (d, $J_{\text{C,P}}$ = 15.6 Hz; CH_{arom}), 147.1 (d, $J_{\text{C,P}}$ = 23.8 Hz; ZrCC), 198.2 (d, $J_{\text{C,P}}$ = 6.2 Hz; ZrC), 201.9 (d, $J_{\text{C,P}}$ = 39.2 Hz; PC=N); elemental analysis calcd for C₄₅H₆₈N₃PSiZr (829.34) (%): C 65.17, H 8.26, N 8.44; found: C 65.21, H 8.29, N 8.50.

8a,b: Ethyl diazoacetate (**6**; 0.053 mL, 0.388 mmol for **8a**; 0.069 mL, 0.500 mmol for **8b**) was added at room temperature to a solution of **1a** (0.215 g, 0.388 mmol) or **1b** (0.357 g, 0.500 mmol) in toluene (5 mL). The mixture was stirred at room temperature for 1 h and then evaporated to dryness. The resulting solid residue was extracted with pentane (15 mL) and filtered. The volatile components were removed from the solution to give the product.

8a: Yellow solid, 0.242 g (88 % yield). MS (FAB/MNBA): m/z : 709 [M^+]; IR (KBr): $\tilde{\nu}$ = 1551 ν (C=N), 1591 ν (C=N), 1715 ν (C=O), 3204 ν (N-H), 3355 cm^{-1} ν (N-H); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): δ = 51.6 (s); ^1H NMR (C_6D_6): δ = 0.87 (t, $J_{\text{H,H}}$ = 7.1 Hz, 3H; OCH₂CH₃), 1.11 (d, $J_{\text{H,H}}$ = 6.7 Hz, 12H; CH₃), 1.21 (d, $J_{\text{H,H}}$ = 6.4 Hz, 12H; CH₃), 3.46 (m, 4H; NCH), 4.16 (q, $J_{\text{H,H}}$ = 7.1 Hz, 2H; CH₂), 5.96 (s, 10H; CH_{CP}), 7.04–7.41 (m, 3H; CH_{arom}), 7.46 (s, 1H; =CH), 8.34 (m, 1H; CH_{arom}), 9.82 (d, $J_{\text{H,P}}$ = 5.3 Hz, 1H; C=NH), 11.29 (br, 1H; =NNH); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): δ = 15.2 (s; OCH₂CH₃), 24.5 (d, $J_{\text{C,P}}$ = 6.0 Hz; CH₃), 24.8 (d, $J_{\text{C,P}}$ = 5.0 Hz; CH₃), 49.3 (d, $J_{\text{C,P}}$ = 11.2 Hz; NCH), 59.6 (s; CH₂), 111.2 (s; CH_{CP}), 122.9, 129.5, 141.3 (s; CH_{arom}), 129.8 (d, $J_{\text{C,P}}$ = 17.4 Hz; CH_{arom}), 139.5 (s; =CH), 147.8 (d, $J_{\text{C,P}}$ = 26.1 Hz; ZrCC), 148.9 (s; C=N), 158.5 (s; =C-O), 160.5 (s; C=O), 198.6 (s; ZrC), 201.3 (d, $J_{\text{C,P}}$ = 38.6 Hz; PC=N); elemental analysis calcd for C₃₅H₅₀N₃O₃PZr (711.00) (%): C 59.12, H 7.08, N 9.85; found: C 59.07, H 7.12, N 9.81.

8b: Orange solid, 0.309 g (71 % yield). IR (KBr): $\tilde{\nu}$ = 1536 ν (C=N), 1561 ν (C=N), 1725 ν (C=O), 3296 ν (N-H), 3325 cm^{-1} ν (N-H); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): δ = 64.0 (s); ^1H NMR (C_6D_6): δ = 0.77–1.94 (m, 43H; CH₂, CH₃), 2.97 (m, 4H; NCH), 4.25 (q, $J_{\text{H,H}}$ = 6.8 Hz, 2H; OCH₂), 6.04 (s, 10H; CH_{CP}), 6.99–7.24 (m, 2H; CH_{arom}), 7.41 (d, $J_{\text{H,H}}$ = 6.8 Hz, 1H; CH_{arom}), 7.47 (s, 1H; =CH), 8.47 (d, $J_{\text{H,H}}$ = 7.1 Hz, 1H; CH_{arom}), 9.88 (d, $J_{\text{H,P}}$ = 5.2 Hz, 1H; C=NH), 10.54 (br, 1H; =NNH); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): δ = 15.2 (s; CH₃), 26.3 (s; CH₃), 27.4 (d, $J_{\text{C,P}}$ = 6.3 Hz; CH₂), 35.6 (br; CH₂), 59.5 (br; NCH), 59.7 (s; OCH₂), 111.3 (s; CH_{CP}), 122.7, 129.5, 141.1 (s; CH_{arom}), 130.2 (d, $J_{\text{C,P}}$ = 13.1 Hz; CH_{arom}), 139.4 (s; =CH), 147.4 (d, $J_{\text{C,P}}$ = 24.1 Hz; ZrCC), 147.6 (s; C=N), 157.9 (s; =C-O), 160.6 (s; C=O), 199.0 (d, $J_{\text{C,P}}$ = 5.0 Hz; ZrC), 201.6 (d, $J_{\text{C,P}}$ = 41.3 Hz; PC=N); elemental analysis calcd for C₄₇H₆₆N₃O₃PZr (871.26) (%): C 64.79, H 7.63, N 8.03; found: C 64.73, H 7.68, N 8.07.

17 and **18:** Diethyl acetylenedicarboxylate (**15**; 0.049 mL, 0.311 mmol for **17**) or methyl propiolate (**16**; 0.071 mL, 0.803 mmol for **18**) was added at room temperature to a solution of **4a** (0.208 g, 0.311 mmol for **17**; 0.537 g, 0.803 mmol for **18**) in toluene (5 mL). The mixture was stirred at room

temperature for 1 h and then evaporated to dryness. The resulting solid residue was extracted with pentane (15 mL) and filtered. The volatile components were removed from the solution to give the product.

17: Yellow solid, 0.242 g (96 % yield). IR (KBr): $\tilde{\nu}$ = 1566 ν (C=N), 1725 ν (C=O), 3335 cm^{-1} ν (N-H); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): δ = 40.9 (s); ^1H NMR (C_6D_6): δ = 0.94–1.18 (m, CH₃, 27H; OCH₂CH₃), 1.35 (d, $J_{\text{H,H}}$ = 6.5 Hz, 6H; CH₃), 3.69 (m, 4H; NCH), 4.22 (q, $J_{\text{H,H}}$ = 7.2 Hz, 2H; CH₂), 4.27 (q, $J_{\text{H,H}}$ = 7.2 Hz, 4H; CH₂), 5.86 (s, 10H; CH_{CP}), 7.02–7.56 (m, 3H; CH_{arom}), 8.49 (m, 1H; CH_{arom}), 10.64 (d, $J_{\text{H,P}}$ = 4.8 Hz, 1H; NH); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): δ = 14.7, 14.9 (s; OCH₂CH₃), 24.5 (d, $J_{\text{C,P}}$ = 6.3 Hz; CH₃), 24.8 (d, $J_{\text{C,P}}$ = 6.6 Hz; CH₃), 49.3 (d, $J_{\text{C,P}}$ = 12.3 Hz; NCH), 60.7, 61.4 (s; CH₂), 111.4 (s; CH_{CP}), 118.7 (s; =C), 123.5, 129.6, 141.6 (s; CH_{arom}), 130.4 (d, $J_{\text{C,P}}$ = 24.4 Hz; CH_{arom}), 146.1 (s; ZrCCO₂Et), 147.3 (d, $J_{\text{C,P}}$ = 30.1 Hz; ZrCC), 164.7, 164.8 (s; C=O), 197.8 (d, $J_{\text{C,P}}$ = 6.7 Hz; ZrC), 202.0 (d, $J_{\text{C,P}}$ = 40.2 Hz; PC=N); elemental analysis calcd for C₄₁H₅₈O₆N₃PZr (811.10) (%): C 60.71, H 7.20, N 5.18; found: C 60.76, H 7.18, N 5.21.

18: Yellow solid, 0.456 g (78 % yield). IR (KBr): $\tilde{\nu}$ = 1561 ν (C=N), 1710 ν (C=O), 3305 cm^{-1} ν (N-H); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): δ = 38.3 (s); ^1H NMR (C_6D_6): δ = 0.97 (d, $J_{\text{H,H}}$ = 6.8 Hz, 6H; CH₃), 1.02 (d, $J_{\text{H,H}}$ = 6.5 Hz, 6H; CH₃), 1.10 (m, CH₃, 9H; OCH₂CH₃), 1.37 (d, $J_{\text{H,H}}$ = 6.5 Hz, 6H; CH₃), 3.57 (s, 3H; OCH₃), 3.82 (m, 4H; NCH), 4.15 (q, $J_{\text{H,H}}$ = 7.1 Hz, 2H; CH₂), 5.88 (s, 10H; CH_{CP}), 6.99–7.53 (m, 3H; CH_{arom}), 7.79 (s; =CH), 8.53 (m, 1H; CH_{arom}), 11.22 (d, $J_{\text{H,P}}$ = 5.0 Hz, 1H; NH); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): δ = 14.9 (s; OCH₂CH₃), 24.7 (d, $J_{\text{C,P}}$ = 6.4 Hz; CH₃), 24.9 (d, $J_{\text{C,P}}$ = 6.6 Hz; CH₃), 49.2 (d, $J_{\text{C,P}}$ = 12.7 Hz; NCH), 51.3 (s; OCH₃), 60.3 (s; CH₂), 111.6 (s; CH_{CP}), 117.3 (s; =CH), 123.2, 129.9, 140.9 (s; CH_{arom}), 130.4 (d, $J_{\text{C,P}}$ = 26.7 Hz; CH_{arom}), 144.7, 145.3 (s; ZrCCO₂Et, =C), 147.5 (d, $J_{\text{C,P}}$ = 32.7 Hz; ZrCC), 163.5, 163.9 (s; C=O), 198.9 (d, $J_{\text{C,P}}$ = 7.9 Hz; ZrC), 201.5 (d, $J_{\text{C,P}}$ = 41.2 Hz; PC=N); elemental analysis calcd for C₃₇H₅₂O₆N₃PZr (725.01) (%): C 61.29, H 7.22, N 5.79; found: C 61.25, H 7.16, N 5.83.

21, 23: Chlorophosphane **20** (0.056 mL, 0.311 mmol) was added at room temperature to a solution of **5a** (0.208 g, 0.311 mmol) in toluene (5 mL). The mixture was stirred at room temperature for 1 h and then evaporated to dryness. The resulting solid residue was extracted with pentane (20 mL) and filtered. The volatile components were removed from the solution to give **21** and **23** as the unique products of the reaction according to ^{31}P NMR spectroscopy. Compound **23** was identified in the reaction mixture by comparison with an authentic sample.^[22] Independently, addition of HCl to **4a** allowed the preparation and isolation of **21** as a yellow solid: IR (KBr): $\tilde{\nu}$ = 1576 ν (C=N), 3305 cm^{-1} ν (N-H); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): δ = 58.2 (s); ^1H NMR (C_6D_6): δ = 1.11 (d, $J_{\text{H,H}}$ = 6.6 Hz, 12H; CH₃), 1.21 (d, $J_{\text{H,H}}$ = 6.6 Hz, 12H; CH₃), 3.27 (d sept, $J_{\text{H,H}}$ = 6.6 Hz, $J_{\text{H,P}}$ = 12.1 Hz, 4H; NCH), 5.90 (s, 10H; CH_{CP}), 7.20 (m, 2H; CH_{arom}), 7.88 (m, 4H; CH_{arom}), 8.25 (m, 1H; CH_{arom}), 10.12 (d, $J_{\text{H,P}}$ = 5.0 Hz, 1H; NH); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): δ = 24.4 (d, $J_{\text{C,P}}$ = 6.8 Hz; CH₃), 25.1 (d, $J_{\text{C,P}}$ = 5.0 Hz; CH₃), 49.6 (d, $J_{\text{C,P}}$ = 11.2 Hz; NCH), 111.3 (s; CH_{CP}), 122.8, 129.8, 141.2 (s; CH_{arom}), 130.1 (d, $J_{\text{C,P}}$ = 14.3 Hz; CH_{arom}), 146.1 (d, $J_{\text{C,P}}$ = 24.1 Hz; ZrCC), 198.6 (d, $J_{\text{C,P}}$ = 6.3 Hz; ZrC), 202.5 (d, $J_{\text{C,P}}$ = 40.6 Hz; PC=N).

X-ray crystal structure analysis of **14** (C₄₄H₄₉N₆O₃PZr·1.5C₆H₆): M_r = 979.72, monoclinic, space group $P2_1/n$, T = 160 K, a = 13.811(6), b = 18.356(5), c = 20.031(8) Å, β = 98.26(5)°, V = 5025(3) Å³, Z = 4, μ = 0.296 mm⁻¹. Of 27371 reflections collected, 6500 were independent; R_{int} = 0.0970; final R indices: $R1$ = 0.0402, $wR2$ = 0.0889 ($I > 2\sigma(I)$), $R1$ = 0.0729, $wR2$ = 0.0992 (all data). Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-149738. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Hybrid Coordination Polymers—Metal Oxide Compounds with Chiral Structures**

Li-Min Zheng, Tabatha Whitfield, Xiqu Wang, and Allan J. Jacobson*

Many investigations of the design and synthesis of metal–organic compounds with porous structures have been motivated by potential applications in molecule recognition, separation, and catalysis.^[1–11] By using rigid, multifunctional ligands, two- or three-dimensional microporous coordination polymers have been obtained with desired pore sizes and shapes.^[3–11] Other metal–organic frameworks have been reported that, though they may have other valuable properties, are not microporous because of lattice interpenetration,^[12] or are unstable on removal or exchange of their guest molecules or ions. Very few examples of either framework or microporous chiral metal–organic structures have been reported.^[13–17]

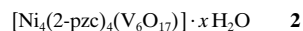
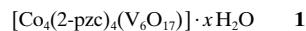
One strategy to enhance the stability with respect to removal of the guest species is to attach the metal–organic components to a thermally more stable inorganic backbone. Several examples of compounds of this type containing both ligated metal cation and metal oxide components are known.^[18–20] These extended lattices are formed in different ways, for example, by connecting a metal oxide cluster with a coordination complex, a metal oxide cluster anion with a coordination polymer, a metal oxide lattice with an isolated coordination complex, and metal oxide lattice with a coordination polymer. All of the inorganic components in the known examples are molybdenum or vanadium oxides.^[18–20]

[*] Prof. A. J. Jacobson, Dr. L.-M. Zheng,^[+] T. Whitfield, Dr. X. Wang
Department of Chemistry
University of Houston
Houston, TX 77204-5641 (USA)
Fax: (+1) 713-743-2787
E-mail: ajacob@uh.edu

[+] Permanent address:
Coordination Chemistry Institute, Department of Chemistry
Nanjing University, Nanjing 210093 (P. R. China)

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We are investigating the combination of coordination polymers with metavanadate chains as a route to stable porous structures. We also have used an unsymmetrical organic ligand, 2-pyrazinecarboxylate (2-pzc)^[21] to introduce chirality into the structures formed. The combination of Co(2-pzc)⁺ and Ni(2-pzc)⁺ with vanadate chains has led to three novel inorganic–organic hybrid compounds **1–3** all of which are noncentric and two of which (**1**, **2**) are chiral.



Compounds **1–3** were prepared by hydrothermal synthesis at 160, 180, and 130 °C, respectively. The structures were determined by single-crystal X-ray diffraction^[21] and the compositions by elemental analysis.^[22] Infrared spectra confirmed the presence of vanadate and coordinated pyrazinecarboxylate. Compounds **1** and **2** are isostructural enantiomers; both crystallize in the chiral space group *I*4. The local coordination geometries of the cobalt and vanadium atoms in **1** are shown in Figure 1. Aspects of the extended chiral framework of **1** are shown in Figures 2 and 3.

Two crystallographically inequivalent Co atoms are present in the structure, each with a distorted octahedral coordination environment. Two nitrogen atoms from two 2-pzc ligands occupy the axial positions. The equatorial positions are filled by four oxygen atoms, two from the carboxylate groups of two 2-pzc ligands and the remaining two from the vanadate chains. The 2-pyrazinecarboxylate serves as a multidentate bridging ligand, as observed in the copper/2-pzc polymeric compounds,^[23, 24] and links the Co1 and Co2 atoms into infinite

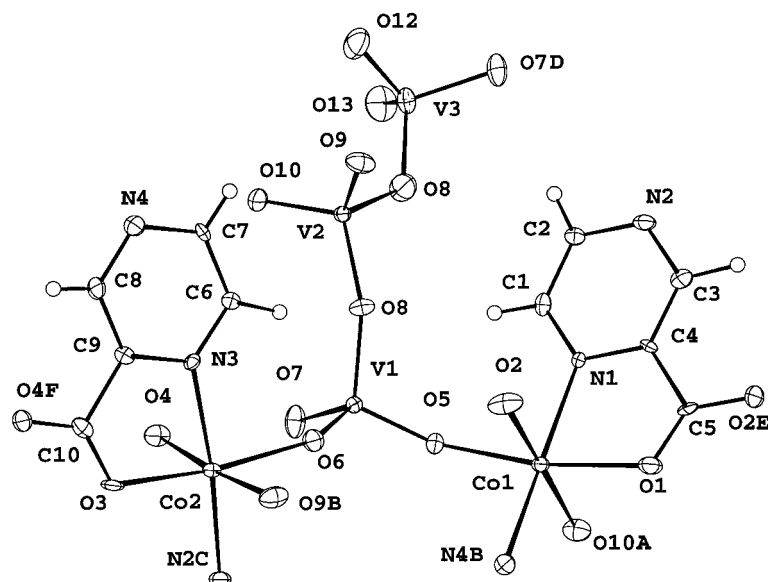


Figure 1. Coordination geometries of cobalt and vanadium in compound **1** with the atom-labeling scheme. Thermal ellipsoids are at the 50% probability level. Selected bond lengths [Å]: Co1–O2 2.038(4), Co1–O10A 2.070(4), Co1–O1 2.093(4), Co1–O5 2.112(4), Co1–N4B 2.116(5), Co1–N1 2.141(4), Co2–O9B 2.044(4), Co2–O4 2.052(4), Co2–O3 2.100(4), Co2–O6 2.104(4), Co2–N3 2.114(4), Co2–N2C 2.122(5), V–O 1.592(5)–1.821(5).

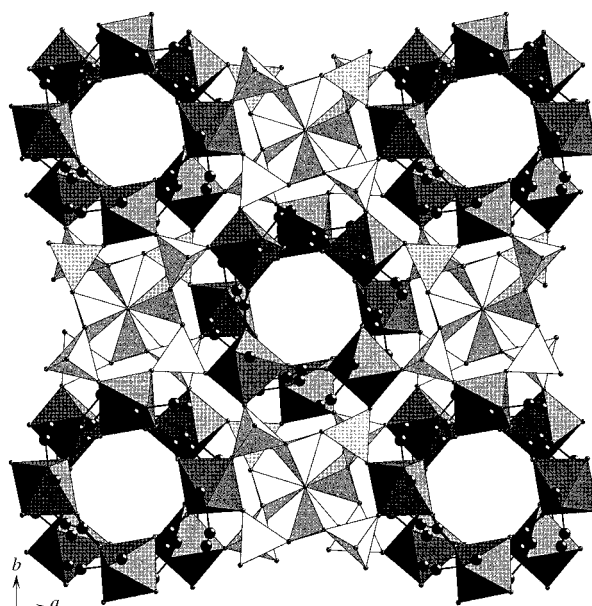


Figure 2. Polyhedral representation of the structure of **1** viewed down [001]; CoO₄N₂ octahedra: dark shaded; VO₄ tetrahedra: light shaded.

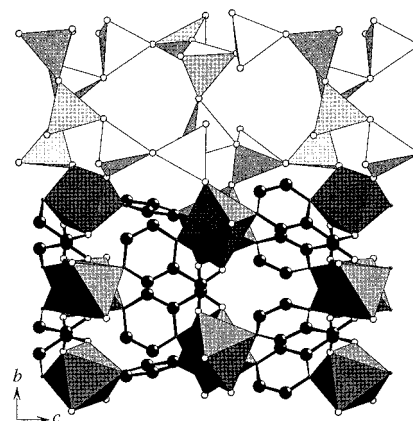


Figure 3. Polyhedral representation of the structure of **1** viewed along [100]; CoO₄N₂ octahedra: dark shaded; VO₄ tetrahedra: light shaded.

helical chains. Each of the equivalent cobalt atoms are connected to each other through the 2-pzc carboxylate group from a neighboring chain. As a result, four Co/2-pzc helical chains form a chiral helical tube of composition $[\{\text{Co}_4(2\text{-pzc})_4\}_n]$ (Figures 2 and 3). Eight oxygen atoms define the window into the tube. The $[\{\text{Co}_4(2\text{-pzc})_4\}_n]$ tubes are stabilized by the vanadium oxide chains through corner-sharing of CoO₄N₂ octahedra with vanadium–oxygen tetrahedra.

Within the vanadium oxide chain, there are three crystallographically distinct tetrahedrally coordinated vanadium(v) atoms (Figure 1). These vanadium tetrahedra share corners with each other through oxygen atoms, forming infinite $[\text{V}_6\text{O}_{17}]_n^{4-}$ double chains along the *c* axis. The chain is bent to give perpendicularly arranged 8-rings (Figure 3). One oxygen atom is only coordinated to vanadium with a bond length significantly shorter (1.592(5) Å) than

those of the other V–O bonds (1.626(4)–1.821(5) Å). This vanadium oxide arrangement is different from the layers of corner-sharing VO₄ units that occur in [Zn(bpy)₂][V₆O₁₇] (bpy = 2,2'-bipyridine).^[19] In **1**, each [[V₆O₁₇]_n]^{4–} chain is connected to four [[Co₄(2-pzc)₄]_n] chains to form a three-dimensional chiral framework structure with 8-ring channels along the *c* axis (Figure 2). The frameworks of **1** and **2** are neutral. Disordered water molecules are found inside the metal–organic tubes.

Compound **3** crystallizes in the space group *Cc* and is closely related to **1** and **2**. The [[Co(2-pzc)]_n] helical tubes in **1** and **3** are comparable but **3** contains single metavanadate chains [[VO₃]_n] instead of [[V₆O₁₇]_n] double chains (Figure 4).

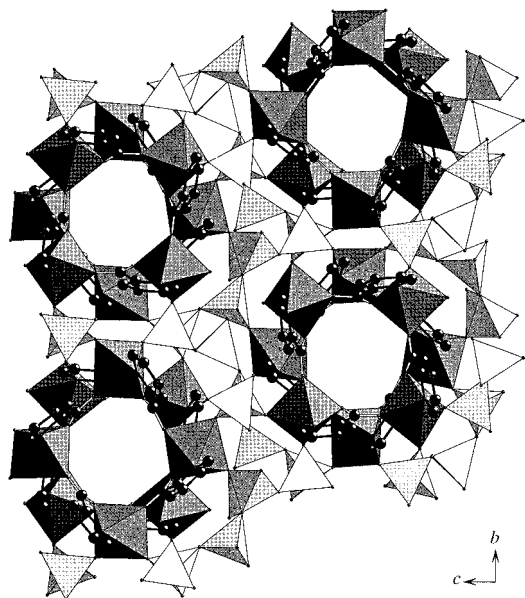


Figure 4. Polyhedral representation of the structure of **3** viewed down [100]; CoO₄N₂ octahedra: dark shaded; VO₄ tetrahedra: light shaded.

Six of the eight cobalt octahedra in **3** are corner-shared by two VO₄ tetrahedra, and two octahedra are connected to only one VO₄ tetrahedron. The octahedral environments of the two distinct Co octahedra are completed by coordinated water molecules. The framework of **3** is anionic and is charge balanced by two NH₄⁺ ions. The NH₄⁺ ions, together with disordered water molecules, reside in the metal–organic tubes.

Coordination of the unsymmetrical bridging ligand 2-pyrazinecarboxylate results in the formation of chiral [[M₄(2-pzc)₄]_n] or [[Co₈(2-pzc)₈(H₂O)₂]_n] helical tubes in **1–3**, each containing four M(2-pzc)⁺ helical chains. Unlike **1** and **2**, compound **3** is not chiral but contains equal numbers of left and right-handed helices. We note that other examples of compounds with tubelike structures have been reported.^[25, 26] The formation of either **1** or **3**, containing different vanadium oxide chains but similar metal–organic components, is mainly dependent on the pH of the reaction mixture. Lower pH (≈4) promotes the formation of [V₆O₁₇] double chains in **1**, whereas higher pH (≈6) favors the metavanadate chains in **3**. Reaction of **1** or **3** with NH₄VO₃ at 160 °C under hydrothermal

conditions gives a higher density phase [Co₂(2-pzc)(H₂O)-(VO₃)₃].^[27]

The presence of the vanadate backbone in **1–3** increases significantly the thermal stability relative to metal pyrazinecarboxylate coordination polymers.^[11, 24] Thermal analysis showed that **1** loses weight from 50 to 160 °C, corresponding to the removal of lattice water. A plateau region is observed from 160 to near 400 °C before the full decomposition occurs between 400 and 440 °C. The thermal decomposition behavior of **2** is very similar to that of **1** except that decomposition begins at 435 °C. For **3**, loss of lattice water, coordinated water, and the ammonium ion occurs below 300 °C, and decomposition below 500 °C. All of the thermogravimetric data agree quantitatively with the analytical compositions.

Compound **1** after dehydration at 200 °C reabsorbs water molecules on cooling in the presence of water vapor. Thermogravimetric analysis shows that the dehydration–hydration process is reversible though some hysteresis is observed. Powder X-ray diffraction confirmed the integrity of the structure after the dehydration and rehydration step.

The temperature-dependent magnetic susceptibilities of **1–3** were measured in a magnetic field of 0.5 T. For **1** and **3**, the effective magnetic moments (μ_{eff}) per Co at 300 K, 4.79 and 4.71 μ_{B} , are typical for Co^{II} compounds. Negative Weiss constants (–10.3 K for **1**, –13.4 K for **3**) indicate antiferromagnetic interactions. For **2**, the μ_{eff} value per molecule at 300 K is 5.88 μ_{B} , slightly higher than the value of 5.66 μ_{B} expected for four isolated Ni^{II} *S* = 1 ions. Assuming that the Ni1–Ni1 and Ni2–Ni2 coupling constants are the same, the data were analyzed by an exchange expression derived for a square arrangement of four *S* = 1 centers. A best fit was obtained with *g* = 2.08, 2*J* = –1.68 cm^{–1}. The small coupling constant indicates very weak antiferromagnetic interactions through the carboxylate bridges.

In conclusion, by using an unsymmetrical organic ligand, 2-pyrazinecarboxylate, three novel inorganic–organic hybrid compounds **1–3** have been prepared, whose framework structures contain tubes formed from four M(2-pzc) helical chains. Compounds **1** and **2** are chiral, whereas **3** contains equal numbers of left- and right-handed tubes. The pH of the syntheses determines the nature of the vanadate chains and consequently the final structure that is adopted. The thermal stabilities of **1** and **2** are among the highest of the metal–organic complexes reported. Further work is in progress to combine asymmetric ligands with other inorganic oxide networks.

Experimental Section

Syntheses were conducted hydrothermally in a Teflon-lined autoclave (23 mL).

1: Co(NO₃)₂ · 6H₂O (0.2918 g, 1 mmol), 2-pzcH (0.1239 g, 1 mmol), NH₄VO₃ (0.0713 g, 0.6 mmol), and H₂O (8 mL), 160 °C, 2 d, single phase, red needlelike crystals, yield about 97 % based on V.

2: Ni(NO₃)₂ · 6H₂O (0.1465 g, 0.5 mmol), 2-pzcH (0.0627 g, 0.5 mmol), NH₄VO₃ (0.0361 g, 0.3 mmol), and H₂O (8 mL), 180 °C, 2 d, single phase, green needlelike crystals, yield 87 % based on V.

3: Co(NO₃)₂ · 6H₂O (0.1437 g, 0.5 mmol), pyrazinamide (0.1134 g, 1 mmol), NH₄VO₃ (0.0589 g, 0.5 mmol), and H₂O (8 mL), 130 °C, 4 d, single phase,

orange flat needles, yield 52 % based on **5**. The hydrolysis of pyrazinamide results in a higher final pH (≈ 6) in the synthesis of **3** than in **1** and **2** (≈ 4).

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- [21] Single-crystal data were collected at 223 K for **1** (293 K for **2,3**) on a Siemens Smart-CCD diffractometer. Crystal data for **1**: tetragonal, space group *I4*, *a* = *b* = 17.6831(7) Å, *c* = 12.4616(7) Å, *V* = 3896.6(3) Å³, *Z* = 4, ρ_{calcd} = 2.250 g cm⁻³, $\mu(\text{MoK}\alpha)$ = 31.28 cm⁻¹. For **2**: tetragonal, space group *I4*, *a* = *b* = 17.671(1) Å, *c* = 12.268(1) Å, *V* = 3831.0(4) Å³, *Z* = 4, ρ_{calcd} = 2.287 g cm⁻³, $\mu(\text{MoK}\alpha)$ = 34.16 cm⁻¹. For **3**: monoclinic, space group *Cc*, *a* = 12.4122(11) Å, *b* = 24.958(2) Å, *c* = 24.949(2) Å, β = 90.102(2)°, *V* = 7728.6(12) Å³, *Z* = 4, ρ_{calcd} = 2.195 g cm⁻³, $\mu(\text{MoK}\alpha)$ = 29.26 cm⁻¹. λ = 0.71073 Å, graphite monochromator, crystal dimen-

sions: **1**, 0.3 × 0.03 × 0.03 mm; **2**, 0.3 × 0.02 × 0.02 mm; **3**, 0.35 × 0.10 × 0.02 mm; unique reflections: **1**, 4455 (*R*_{int} = 0.0485); **2**, 3930 (*R*_{int} = 0.1032); **3**, 13 544 (*R*_{int} = 0.0548). Empirical absorption corrections and extinction corrections are applied. The structures were solved by direct methods and refined on *F*² by full-matrix least-squares by using the SHELXTL program. All non-hydrogen atoms except the oxygen atoms (Ow) of lattice water were refined anisotropically. The disordered Ow and H atoms were refined isotropically. The H atoms attached to Ow of the lattice and coordinated water molecules or the ammonium nitrogen atom were not located. Absolute structure parameter: 0.03(2) for **1**, 0.07(3) for **2**, and 0.56(3) for **3**. ($\Delta\rho$)_{max,min}: **1**, 1.157, −0.523 e Å⁻³; **2**, 1.075, −0.675 e Å⁻³; **3**, 1.461, −0.663 e Å⁻³. Final *R* indices (*I* > 2σ(*I*)) (*R*₁, *wR*₂): **1**, 0.0424, 0.0833; **2**, 0.0678, 0.1111; **3**, 0.0517, 0.1171. GOF: **1**, 1.082; **2**, 1.055; **3**, 0.993. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-147014 (**1**), CCDC-147015 (**2**), and CCDC-147016 (**3**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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[HC((CMe)(NAr))₂Al][NSiMe₃)₂N₂] (Ar = 2,6-*i*Pr₂C₆H₃): The First Five-Membered AlN₄ Ring System**

Chunming Cui, Herbert W. Roesky,*
Hans-Georg Schmidt, and Mathias Noltemeyer

Dedicated to Professor Pierre Braunstein

Main group heterocycles have attracted much attention from the pharmaceutical, agrochemical, and material science communities.^[1] Among the varieties of ring systems, tetrazoles are of current interest. The discovery of the antiallergic, antibiotic activities and the important roles in biological systems of tetrazoles has stimulated extensive research on their chemistry in recent years.^[2] In contrast, very little is

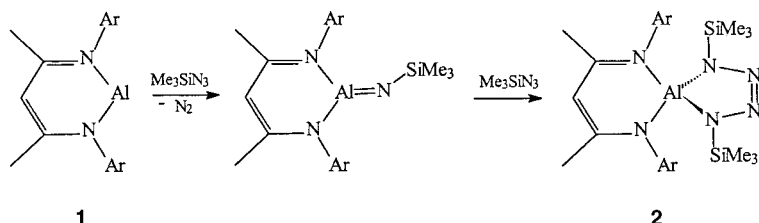
[*] Prof. H. W. Roesky, Dipl.-Chem. C. Cui, Dr. H.-G. Schmidt, M. Noltemeyer
Institut für Anorganische Chemie der Universität Göttingen
Tammannstrasse 4, 37077 Göttingen (Germany)
Fax: (+49) 551-393373
E-mail: hroesky@gwdg.de

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known about the other main group element analogues of tetrazoles. To the best of our knowledge only a few heavier Group 14 elements containing MN_4 ($M = Si, Ge, Sn$) cyclic compounds have been reported.^[3] Similarly, for Group 13 elements, with the exception of boron,^[4] metalloid-containing MN_4 (Al, Ga, In, Tl) cyclic tetrazoles are hitherto unknown. Structurally authenticated heterocycles of this type are limited to only a handful of carbon-containing CN_4 rings.^[2]

In the course of our study on the chemistry of bulky β -diketoiminate aluminum compounds, we prepared the first stable monomeric Al^I compound $[HC\{(CMe)(NAr)\}_2]Al$ (**1**) ($Ar = 2,6\text{-}iPr_2C_6H_3$).^[5] Being interested in $Al-N$ binary systems, we began to explore the reactivity of this compound towards a few azides. Herein we report on the reaction of **1** with Me_3SiN_3 and the first example of a structurally characterized Group 13 element containing tetrazole MN_4 ring system $[HC\{(CMe)(NAr)\}_2]Al[(NSiMe_3)_2N_2]$ (**2**).

The aluminum-containing tetrazole heterocycle **2** was synthesized by the reaction of the monomeric Al^I compound **1** with two equivalents of Me_3SiN_3 from low to room temperature (Scheme 1). The reaction of $[(Cp^*Al)_4]$ ($Cp^* = C_5Me_5$) with various organic azides has been reported to yield four-membered Al_2N_2 ring systems at elevated temperatures.^[6] The higher reactivity of **1** compared to $[(Cp^*Al)_4]$ toward the azide may be attributed to the monomeric nature of **1** even at low temperature. The formation of the cyclic aluminum-containing tetrazole **2** is particularly interesting since it has been reported that the heavier Group 14 elements containing cyclic tetrazoles are prepared by a $[2+3]$ cyclo-



Scheme 1. Synthesis of **2**. $Ar = 2,6\text{-}iPr_2C_6H_3$.

addition of the $R_2M=NR'$ ($M = Si, Sn$; $R, R' =$ organic group) unit with organic azides.^[3] Similarly we therefore assume that the reaction of **1** with Me_3SiN_3 proceeds via the $[HC\{(CMe)(NAr)\}_2]Al=NSiMe_3$ intermediate, although stable compounds containing an $Al=N$ unit have not yet been reported (Scheme 1). It is noted that even when one equivalent of Me_3SiN_3 was employed, the only isolated product is **2** and the unreacted **1** could be detected, indicating that the $[HC\{(CMe)(NAr)\}_2]Al=NSiMe_3$ intermediate is highly reactive. No dimerization of $[HC\{(CMe)(NAr)\}_2]Al=NSiMe_3$ was observed, presumably due to the bulky chelating β -diketoiminate ligand preventing association.

Compound **2** was characterized by ^1H , ^{13}C , and ^{29}Si NMR spectroscopy, EI mass spectrometry, and elemental analysis. The EI mass spectrum shows a molecular ion which is consistent with the formulation of **2**. The NMR spectra all give two distinct singlets for the $SiMe_3$ groups, indicating the two groups in different environments. This is also reflected in the ^1H NMR pattern of the β -diketoiminate ligand, in which two

sets of signals for $\gamma\text{-CH}$ and four doublets for $CHMe_2$ groups are observed. In order to confirm the bonding in the AlN_4 ring system, an X-ray single-crystal analysis was undertaken using yellow crystals of **2** obtained from toluene at $-30^\circ C$ (Figure 1). Compound **2** crystallizes in the monoclinic system, space group $P2_1/n$.^[7] The aluminum atom is coordinated by

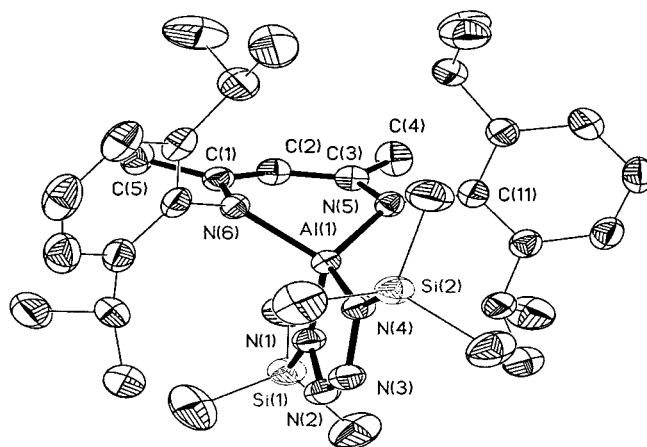


Figure 1. Molecular structure of **2** in the crystal. Hydrogen atoms are omitted for clarity. Selected bond lengths [pm] and angles $^\circ$: $Al(1)\text{--}N(1)$ 185.1(2), $Al(1)\text{--}N(4)$ 181.52(15), $Al(1)\text{--}N(5)$ 189.6(2), $Al(1)\text{--}N(6)$ 189.97(14), $N(1)\text{--}N(2)$ 141.4(2), $N(2)\text{--}N(3)$ 126.3(2), $N(3)\text{--}N(4)$ 141.4(2), $Si(1)\text{--}N(1)$ 174.9(2); $N(1)\text{--}Al(1)\text{--}N(4)$ 87.15(7), $N(5)\text{--}Al(1)\text{--}N(6)$ 96.50(6), $N(1)\text{--}Al(1)\text{--}N(5)$ 117.01(7), $N(4)\text{--}Al(1)\text{--}N(6)$ 117.70(7), $N(2)\text{--}N(1)\text{--}Al(1)$ 109.10(11), $N(2)\text{--}N(1)\text{--}Si(1)$ 108.20(11), $Si(1)\text{--}N(1)\text{--}Al(1)$ 142.66(9), $N(3)\text{--}N(2)\text{--}N(1)$ 116.68(15), $N(2)\text{--}N(3)\text{--}N(4)$ 116.44(14).

four nitrogen atoms and connects two fused rings with a distorted tetrahedral arrangement. The $Al(1)\text{--}N(5)$ (189.6(2) pm) and $Al(1)\text{--}N(6)$ (189.97(14) pm) distances fall within the range of those in β -diketoiminate aluminum derivatives.^[8] The $Al(1)\text{--}N(4)$ distance (181.52(15) pm) is shorter (ca 3.6 pm) than that of $Al(1)\text{--}N(1)$ (185.1(2) pm), which is in line with the NMR spectral data for the $SiMe_3$ groups, indicating the solid-state structure of **2** is maintained in solution. Similar short $Al\text{--}N$ bond lengths have been found only in three-coordinate aluminum amide complexes^[9] and a few four-coordinate aluminum amide dihalides.^[10] However they are unusual for a very crowded molecule such as **2**. The shortening of the $Al\text{--}N$ bond length and the $N(1)\text{--}Al(1)\text{--}N(4)$ angle (87.15(7) $^\circ$) are an indication of the increased ionic interactions between the Al center and the N_4 unit. The $N(2)\text{--}N(3)$ bond length (126.3(2) pm) is consistent with a $N=N$ double bond. The $N(1)\text{--}N(2)$ and $N(3)\text{--}N(4)$ (141.4(2) pm) bond lengths are slightly shorter than a $N\text{--}N$ single bond (ca 150 pm).

Interestingly, the AlN_4 five-membered ring is essentially planar and the geometries at $N(1)$ and $N(4)$ are both trigonal planar (sums of angles 360.0 $^\circ$ and 359.7 $^\circ$, respectively). This planar arrangement of the AlN_4 ring may be comparable to Group 13 element diazabutadiene complexes which feature planar C_2N_2M ($M = B, Ga$) five-membered rings. In the latter case, a 6π -electron system for the C_2N_2M ring is suggested to explain the bonding situation of the diazabutadiene derivatives.^[11]

In summary, we have prepared and structurally characterized a five-membered AlN_4 ring compound. Evidently, compound **2** represents the first structurally characterized Group 13 element MN_4 system, although various binary M–N (M = Group 13 element) rings are known which show unique structural features.^[12] The synthesis of **2** under mild conditions indicates that **1** is very reactive toward organic azides. Stimulated by this result, we are investigating the reaction of **1** with more bulky organic azides in an effort to prepare stable compounds containing $\text{Al}=\text{N}$ double-bond systems.

Experimental Section

All manipulations were carried out under an argon atmosphere by using Schlenk line techniques.

2: Neat Me_3SiN_3 (0.23 g, 2 mmol) was added to a solution of **1** (0.44 g, 1 mmol) in toluene (20 mL) at -78°C leading to an immediate color change from red to orange-yellow. The mixture was allowed to warm to room temperature, and stirred for 1 h. The yellow solution was concentrated (ca. 5 mL) and stored at -30°C overnight to give yellow crystals of **2** (0.37 g, 58%). M.p. 130°C (decomp); ^1H NMR (C_6D_6): δ = 7.08 (m, 6H; Ph), 5.04 (s, 1H; γ -CH), 3.48 (sept, 2H, J = 6.8 Hz; CHMe_2), 3.02 (sept, 2H, J = 6.8 Hz; CHMe_2), 1.52 (s, 6H; Me), 1.30 (d, 6H; CHMe_2), 1.20 (d, 6H; CHMe_2), 1.14 (d, 6H; CHMe_2), 1.02 (d, 6H; CHMe_2), 0.48 (s, 9H; SiMe_3), -0.14 (s, 9H; SiMe_3); ^{13}C NMR (C_6D_6): δ = 172.49 (CN), 145.78, 142.68, 140.11, 125.67, 124.28 (Ph), 101.08 (γ -C), 28.91, 28.83, 25.22, 25.20 (CHMe_2), 24.07 (Me), 1.64, 0.62 (SiMe_3); ^{29}Si NMR (C_6D_6): δ = 4.89, 3.08 (SiMe_3); EI-MS: m/z (%): 646 (20, $[\text{M}^+]$), 631 (100, $[\text{M}^+ - \text{Me}]$).

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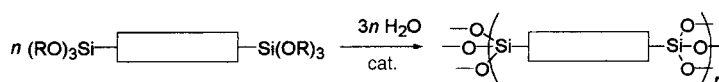
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Synthesis of Stable Organo(bis-silanetriols): X-Ray Powder Structure of 1,4-Bis(trihydroxysilyl)benzene**

Geneviève Cerveau,* Robert J. P. Corriu,* Bertrand Dabiens, and Jean Le Bideau

In the course of our work concerning nanostructured hybrid organic–inorganic materials,^[1] we have been interested in finding an alternative route to the commonly used hydrolytic polycondensation (the sol–gel process).^[2] This general method permits access to materials through a procedure that is very convenient from a practical point of view (Scheme 1). Moreover it allows easy processing of the compounds obtained during the sol step. However, the mechanism is highly complex since the polysubstitutions and polycondensations are concurrent with other reactions, and many points of this process are not well understood.^[3] For these reasons we have examined the possibility of preparing and isolating stable silanetriols that contain at least two $\text{Si}(\text{OH})_3$ groups in the



Scheme 1. The hydrolytic polycondensation process. The rectangle represents the bridging organo group.

[*] Prof. Dr. G. Cerveau, Prof. Dr. R. J. P. Corriu, B. Dabiens, J. Le Bideau
Laboratoire de Chimie Moléculaire et Organisation du Solide
UMR 5637, Université Montpellier II
Place E. Bataillon, 34095 Montpellier Cedex 5 (France)
Fax: (+33) 4-67-14-38-52
E-mail: gcerveau@crit.univ-montp2.fr
corriu@crit.univ-montp2.fr

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

same molecule in order to find a new route for accessing nanostructured hybrid materials.

The chemistry of silanetriols is relatively new, compared to monosilanols and silanediols.^[4] The use of this class of compounds as building blocks in material science and particularly for the synthesis of crystalline metasiloxanes is a growing field of interest.^[4] However the easy self-condensation of silanetriols^[5] has considerably hindered their development. All the stable silanetriols reported in the literature possess one Si(OH)₃ group^[6–18] and are stabilized by a balance between steric and electronic factors.

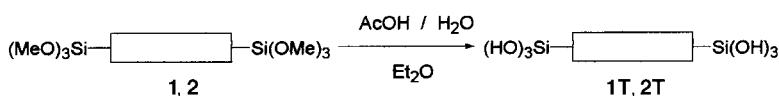
The relative stability of different types of silanetriols is due to their molecular association in the solid state through hydrogen bonding to form a variety of structures:

- 1) a double-sheet structure where the alkyl and the OH groups form alternating hydrophobic and hydrophilic double sheets respectively,^[8, 9, 10]
- 2) tetrameric structures,^[5]
- 3) hexameric and octameric cage structures for sterically hindered silanetriols,^[11, 12]
- 4) tube structures with hydrophilic inside walls and hydrophobic outside walls.^[13]

We have investigated the formation of silanetriols containing two Si(OH)₃ groups in the same molecule. We report here the synthesis and structure of two bis-silanetriols that contain either a phenyl group (**1T**) or a diphenyl group (**2T**) as a bridging moiety.

Two main routes to organosilanetriols are reported in the literature. The first one consists of a controlled hydrolysis of trimethoxysilane precursors in aqueous acetic acid at low temperature.^[6] The second is the hydrolysis of trichlorosilanes in the presence of base (triethylamine or pyridine) at 0 °C; this is used in the case of bulky organic or aromatic groups.^[8, 9, 11, 14, 16–18] It has been previously shown that organoalkoxysilanes are especially useful for the preparation of silanols since the cleavage products which are formed do not accelerate the condensation of the silanols. The two compounds **1T** and **2T** presented herein were prepared according to Scheme 2, which consisted of a controlled hydrolysis of **1** and **2**^[19] under mild conditions. We modified the procedure reported^[6] since it led to silsesquioxane material. This reaction is easily explained by the presence of six possible directions for substitution at silicon. The bis-silanetriols **1T** and **2T** were obtained, after 24 hours in a biphasic medium (diethyl ether/water), as white powders in high yields (93–95 %).

These solids were insoluble in the usual organic solvents and were soluble only in DMSO, because of the strong hydrogen bonding between DMSO and the OH groups. **1T** and **2T** were thermally stable up to 100 and 70 °C, respectively. The differential scanning calorimetry (DSC) curves exhibited very broad and highly asymmetric endothermic signals between 100 and 240 °C with a maximum at 196 °C for **1T**, and between 70 and 200 °C with a maximum at 152 °C for **2T**;



Scheme 2. Synthesis of **1T** and **2T**. In compounds **1** and **1T** the rectangle represents a bridging phenyl group, in compounds **2** and **2T** it represents a diphenyl group.

these signals correspond to the polycondensation reactions. **1T** and **2T** were identified and characterized by elemental analysis, IR, ¹H, ¹³C, and ²⁹Si NMR spectroscopy, and X-ray powder diffraction studies.

In both cases, the FTIR spectra (KCl pellets) exhibited a broad absorption band centered at 3200 cm^{−1}, attributed to hydrogen-bonded OH groups (no free O–H vibration modes were observed), and a broad band centered around 890 cm^{−1}, which was due to the Si–O(–H) bonds vibration modes. Moreover no absorption bands corresponding to Si–O–Si vibrations were detected around 1050 cm^{−1}. The NMR data were collected in deuterated DMSO. ¹H and ¹³C NMR spectra of **1T** and **2T** displayed the expected resonances for the organic moiety in each case. The ¹H NMR spectra displayed a singlet at 6.36 or 6.42 ppm, respectively, which was assigned to the OH groups in the liquid medium. The ²⁹Si NMR spectra exhibited a sharp resonance at −54.3 ppm, in agreement with the chemical shifts already reported for silanetriols.^[20]

From their X-ray powder diffraction patterns, the solids were indexed with the following cell parameters: *a* = 10.056(2), *b* = 6.879(2), *c* = 6.937(2) Å, β = 95.71(3)° (*M*(18) = 34^[21]) for **1T**, and *a* = 14.030(7), *b* = 6.953(4), *c* = 6.86(1) Å, β = 92.80(8)° (*M*(18) = 9^[21]) for **2T**. The parameters describing the plane of the oxygen and silicon atoms, that is, *b* and *c*, are similar for both cells. The *a* parameter, along the stacking axis, depends on the length of the organic group. Even though the pattern of **2T** exhibited much lower crystallinity than that of **1T**, the figure of merit obtained for **2T** with 18 peaks shows some reliability for this indexation. From these results, as well as those obtained from NMR and IR spectra, we could infer that the crystallographic arrangements for **1T** and **2T** would be highly similar.

The crystallographic structure determination of **1T** has been carried out with the Rietveld method^[22] (Figure 1). This

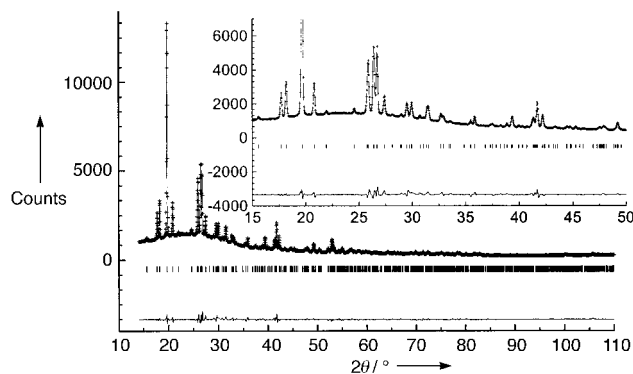


Figure 1. Experimental pattern (+), Rietveld-calculated pattern (solid line), difference (lower solid line), and Bragg positions (|) for the X-ray powder diffraction on **1T**.

structure, determined without any predefined molecular model (ab initio), is highly reliable, despite the *R*_{wp} value of 14.4 (versus an *R*_{exp} of 13.8, Ref. [23] and Table 1), with regard to the arrangement and distances directly obtained; these are consistent with the chemistry and the literature.

As seen in Figure 2, **1T** is arranged in sheets and each molecule is eclipsed (Figure 3). The two triol

Table 1. Fractional atomic parameters and equivalent isotropic displacements for **1T**.^[a]

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> _{eq} ^[b] [Å ²]
Si	0.6963(3)	−0.0231(5)	0.2790(5)	1.0
O1	0.6210(6)	0.1426(8)	0.3858(10)	1.0
O2	0.6341(6)	−0.2492(10)	0.2900(10)	1.0
O3	0.6963(5)	0.0156(11)	0.0469(11)	1.0
C1	0.875(1)	−0.012(2)	0.417(2)	2.0
C2	0.981(1)	−0.056(2)	0.295(1)	2.0
C6	0.893(1)	0.046(2)	0.592(2)	2.0

[a] In each case the site was 4e. [b] $B_{eq} = 8\pi^2 \times U_{eq}$.

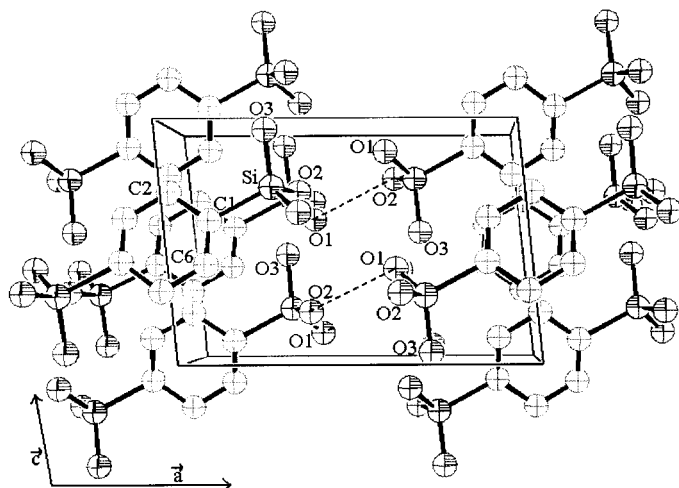


Figure 2. View along the *b* axis of two sheets of **1T**. Dashed lines represent hydrogen bonding at the interface between two sheets (hydrogen bonds in which one atom is not shown in the figure and hydrogen bonds within sheets have been omitted for clarity).

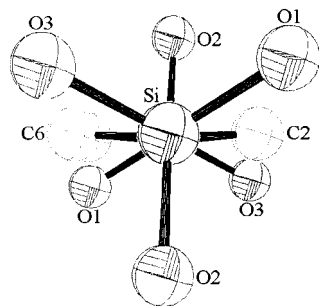


Figure 3. Topside view of a molecule of **1T** showing the eclipsed arrangement of the two triol end groups.

end groups form hydrophilic layers, whereas the body of the sheets form hydrophobic layers. It is interesting to examine more closely the hydrogen-bonding network which stabilizes the whole structure.

Within a sheet, we observed two types of hydrogen-bond distance. Starting from the O3 oxygen atom of one molecule, we found one oxygen atom O1 of a neighboring molecule at 2.68(1) Å and one oxygen atom O2 of another neighboring molecule at 2.59(1) Å (Table 2, Figures 2 and 4). It is noteworthy that this second hydrogen-bond distance is consistently shorter than the first one, but we observed also that the Si–O2 distance is 1.68(1) Å whereas the Si–O1 distance is 1.59(1) Å. The main point is that O3 has two hydrogen bonds within a sheet, and this implies, as observed, that the silicon –

Table 2. Selected bond lengths [Å] and angles [°] for **1T**.

Si–O1	1.59(1)	Intrasheet hydrogen bonds	
Si–O2	1.68(1)	O3 ... O1 ^[a]	2.68(1)
Si–O3	1.63(1)	O3 ... O2 ^[b]	2.59(1)
Si–C1	1.95(1)	Intersheet hydrogen bonds	
Phenyl (C–C) = 1.38(9)		O1 ... O2 ^[c]	2.82(1)
O1–Si–O2	116.4(4)	C1–Si–O1	101.6(5)
O1–Si–O3	112.9(4)	C1–Si–O2	110.0(5)
O2–Si–O3	103.4(4)	C1–Si–O3	112.8(5)

[a] Symmetry code: $x, \frac{1}{2} - y, z - \frac{1}{2}$. [b] Symmetry code: $x, -y - \frac{1}{2}, z - \frac{1}{2}$.

[c] Symmetry code: $1 - x, \frac{1}{2} + y, \frac{1}{2} - z$.

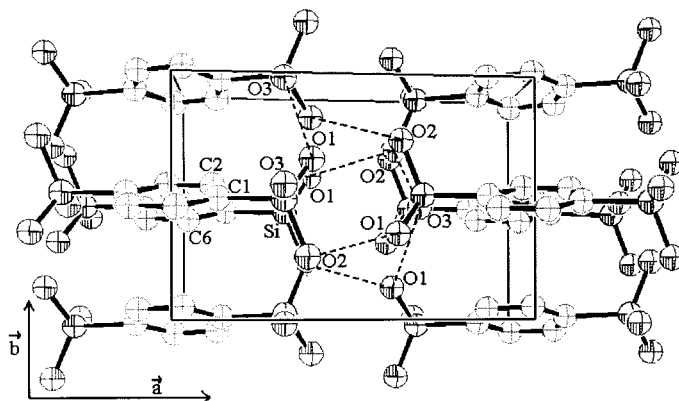


Figure 4. View along the *c* axis of two sheets of **1T**. Dashed lines represent selected hydrogen bonds at the interface.

silicon axis of these flat molecules is inclined by 29° with respect to the *a* axis: thus, the Si–O3 bond points within its layer, whereas the Si–O1 and the Si–O2 bonds point toward the neighboring layer. The phenyl rings are mainly in the *ac* plane, even though they are tilted away from this plane alternately by +21.8° and −21.8° when moving along the *b* axis in Figure 4. Consequently, the phenyl rings make a 43.6° angle with respect to their four neighbors within a sheet, the closest of which is at 5.24(1) Å on the basis of silicon to silicon distances; the closest parallel phenyl rings within a sheet are 7.24(1) Å apart on the same basis.

Between two sheets, the structure is stabilized by hydrogen bonding (2.82(1) Å) between the O1 and O2 atoms (Figures 4 and 5). The oxygen atom O3 is not involved in any hydrogen bonding between two sheets.

So, in this compound each of the oxygen atoms supplies two hydrogen bonds. The hydrogen-bond distances are consistent with those found in the literature.^[5] As previously mentioned, we can infer that **2T** presents the same type of structural arrangement as **1T**, with a thickness of 14.01 Å for a sheet, as observed in its X-ray powder diffraction pattern.

Layered structures have also been reported for bulky monosilanetriols, such as cyclohexylsilanetriol^[10] or *tert*-butylsilanetriol.^[8] A bilayer arrangement with distances directly connected with the lengths of the alkyl chains has also been proposed in the case of alkylsilanetriols containing long-chain alkyl groups.^[24] In these structures, as well as those observed in some layered phosphonates,^[25] the molecules are arranged in a head-to-tail manner, in a such way that we have alternately hydrophobic and hydrophilic interactions. In our

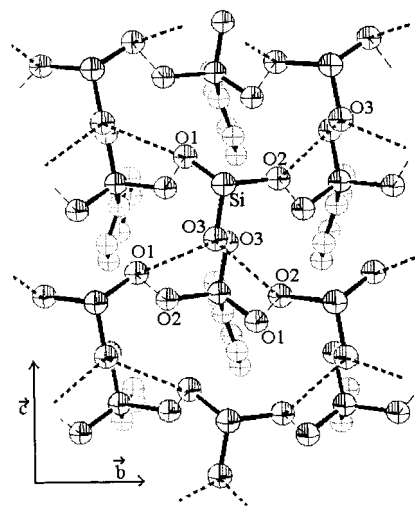


Figure 5. View along the *a* axis of one interface between two sheets of **1T**. Silicon atoms which are connected to carbon atoms belong to the inferior sheet whereas silicon atoms where the phenyl ring has been omitted belong to the superior sheet; bold dashed lines represent hydrogen bonding at the interface within the superior sheet; thin dashed lines represent hydrogen bonding at the interface between the two sheets (hydrogen bonds at the interface within the inferior sheet have been omitted for clarity).

cases, the two triol groups in *para* positions form the two hydrophilic ends of hydrophobic pillars.

We propose that the stabilization of ordered structures is the result of the formation of a strongly hydrogen-bonded network which induces the organisation of hydrophobic organic groups. There is a competition between the polycondensation reaction leading to polysiloxanes and the formation of hydrogen bonds. The formation of bis-silane-triols was possible thanks to the biphasic medium which allowed and favored the formation of the hydrophilic layer (strong hydrogen-bonded network) in the aqueous phase, while the polycondensation reaction with elimination of water was strongly slowed down. Moreover the hydrophobic interactions between organic groups certainly assisted the formation of ordered structures as shown in the case of alkyl long-chain systems.^[24]

We have reported here the preparation of the first stable bis-silane-triols. The first structure of such a compound has been presented and provides evidence for the importance of the hydrogen bonding between neighboring silanol groups. The simultaneous presence of hydrophilic and hydrophobic interactions could be a driving force in the formation of ordered structures. The distance between the hydrophilic sheets was directly connected to the length of the organic group. When the organic pillar was shorter, the bis-silane-triol appeared more organized than with the longer one. The properties of this new class of solids are currently being studied. The first attempts show that the hybrid materials obtained by polycondensation exhibit different properties from those obtained by the usual route.^[1]

Experimental Section

A mixture of 1,4-bis(trimethoxysilyl)benzene (1.6 g, 5 mmol) in 3 mL of diethyl ether and 2.2 mL of an aqueous solution of AcOH (0.5 vol %) was vigorously stirred at room temperature for 24 hours. During this period a

white solid gradually precipitated out and was then separated by filtration. The white powder was washed with ethanol (3 × 20 mL), acetone (3 × 20 mL) and diethyl ether (3 × 20 mL). The resulting solid was dried under vacuum (2 mbar) at 20 °C during 24 hours to yield 93 % of **1T** (1.09 g, 4.65 mmol) as a white powder. Elemental analysis: calcd for C₆H₁₀O₆Si₂: C 30.16, H 4.30, Si 23.95; found: C 30.86, H 4.39, Si 24.20; ¹H NMR (DMSO-d₆): δ = 6.36 (s, 6H), 7.55 (s, 4H); ¹³C NMR (DMSO-d₆): δ = 133.7, 138.7, 135.5, 136.8, 141.2; ²⁹Si NMR (DMSO-d₆): δ = −54.3.

Compound **2T** was obtained from **2** by a similar method as a white powder with a 95 % yield. Elemental analysis: calcd for C₁₂H₁₄O₆Si₂: C 46.44, H 4.54, Si 18.09; found: C 45.44, H 4.70, Si 18.20; ¹H NMR (DMSO-d₆): δ = 6.43 (s, 6H), 7.63 (d, 4H), 7.69 (d, 4H); ¹³C NMR (DMSO-d₆): δ = 126.3, 135.5, 136.8, 141.2; ²⁹Si NMR (DMSO-d₆): δ = −54.3.

X-ray powder diffraction patterns were recorded on an INEL diffractometer equipped with a CPS 120 detector, with CuKα₁ radiation (40 kV × 30 mA) in transmission mode. The sample was sieved (particles size below 63 nm) and placed in a 0.3 mm diameter capillary. Peak positions were determined using the WinPLOTR package^[26] and the pattern was indexed with DICVOL91.^[27] Si, O, and C atom positions were determined by a direct method with the SIRPOW92 package.^[28] Rietveld refinements were carried out with FULLPROF software^[29], with fixed thermal parameters. R_{wp} = 13.8 reflects the quality of the pattern. The refinement with 603 Bragg peaks led finally to R_{wp} = 14.4, R_B = 10.8, and χ² = 1.09. (See Supporting Information for more details.)

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$\lambda(\text{Cu-K}\alpha_1) = 1.5406 \text{ \AA}$, $\mu = 32.27 \text{ cm}^{-1}$. The pattern was collected with transmission geometry, from 14 to 110° (2θ). 603 reflections (206 independent) were used with 25 structural parameters and 8 profile parameters. $R_{\text{exp}} = [(N-P)/\sum w_i(y_{\text{obs}}^2)^{1/2}]^{1/2} = 13.8$, $R_p = \sum |y_{\text{obs}} - y_{\text{calc}}| / \sum y_{\text{obs}} = 17.3$, $R_{\text{wp}} = [\sum w_i |y_{\text{obs}} - y_{\text{calc}}|^2 / \sum w_i (y_{\text{obs}}^2)^{1/2}]^{1/2} = 14.4$, $R_B = \sum |I_{\text{obs}} - I_{\text{calc}}| / \sum I_{\text{obs}} = 10.8$, $\text{GOF} = (R_{\text{wp}}/R_{\text{exp}})^2 = 1.09$. Details about the structure solution and refinement are given in the Supporting Information. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-147044. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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The Essential Role of a Zn^{II} Ion in the Disproportionation of Semiquinone Radical Anion by an Imidazolate-Bridged $\text{Cu}^{\text{II}}-\text{Zn}^{\text{II}}$ Model of Superoxide Dismutase**

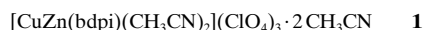
Hideki Ohtsu and Shunichi Fukuzumi*

Copper–zinc superoxide dismutase (Cu,Zn-SOD) contains an imidazolate-bridged $\text{Cu}^{\text{II}}-\text{Zn}^{\text{II}}$ heterodinuclear metal center in its active site.^[1–6] This enzyme catalyzes a very rapid two-step dismutation of toxic superoxide ($\text{O}_2^{\cdot-}$) into dioxygen and hydrogen peroxide by alternating reduction and oxidation of the copper ion of the active site.^[5–7] Superoxide first reduces the Cu^{II} center of Cu,Zn-SOD to produce O_2 , and then another molecule of superoxide oxidizes the Cu^{I} center to produce H_2O_2 .^[6, 7]

Since the oxidation potential of $\text{O}_2^{\cdot-}$ ($E_{\text{ox}}^0 = -0.16 \text{ V}$ versus the normal hydrogen electrode (NHE) at pH 7.0)^[8] is more negative than the reduction potential of Cu,Zn-SOD ($E_{\text{red}}^0 = 0.36 \text{ V}$ versus NHE at pH 7.4),^[9] the oxidation of $\text{O}_2^{\cdot-}$ may occur rapidly by electron transfer from $\text{O}_2^{\cdot-}$ to the Cu^{II} center of Cu,Zn-SOD, whereas outer-sphere electron transfer from

the Cu^{I} center of Cu,Zn-SOD to $\text{O}_2^{\cdot-}$ is highly endergonic. Therefore, coordination of $\text{O}_2^{\cdot-}$ to the Zn^{II} center of Cu,Zn-SOD was suggested to be essential to accelerate electron transfer from the Cu^{I} center of Cu,Zn-SOD to $\text{O}_2^{\cdot-}$ which is bound to the Zn^{II} center.^[10] A central issue in bioinorganic chemistry is to clarify how the Zn^{II} ion can facilitate both the oxidation and reduction of $\text{O}_2^{\cdot-}$ in Cu,Zn-SOD.^[1–7] However, the instability of the reaction intermediates and the lack of appropriate model complexes which contain the substrate binding sites has so far precluded studying the essential role of the Zn^{II} ion in Cu,Zn-SOD.^[1–7, 9–11]

We succeeded in preparing the imidazolate-bridged $\text{Cu}^{\text{II}}-\text{Zn}^{\text{II}}$ heterodinuclear and $\text{Cu}^{\text{II}}-\text{Cu}^{\text{II}}$ homodinuclear complexes **1** and **2** by using the novel dinucleating ligand Hbdpi



(Hbdpi = 4,5-bis(di(2-pyridylmethyl)aminomethyl)imidazole; Figure 1).^[10] In these SOD model complexes, each metal center has a pentacoordinate environment that includes a

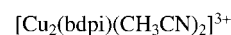
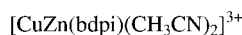
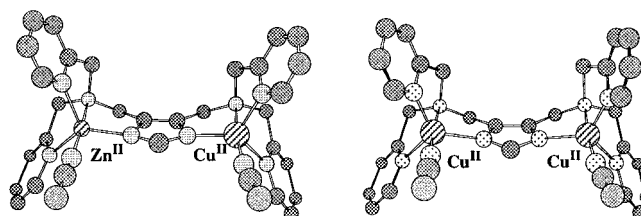


Figure 1. Structures of **1** and **2**.

solvent molecule which can be readily replaced by a substrate. Here we report on the dramatic difference in the stoichiometric disproportionation of semiquinone radical anion ($\text{Q}^{\cdot-}$) with the imidazolate-bridged $\text{Cu}^{\text{II}}-\text{Zn}^{\text{II}}$ heterodinuclear (**1**) and $\text{Cu}^{\text{II}}-\text{Cu}^{\text{II}}$ homodinuclear (**2**) complexes in propionitrile (EtCN). The presence of Zn^{II} ion is shown to be essential for disproportionation of $\text{Q}^{\cdot-}$ to occur. The use of $\text{Q}^{\cdot-}$ instead of $\text{O}_2^{\cdot-}$ provides valuable mechanistic insights into the essential role of the Zn^{II} ion in Cu,Zn-SOD, since the reaction intermediates are stable enough to be well characterized.

The addition of less than one equivalent of $\text{Q}^{\cdot-}$ to a deaerated solution of **1** in EtCN results in the appearance of a new absorption band at 585 nm, the absorbance of which increases linearly with increasing concentration of $\text{Q}^{\cdot-}$ (Figure 2a). The addition of more than two equivalents of $\text{Q}^{\cdot-}$ results in no further increase in the absorption band at 585 nm, but decay of the absorption band due to $\text{Q}^{\cdot-}$ ($\lambda_{\text{max}} = 422 \text{ nm}$) is observed (Figure 2a). The decay rate obeys first-order kinetics (Figure 2a, inset), and the rate constant k_{obs} at 193 K is 0.4 s^{-1} . Further addition of $\text{Q}^{\cdot-}$ after the initial disappearance of $\text{Q}^{\cdot-}$ resulted in reappearance of the absorption band due to $\text{Q}^{\cdot-}$, which was stable under the present experimental conditions.

[*] Prof. S. Fukuzumi, H. Ohtsu
 Department of Material and Life Science
 Graduate School of Engineering, Osaka University
 CREST, JAPAN Science and Technology Corporation
 2-1 Yamada-oka, Suita, Osaka 565-0871 (Japan)
 Fax: (+81) 6-6879-7370
 E-mail: fukuzumi@chem.eng.osaka-u.ac.jp

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

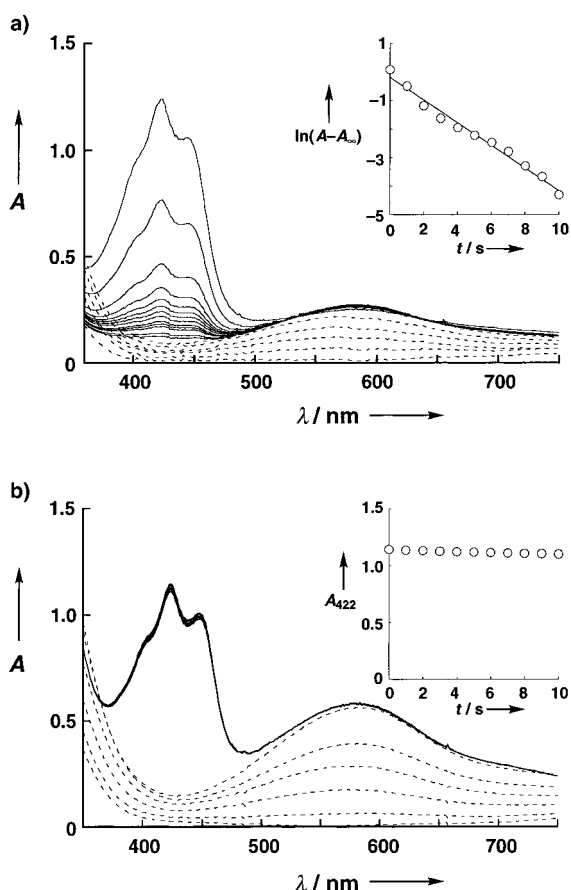


Figure 2. Changes in absorbance A observed on addition of $Q^{\bullet-}$ (0, 0.2, 0.4, 0.6, 0.8, 1.0 equiv for **1**; 0, 0.4, 0.8, 1.2, 1.6, 2.0 equiv for **2**) to an EtCN solution of a) **1** (0.1×10^{-4} M, dotted line) and b) **2** (0.1×10^{-4} M, dotted line) and spectral changes observed upon further addition of 2 equiv $Q^{\bullet-}$ to the resulting solution for a) **1** (solid line) and b) **2** (solid line) at 193 K; 1 s intervals. Inset: first-order plot of the change in absorbance for **1** and time course of the change in absorbance for **2** at 422 nm.

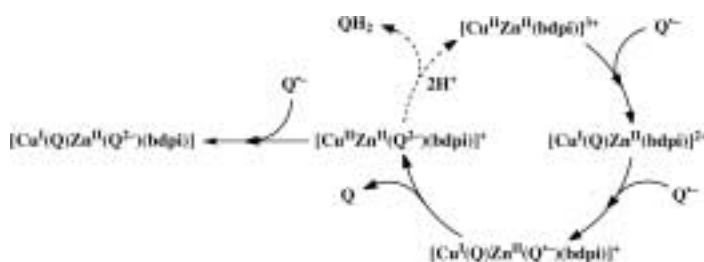
Appearance of the same absorption band ($\lambda_{\max} = 585$ nm) is observed when **1** is replaced by **2** in the reaction with $Q^{\bullet-}$. In the case of **2**, however, the absorbance at 585 nm increases linearly with an increase in the $Q^{\bullet-}$ concentration up to two equivalents of $Q^{\bullet-}$ (Figure 2b). The addition of more than two equivalents of $Q^{\bullet-}$ results in appearance of the absorption band due to $Q^{\bullet-}$, which is stable in contrast to the case of **1** (Figure 2b).

Since the one-electron oxidation potential (E_{ox}^0 versus the saturated calomel electrode (SCE)) of $Q^{\bullet-}$ (-0.51 V)^[12] is more negative than the one-electron reduction potentials (E_{red}^0 versus SCE) of the Cu^{II} center of **1** (-0.03 V)^[10] and **2** (-0.03 V),^[10] an electron transfer from $Q^{\bullet-}$ to the Cu^{II} center should occur to yield the $\text{Cu}^{\text{I}}\text{-Q}$ complex **3**, which may have a metal-to-ligand charge transfer (MLCT) band at 585 nm. In the case of **2**, two equivalents of $Q^{\bullet-}$ can be reduced, since the E_{ox}^0 value of $Q^{\bullet-}$ is still more negative than the second one-electron reduction potential for the other Cu^{II} center in **2** (-0.31 V).^[10] This may be the reason why two equivalents of $Q^{\bullet-}$ can react with **2** to give the 2:1 complex between **Q** and the Cu^{I} center **4**, which has the same absorption band at 585 nm, and why the absorbance is twice as large as that of **3**. The $\text{Cu}^{\text{I}}\text{-Q}$ complexes **3** and **4** should be diamagnetic, and it

was confirmed that the reaction mixtures of one and two equivalents of $Q^{\bullet-}$ with **1** and **2**, respectively, were ESR silent at 77 K.

The resonance Raman spectra of **3** and **4** in acetonitrile (CH_3CN) at 298 K (laser excitation wavelength 632.8 nm) revealed the same strong resonance-enhanced Raman bands at 1580 cm^{-1} . The resonance Raman frequency is significantly higher than that reported for the CO stretching vibration of free $Q^{\bullet-}$ (1435 cm^{-1}), for which the CO bond order was estimated from the Raman data to be 1.5,^[13] but lower than that of neutral *p*-benzoquinone (1651 cm^{-1}).^[14, 15] Hence, the resonance Raman bands of **3** and **4** at 1580 cm^{-1} can be assigned with reasonable certainty to a CO stretching vibration in the $\text{Cu}^{\text{I}}\text{-Q}$ complexes $[\text{Cu}^{\text{I}}(\text{Q})\text{Zn}^{\text{II}}(\text{bdpi})]^{2+}$ and $[\text{Cu}^{\text{I}}(\text{Q})\text{Cu}^{\text{I}}(\text{Q})(\text{bdpi})]^+$, respectively.

The drastic difference in the reactions of $Q^{\bullet-}$ with **1** and **2** (Figure 2) may be ascribed to the presence in **1** of a Zn^{II} ion which can bind $Q^{\bullet-}$. Since the binding of $Q^{\bullet-}$ to an Mg^{II} ion is known to facilitate the electron-transfer reduction of $Q^{\bullet-}$,^[16] the complexation of $Q^{\bullet-}$ with Zn^{II} ion in $[\text{Cu}^{\text{I}}(\text{Q})\text{Zn}^{\text{II}}(\text{bdpi})]^{2+}$ may also facilitate an intramolecular electron transfer from the Cu^{I} center, which is produced by an electron transfer from $Q^{\bullet-}$ to the Cu^{II} center, to $Q^{\bullet-}$ bound to the Zn^{II} center (Scheme 1). In **2**, however, there is no Zn^{II} center available to



Scheme 1. Mechanism for the disproportionation of $Q^{\bullet-}$ by the imidazole-bridged $\text{Cu}^{\text{II}}\text{-Zn}^{\text{II}}$ complex.

facilitate the reduction of $Q^{\bullet-}$. In an aprotic solvent such as EtCN, the final product of the stoichiometric disproportionation of $Q^{\bullet-}$ is $[\text{Cu}^{\text{I}}(\text{Q})\text{Zn}^{\text{II}}(\text{Q}^{2-})(\text{bdpi})]$ (Scheme 1), which was confirmed by the ESI mass spectrum (see Supporting Information; Figure S1). In the presence of H^+ , **1** can act as a catalyst for the disproportionation of $Q^{\bullet-}$ to **Q** and QH_2 (broken arrow in Scheme 1).

We successfully detected spectroscopically the $\text{Zn}^{\text{II}}\text{-Q}^{\bullet-}$ complex by using the mononuclear Zn^{II} complex $[\text{Zn}\{\text{MeIm}(\text{Py})_2\}(\text{CH}_3\text{CN})](\text{ClO}_4)_2$ (**5**; $\text{MeIm}(\text{Py})_2 = (1\text{-methyl-4-imidazolylmethyl})\text{bis}(2\text{-pyridylmethyl})\text{amine}$).^[17] The addition of a large excess of **5** to a solution of $Q^{\bullet-}$ in EtCN at 193 K resulted in the appearance of a new absorption band at 570 nm (see Supporting Information; Figure S2), which disappeared at higher temperatures.^[18] We previously reported that the $\text{Mg}^{\text{II}}\text{-Q}^{\bullet-}$ complex has a characteristic absorption band at 590 nm due to a red-shifted $\pi\text{-}\pi^*$ transition of $Q^{\bullet-}$.^[16, 19] Hence the absorption band at 570 nm of the $\text{Zn}^{\text{II}}\text{-Q}^{\bullet-}$ complex, which is slightly red-shifted relative to that of the $\text{Mg}^{\text{II}}\text{-Q}^{\bullet-}$ complex, can be assigned to a $\pi\text{-}\pi^*$ transition of $[\text{Zn}(\text{Q}^{\bullet-})\{\text{MeIm}(\text{Py})_2\}]^+$. One of the decomposition products was confirmed by the ESI mass spectrum, which exhibited a

signal at m/z 466 (see Supporting Information; Figure S3). The observed mass and isotope patterns are consistent with the ion $[\text{Zn}(\text{QH})\{\text{MeIm}(\text{Py})_2\}]^+$, which is the protonated form of the reduced species of $[\text{Zn}(\text{Q}^{\cdot-})\{\text{MeIm}(\text{Py})_2\}]^+$.

In conclusion, the Zn^{II} ion in the SOD model complex has been shown to play the essential role in facilitating the reduction of $\text{Q}^{\cdot-}$ by coordination of $\text{Q}^{\cdot-}$ to the Zn^{II} ion. The oxidation of $\text{Q}^{\cdot-}$ is also facilitated by the Zn^{II} ion, since the reduction potential of the Cu^{II} center in the imidazolate-bridged $\text{Cu}^{\text{II}}-\text{Zn}^{\text{II}}$ heterodinuclear complex **1** is shifted to a more positive value (0.21 V) relative to that without a Zn^{II} ion.^[10] Thus, the Zn^{II} ion can facilitate both the oxidation and reduction of $\text{Q}^{\cdot-}$. Essentially the same mechanism may also be applied to the disproportionation of $\text{O}_2^{\cdot-}$ catalyzed by Zn,Cu-SOD .

Experimental Section

To a deaerated solution (25 mL) of *p*-benzoquinone (8.10 mg) in EtCN and hydroquinone (8.25 mg) was added two equivalents of 1M $\text{Bu}_4\text{NOH} \cdot \text{MeOH}$ solution (72 μL) to make the stock solution of *p*-benzoquinone radical anion $\text{Q}^{\cdot-}$ ($6.0 \times 10^{-3} \text{ M}$). The reactions of imidazolate-bridged $\text{Cu}^{\text{II}}-\text{Zn}^{\text{II}}$ heterodinuclear and $\text{Cu}^{\text{II}}-\text{Cu}^{\text{II}}$ homodinuclear SOD model complexes and semiquinone radical anion were performed in a UV/Vis cell (path length 1 cm) which was held in a Unisoku temperature-controlled ($\pm 0.5^\circ\text{C}$) cell holder designed for low-temperature experiments. After the deaerated solution of the SOD model complexes ($0.1 \times 10^{-4} \text{ M}$) in the cell had been kept at the desired temperature for several minutes, semiquinone radical anion was added by syringe. Formation of the $\text{Cu}^{\text{I}}-\text{Q}$ complexes was followed by monitoring the absorption change at 585 nm. The rate constant k_{obs} for the stoichiometric disproportionation of $\text{Q}^{\cdot-}$ was determined by monitoring the decrease in the absorption band due to $\text{Q}^{\cdot-}$ ($\lambda_{\text{max}} = 422 \text{ nm}$).

Frozen-solution ESR spectra were recorded on a JEOL JES-RE1X X-band spectrometer equipped with a standard low-temperature apparatus. All spectra were recorded at 77 K in quartz tubes with 4 mm inner diameters. The g values were calibrated with a Mn^{II} marker as a reference.

Resonance Raman spectra were excited at 632.8 nm with an He–Ne laser and detected with a JASCO NR-1800 triple polychromator equipped with a liquid-nitrogen-cooled Princeton Instruments CCD detector. Raman measurements were carried out with a spinning cell, and the laser power was adjusted to 50 mW at the sample point. Raman shifts were calibrated with acetonitrile; the accuracy of the peak positions of the Raman bands was $\pm 1 \text{ cm}^{-1}$.

ESI mass spectra were obtained with an API 150 triple quadrupole mass spectrometer (PE-Sciex) in positive-ion detection mode, equipped with an ion-spray interface. The sprayer was held at a potential of 5.0 kV, and compressed N_2 was employed to assist liquid nebulization. The positive-ion ESI mass spectra were measured in the range m/z 100–1000.

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New Family of Cyclopropanating Reagents: Synthesis, Reactivity, and Stability Studies of Iodomethylzinc Phenoxides**

André B. Charette,* Sébastien Francoeur, Jonathan Martel, and Nicole Wilb

The cyclopropanation of olefins is a very useful process in synthetic organic chemistry. Cyclopropane moieties are found in many natural^[1] and unnatural products^[2] possessing interesting biological activities.^[3] These units are also very useful synthons for further synthetic transformations.^[4]

Amongst the different methods of cyclopropanation, the Simmons–Smith reaction^[5] has stimulated a considerable

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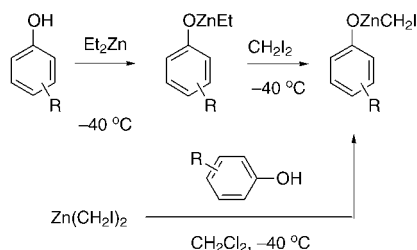
[*] Prof. A. B. Charette, S. Francoeur, J. Martel, N. Wilb
Département de Chimie
Université de Montréal
P.O. Box 6128, Station Downtown, Montréal (Canada)
Fax: (+1) 514-343-5900
E-mail: andre.charette@umontreal.ca

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amount of interest within the chemical community.^[6] Although the initial method for the preparation of zinc carbenoids with a zinc/copper couple was cumbersome, several synthetically more accessible and reproducible methods quickly followed.^[7] Notable amongst these is the protocol of Furukawa et al. which prepares a zinc carbenoid from Et₂Zn and CH₂I₂.^[8] This landmark observation allowed for the reaction to take place in noncomplexing solvents, such as dichloromethane or 1,2-dichloroethane,^[9] which greatly improved the reactivity of zinc carbenoids for the cyclopropanation of olefins.

Despite the fact that zinc carbenoids have been studied quite extensively, very little work has been done to modify the nature of the R group of the zinc reagent “RZnCH₂X” and to compare the reactivity of the new reagents to the classical ones (when R = I, Et, CH₂X). A few years ago, we demonstrated that a new class of reagents “ROZnCH₂I” (R = alkyl or allyl) are unreactive towards olefins in the absence of a Lewis acid.^[10] Recently, Shi and co-workers reported^[11] that a reagent prepared by mixing stoichiometric quantities of Et₂Zn, trifluoroacetic acid, and CH₂I₂ (which presumably form “RCOOZnCH₂I”) displayed an increased reactivity towards stilbene, a substrate that is often unreactive using previously reported Simmons–Smith protocols. In this communication, we report that some reagents of general structure “ArOZnCH₂I” are very reactive species for the cyclopropanation of unfunctionalized olefins. This synthetically useful observation is a first step towards the development of an enantioselective cyclopropanation method for unfunctionalized olefins.

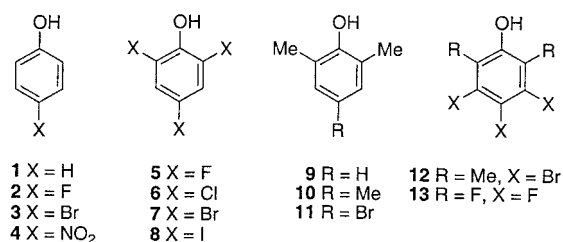
The carbenoids ArOZnCH₂I can be easily prepared by two different methods (Scheme 1). The phenol can be deprotonated upon treatment with Et₂Zn, and a subsequent metal–halogen exchange process with CH₂I₂ leads to the formation



Scheme 1. Two methods for the synthesis of ArOZnCH₂I from the corresponding phenol.

of the cyclopropanating reagent. Alternatively, one equivalent of the phenol can be treated with one equivalent of Zn(CH₂I)₂.^[8] The first method is generally preferred since it uses only one equivalent of diiodomethane and it does not necessitate the prior formation of the less stable bis(iodomethyl)zinc.

Several phenols (**1**–**13**) possessing different substitution patterns and acidities were screened (Scheme 2). To test the reactivity of these reagents, three relatively unreactive olefins were selected: α -methylstyrene (**14**), styrene (**15**), and indene (**16**). These unfunctionalized olefins were treated with the zinc phenoxide reagents and the results are shown in Table 1.^[12]



Scheme 2. Phenolic precursors for the cyclopropanating reagents.

Table 1. Reactivity of ArOZnCH₂I with aryl-substituted alkenes.

Entry	Phenol	Equiv	Conversion of alkenes [%] ^[a]		
			14 ^[b]	15 ^[c]	16 ^[d]
1	1	2.0	22	10	< 1
2	2	2.0	45	20	24
3	3	2.0	60	26	35
4	4	2.0	–	–	44
5	5	2.0	> 95	> 95	> 95
6	6	2.0	> 95 (94)	> 95 (91)	> 95 (94)
7	7	2.0	> 95	95	88
8	8	2.0	57	30	45
9	9	2.0	87	27	20
10	10	2.0	66	18	15
11	11	2.0	> 95	85	91
12	11	1.1	89	73	95
13	12	2.0	> 95	95	94
14	12	1.1	> 95	79	94
15	13	2.0	> 95	89	> 95
16	EtZnCH ₂ I	2.0	85	50	58
17	Zn(CH ₂ I) ₂	1.0	86	47	50
18	Zn(CH ₂ Cl) ₂	1.0	> 95	> 95	93

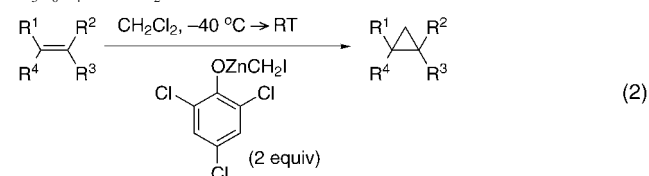
[a] Yields of isolated, analytically pure cyclopropanes are in parentheses. [b] **14**: R¹ = CH₃, R² = R³ = H. [c] **15**: R¹ = R² = R³ = H. [d] **16**: R¹ = R² = H, R³ = CH₂-Ar.

The reactivity of the reagents is highly dependant upon the position and nature of the substituents on the aromatic ring. The first observation that can be made is the apparent need for two *ortho* substituents to achieve high conversion into the corresponding cyclopropyl derivative (entries 1–3 versus entries 5, 7, 11). This trend is a consequence of the self-destruction of the reagent by an intramolecular electrophilic aromatic substitution,^[13] which is otherwise prevented by *ortho* substitution. Another striking feature of these reagents is the beneficial effect of having a strong electron-withdrawing group at the para position (entries 9–11). Although a clear trend between the pK_a of the phenol and the yield of cyclopropanation has not been established, it is obvious that having several electron-withdrawing groups is suitable. For example, 2,4,6-trihalophenol-derived reagents **5**–**7** (when X = F, Cl, and Br, respectively) produced very high yields of cyclopropanes. This higher reactivity could result from an increase in the electrophilicity of the zinc carbenoid and a decrease in the nucleophilicity of the aromatic ring (thus

preventing the rapid decomposition of the carbenoid). Of special interest are the high reactivities of the carbenoids prepared from phenols **11** and **12**. Excellent conversions are observed even when only 1.1 equivalents of the carbenoid are used (entry 11 versus 12, and 13 versus 14). It is important to note that the phenol by-product can be easily recovered in nearly quantitative yield by flash chromatography or by acid-base extraction after the reaction. The reagent derived from 2,4,6-trichlorophenol was more extensively studied since it displayed a very high reactivity for the cyclopropanation reaction and the precursor is cheap and readily available. Direct comparison between the reactivity of traditional zinc reagents and the 2,4,6-trichlorophenol-derived reagent indicates that the latter is an equal or a better reagent for the cyclopropanation of unfunctionalized olefins when the same number of active methylene group equivalents are used (entry 6 versus 16–18). The zinc phenoxide reagent is typically more reactive than Furukawa et al.'s reagent^[8] and bis(iodomethyl)zinc and of comparable reactivity to Denmark and Edwards' bis(chloromethyl)zinc.^[9]

In addition to aryl-substituted olefins, alkyl-substituted alkenes are also converted into the corresponding cyclopropane in high yields (Table 2). The yields are excellent in all

Table 2. Cyclopropanation of alkyl-substituted alkenes with 2,4,6- $\text{Cl}_3\text{C}_6\text{H}_2\text{OZnCH}_2\text{I}$.



Entry	Alkene	Conversion [%] ^[a]
1		> 95 (90)
2		> 95 (97)
3		> 95 (93) ^[b]
4		> 95 (96)
5 ^[c]		> 95 (98)
6 ^[c]		92 (92 ^[d] , 85 ^[e])

[a] Yields of isolated products are given in parentheses. [b] In this case, 3 equiv of the carbenoid were used. [c] Bn = Benzyl. [d] 1 equiv of the carbenoid was used (see text). [e] 4 equiv of the carbenoid was used (see text).

cases. As observed with other zinc reagents, it is also possible to achieve a chemoselective monocyclopropanation of an allylic ether in the presence of a trisubstituted olefin when only one equivalent of the reagent is used (entry 6). The use of an excess of the reagent led to the dicyclopropane derivative. Although we have not been able to characterize the reagent by NMR spectroscopy yet, we have shown that a solution of 2,4,6- $\text{Cl}_3\text{C}_6\text{H}_2\text{OZnCH}_2\text{I}$ in CH_2Cl_2 is stable for at least one hour at 0 °C, which indicates that the reagent is quite stable. However, a significant drop in the yield was observed when a solution of the reagent that was four hours old was used.

In conclusion, the iodomethylzinc phenoxides offer a valuable alternative to the traditional Simmons–Smith reagents due to their ease of preparation, high reactivities towards unfunctionalized olefins, and good stabilities. Further work is currently underway to study the behavior of these carbenoids in directed reactions and to develop an efficient asymmetric cyclopropanation of unfunctionalized olefins.^[14, 15]

Experimental Section

Typical procedure for the cyclopropanation reaction using iodomethylzinc 2,4,6-trichlorophenoxide: Et_2Zn (103 μL , 1.0 mmol) was added to a solution of 2,4,6-trichlorophenol (0.197 g, 1.0 mmol) in CH_2Cl_2 (10 mL) at -40°C . The solution was stirred for 15 min at that temperature and then CH_2I_2 (81 μL , 1.0 mmol) was added. After stirring for an additional 15 min, α -methylstyrene (65 μL , 0.5 mmol) was added. The cold bath was removed and the mixture was stirred for 12 h. Pentane (25 mL) was added and the organic phase was washed sequentially with 10% aq HCl (2 $\times$ 25 mL), saturated aq NaHCO_3 (2 $\times$ 25 mL), saturated aq Na_2SO_3 (25 mL), and saturated aq NaCl (25 mL). The organic layer was dried over MgSO_4 and concentrated to approximately 300 μL under reduced pressure. Flash chromatography (100% pentane) afforded the desired cyclopropane (62 mg, 94%) as a colorless liquid: R_f = 0.47 (pentane); ^1H NMR (400 MHz, CDCl_3): δ = 7.39 (m, 4H), 7.28 (m, 1H), 1.54 (s, 3H), 0.99 (dd, J = 5.9, 4.4 Hz, 2H), 0.85 (dd, J = 6.1, 4.2 Hz, 2H); ^{13}C NMR (100 MHz, CDCl_3): δ = 147.1, 128.3, 126.8, 125.5, 25.8, 19.8, 15.7; HR-MS: calcd for $\text{C}_{10}\text{H}_{12}$: 132.093900, found: 132.092033; elemental analysis: calcd for $\text{C}_{10}\text{H}_{12}$: C 90.85, H 9.15; found: C 90.87, H 9.30.

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A Near-Infrared Luminescent Label Based on Yb^{III} Ions and Its Application in a Fluoroimmunoassay**

Martinus H. V. Werts, Richard H. Woudenberg,
Peter G. Emmerink, Rob van Gassel,
Johannes W. Hofstra, and Jan W. Verhoeven*

There has been a great deal of interest in luminescent lanthanide complexes since Weissman's discovery that in these complexes "the excitation may be accomplished, under suitable conditions, through light absorption by other constituents of the rare earth compound with subsequent transfer of energy to the rare earth ion".^[1] The energy transfer from organic chromophores to lanthanide ions that he described provides an effective way to excite the long-lived, sharply spiked emission. Direct excitation of lanthanide ions is difficult because of the forbidden nature of the electronic transitions in these ions. One of the most important applications of lanthanide complexes is as luminescent labels in clinical diagnostics where they provide an alternative to radioactive probes.^[2–5] Since lanthanide luminescence decays much more slowly than the autofluorescence from the biological material, the latter is suppressed when using time-gated detection.

Research on luminescent lanthanide complexes has been almost exclusively devoted to europium(III) and terbium(III) complexes.^[6–8] The energetics of sensitized luminescence, however, dictate that those complexes must be excited with ultraviolet light,^[9] which is not desirable when working with vital biosystems, requires special optics, and causes extensive scattering in inhomogeneous media. Although some recent work has demonstrated that the excitation window for Eu^{III} complexes can be extended to the violet edge of the visible region^[10] or even to the blue region,^[11] it would be desirable to use significantly longer wavelengths for excitation. Lanthanide ions that emit in the near-infrared, such as ytterbium(III),^[12] neodymium(III),^[13] and erbium(III), may be excited through organic dyes that absorb at wavelengths extending towards the red region of the spectrum. With their potential use as luminescent labels in mind we designed and studied well-defined, water-soluble near-infrared luminescent lanthanide complexes containing organic dyes as the light-absorbing unit.^[14] Recently, we found that 4',5'-bis[*N,N*-bis(carboxymethyl)aminomethyl]-fluorescein (fluorexon, Fx) is a very efficient sensitizer and a strongly binding ligand for near-infrared-luminescent lanthanide ions.^[15] The thermodynamic stability of the $[\text{Yb}(\text{Fx})]$ complex is comparable to that of $[\text{Yb}(\text{EDTA})]$ (EDTA = ethylenediaminetetraacetate).

Here we report on the ligand FxITC (**5**; Scheme 1), which is structurally similar to Fx but carries an isothiocyanate group (ITC) that is reactive towards amine groups and so can be coupled to proteins. The iminodiacetic acid groups ensure firm complexation of lanthanide ions (like they do in $[\text{Yb}(\text{Fx})]$), and the dichlorofluorescein chromophore acts as a sensitizer to near-infrared lanthanide luminescence.

The synthesis of FxITC (Scheme 1) reflects the idea of combining the syntheses of Fx^[16] and fluorescein isothiocyanate.^[17] 2',7'-Dichloro-4-nitrofluorescein diacetate was obtained by condensing 4-nitrophthalic acid with 4-chlororesorcinol at 240 °C and was separated from the 5-nitro isomer by fractional crystallization from acetic acid.^[18] The dichloro derivative was chosen to prevent formation of isomers during the introduction of the methyleneiminodiacetic acid groups. Moreover, the "heavy" chlorine atoms might enhance intersystem crossing in the chromophore which is favorable for sensitizing lanthanide luminescence.^[15]

Direct introduction of the methyleneiminodiacetic acid groups, as used in the production of Fx,^[16] was not successful. Reaction of iminodiacetic acid dimethyl ester^[19] with **2**^[20] and subsequent hydrolysis of the ester yielded **3**, the desired nitro precursor to FxITC. Reduction of **3** using $\text{H}_2/\text{Raney Ni}$ proceeded cleanly, but gave rise to the dinickel complex of **4**, which can be observed with electrospray mass spectrometry. Therefore, **3** was reduced using $\text{Na}_2\text{S}/\text{NaHS}$ in dilute NaOH . After purification by preparative HPLC (C_8 column, eluent: water:acetonitrile:formic acid (155:45:1)), **4** was converted into FxITC using thiophosgene in acetone.

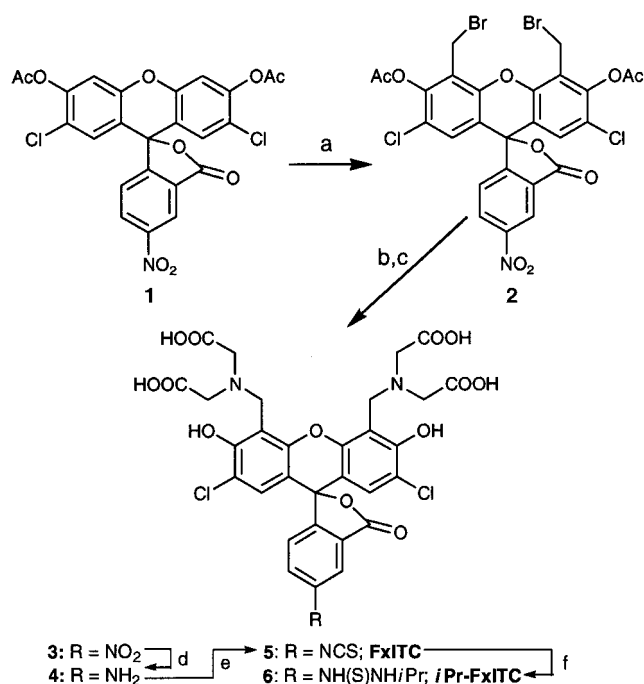
The isopropylamine adduct **6** shows the same complexation behavior towards lanthanide ions as Fx does.^[15] Fluorimetric titrations with Yb^{III} ions indicate formation of a 1:1 complex as long as there is no excess Yb^{III} ions. Excess lanthanide ions leads to the formation of poorly emitting aggregates that so far have escaped further characterization. Such aggregates,

[*] Prof. Dr. J. W. Verhoeven, M. H. V. Werts
Laboratory of Organic Chemistry
University of Amsterdam
Nieuwe Achtergracht 129, 1018 WS Amsterdam (The Netherlands)
Fax: (+31)20-525-5670
E-mail: jwv@org.chem.uva.nl

Dr. R. H. Woudenberg, P. G. Emmerink, R. van Gassel
Akzo Nobel Central Research
P.O. Box 2300, 6800 SB Arnhem (The Netherlands)

Prof. Dr. J. W. Hofstra
Philips Research, Prof. Holstlaan 4
5656 AA Eindhoven (The Netherlands)

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Scheme 1. Synthesis of FxITC. Reagents and conditions: a) paraformaldehyde (90 equiv), 33% HBr in acetic acid, 110 °C, 2 h; acetic anhydride (100 equiv) added dropwise at 20 °C, reflux, 1 h, 81%; b) iminodiacetic acid dimethyl ester (15 equiv) in dichloromethane, 90 h at 20 °C, 69%; c) methanol/50% NaOH, 16 h at 20 °C, 99%; d) Na₂S, NaHS in 0.6 M NaOH, reflux 15 min; e) thiophosgene (200 equiv) in acetone/water, 30 min at 20 °C; f) isopropylamine, 8 h at 20 °C.

however, are not formed when FxITC is bound to avidin. Titrating these protein conjugates with Yb^{III} ions yields a curve typical for the exclusive formation of the 1:1 complex.

Excitation of the avidin conjugate of the Yb^{III} complex leads to a metal-centered near-infrared luminescence with a quantum yield equal to that of [Yb(Fx)]. The corresponding excitation spectrum closely matches the absorption spectrum of the complex (Figure 1). No quenching of the luminescence

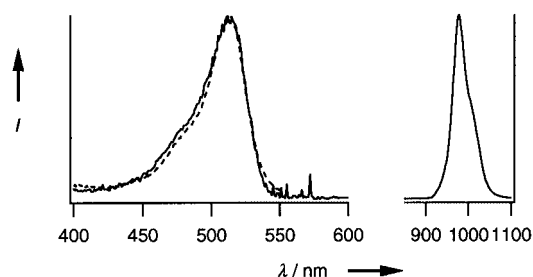


Figure 1. Luminescence spectra of [Yb(FxITC)]-labeled avidin in saline Tris-HCl buffer (pH 8.3). Left: excitation spectrum ($\lambda_{\text{em}} = 980$ nm). The visible absorption profile is shown as a dotted line. Right: emission spectrum ($\lambda_{\text{exc}} = 510$ nm).

by dissolved molecular oxygen was observed, which demonstrates that the transfer of excitation energy from the organic chromophore to the lanthanide ion is a rapid and efficient process. These observations show that the photoluminescence of the label is not adversely affected by conjugation to a protein, which furthermore has the desirable consequence

that addition of excess lanthanide ions does not influence the 1:1 antenna:lanthanide stoichiometry in the complex.

The complexes and the avidin conjugates have appreciable extinction coefficients (Table 1). Their quantum yields and luminescence lifetimes also compare well to those of [Yb(Fx)]. The absolute quantum yields, however, are low.

Table 1. Photophysical data for *iPr*-FxITC, its Yb^{III} complex, and the protein conjugates of [Yb(FxITC)] in aqueous buffer.

	$\Phi_{\text{rel}}^{[a]}$	$\epsilon_{\text{max}} [\text{M}^{-1} \text{cm}^{-1}]^{[b]}$	$\lambda_{\text{max}} [\text{nm}]^{[c]}$	$\tau_{\text{NIR}} [\mu\text{s}]^{[d]}$
[Yb(Fx)] ^[15]	1 ^[e]	125 000	502	1.9
<i>iPr</i> -FxITC	–	125 000	509	–
[Yb(<i>iPr</i> -FxITC)]	0.6	145 000	516	1.8
avidin-[Yb(FxITC)] (3.2 l/p) ^[f]	1.0	–	514	2.0
avidin-[Yb(FxITC)] (5.2 l/p) ^[f]	0.8	–	514	2.0
anti-hCG-[Yb(FxITC)]	–	–	–	2.2

[a] Φ_{rel} = near-infrared luminescence quantum yield relative to that of [Yb(Fx)]. [b] ϵ_{max} = molar extinction coefficient. [c] λ_{max} = absorption maximum. [d] τ_{NIR} = near-infrared luminescence lifetime monitored at 980 nm. [e] The absolute quantum yield of [Yb(Fx)] in Tris is 7×10^{-4} .^[15] [f] l/p means labels per protein.

This is caused by radiationless deactivation of excited lanthanide ions by molecular vibrations (especially C–H and O–H oscillators) in the surrounding matrix,^[21] a process which is especially effective with near-infrared-luminescent lanthanide ions.^[22–25] Very recently, Hasegawa et al. succeeded in pushing the luminescence quantum yield of Nd^{III} ions in organic complexes up as high as 0.03 by careful design of the ligands,^[26] but such (relatively) high quantum yields have thus far only been achieved in organic solvents. In this respect, it should be noted that closely diffusing O–H oscillators (such as water) form a particularly important contribution to the nonradiative deactivation of the luminescent state of Yb^{III} complexes.^[27]

Although the luminescence quantum yield Φ is an important parameter it is surely not the only one determining the detectability of a luminescent label. The molar extinction coefficient ϵ of the label is equally important, and when these two are combined it is apparent that [Yb(FxITC)] performs quite well in comparison to, for example, the Eu^{III} cryptate that is used in a commercial diagnostic test.^[2] The product $\epsilon\Phi$ for the cryptate in water amounts to approximately $100 \text{ M}^{-1} \text{cm}^{-1}$ when light from a N₂ laser with a wavelength of 337 nm is used for excitation.^[28] The avidin conjugate of [Yb(FxITC)] achieves the same $\epsilon\Phi$ value for light with a wavelength of 514 nm.

The robustness of the label was tested in an archetypal medical diagnostic test. The format chosen was that of a simple heterogeneous noncompetitive immunoassay,^[5] in which the analyte to be detected was “sandwiched” between an immobilized and a labeled antibody. Antibodies to hCG (anti-hCG 147b) were immobilized on a small region (diameter 3 mm) of a nitrocellulose membrane by impregnation of 0.5 μL of a 2.7 g L^{-1} solution. After drying the membrane, a mixture of solutions of the [Yb(FxITC)]-labeled antibodies (anti-hCG 293a, 2.7 g L^{-1}) and of the sample to be assayed for hCG (total 20 μL) was spotted onto a different place on the membrane. This spot was eluted with buffer (100 μL) so that

eventually the labeled antibodies and the sample passed the immobilized anti-hCG 147b. The experiment was done both with a positive sample containing 5000 mIU of hCG plus labeled antibodies and a blank that only contained the labeled antibodies. The traces generated by a home-built scanning near-infrared luminescence microscope clearly showed that a luminescence signal is generated in the case of a "positive" test result (Figure 2).

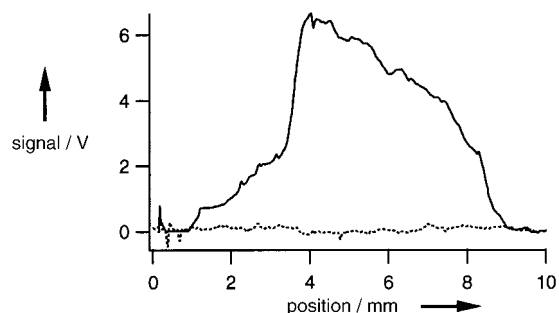


Figure 2. One-dimensional near-infrared fluorescence microscopy scan of the region of the nitrocellulose membrane where anti-hCG is immobilized. The solid line is the near-infrared luminescence signal generated after elution of a solution of labeled anti-hCG and hCG. The dotted line is the signal generated by a solution containing only the labeled anti-hCG.

It has been shown that the protein conjugates of [Yb-(FxITC)] retain photophysical characteristics that are similar to [Yb(Fx)], and that they can be applied in a model medical diagnostic test. Their luminescence is long-lived (microseconds) compared to the optical interferences encountered in biological matrices, such as fluorescence and scatter, and resides in the near-infrared region where biological material is essentially transparent. Most CCD detectors can readily detect the luminescence of Nd^{III} ions at 880 nm and that of Yb^{III} ions at 980 nm. These factors, together with the possibility of exciting these labels with visible light, make them an attractive new class of luminescent labels. However, they would benefit from an improvement of their overall luminescence quantum yield by the suppression^[24] of the nonradiative deactivation^[27] of their luminescent states.

Experimental Section

Conjugation to avidin: A 2 g L⁻¹ solution of FxITC in dry DMSO (40 µL or 80 µL) was added to a 2 g L⁻¹ solution of avidin (250 µL, Sigma) in 0.1 M carbonate buffer (pH 9.5). The mixture was incubated for 3 h at room temperature, with shaking. The solution was diluted to 2.5 mL with a saline 0.05 M Tris-HCl buffer (pH 8.3, 0.15 M NaCl; Tris = tris(hydroxymethyl)aminomethane) and purified by gel chromatography on a PD-10 column (Amersham Pharmacia Biotech) using the saline Tris buffer as an eluent. The number of labels per protein was determined by means of UV/Vis spectrometry using the extinction coefficients of the species at 280 and 514 nm.

Optical spectroscopy: The equipment and experimental procedures have been described in detail previously.^[15] Fluorimetry was carried out using a modified PTI Alphascan apparatus with a Ge detector for the near-infrared and using a modified Spex Fluorolog 3 with a CCD detector for the near-infrared measurements.

Fluorescence microscopy: The excitation light from an Ar⁺ laser (488 nm) was modulated using a mechanical chopper (40 Hz) and delivered to the sample via a dichroic mirror and the microscope objective. The emitted light was detected through the objective by a Ge detector (North Coast

817L) coupled to a lock-in amplifier (Stanford Research SRS530) after passing it through the dichroic mirror and optical filters to remove the scattered excitation light. The sample was moved with respect to the excitation/detection spot using a computer-controlled stage.

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Removal of Benzyl Protecting Groups from Solid-Supported Compounds by Hydrogenolysis Using Palladium Nanoparticles**

Osamu Kanie,* Gijsbert Grotenbreg, and Chi-Huey Wong*

One of the major problems currently encountered in solid-phase synthesis, especially in the synthesis of oligosaccharides, is the lack of a mild and effective debenzyl reaction on solid supports. The benzyl protecting group has been widely used in organic synthesis as a result of its stability and ease of mild and selective deprotection by catalytic hydrogenolysis.^[2] Hydrogenolysis using particle catalysts, however, can not be applied to solid-phase organic synthesis because of steric and mass-transport problems.^[3] Although palladium(II) acetate has been successfully used in the hydrogenolysis of benzyl groups in peptide synthesis, the Pd(0) particles formed in situ immediately precipitate in the Merrifield-type resin. In addition, there are no alternative mild methods available for the deprotection of the benzyl group under conditions compatible with acid-sensitive acetal groups and also that are applicable to the changes in the polarity of the substrate in the final stage of the synthesis.^[4, 5] Current efforts to address this issue focus on replacing the benzyl group with substituted benzyl ethers, which are removable by relatively mild conditions, such as acid^[6] or oxidative^[7] hydrolysis, reaction with a Lewis acid,^[8] a two-step reduction–oxidation process^[9], or treatment with fluoride anions.^[7b] However, these new substituted benzyl ethers are unstable under certain reaction conditions, which makes synthetic planning sometimes troublesome.

In our endeavor to find a suitably mild benzyl-deprotection method applicable to solid-phase synthesis, we considered Pd nanoparticles with a size equivalent to small organic molecules (10–40 Å diameter) as potential catalysts for the hydrogenolysis reaction. Recent developments in Pd nanoparticles^[10] have proven to be successful in making monodispersed Pd nanoparticles with a controlled size that could be used in the hydrogenation of olefins,^[10c, 11, 12] although no hydrogenolysis of benzyl groups was reported. One of the

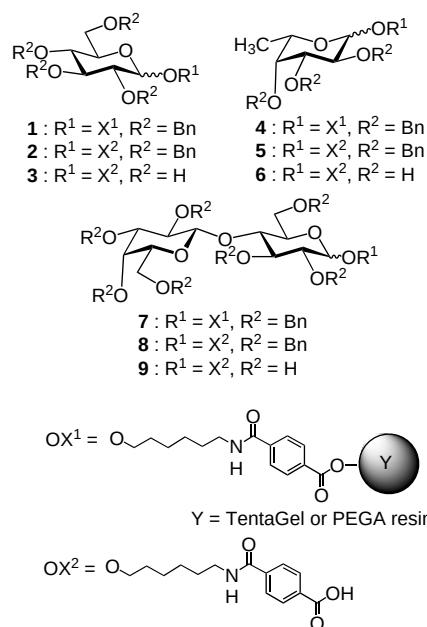
problems of using such metal clusters is that the small particles become unstable as they aggregate together. To overcome this problem, polymers, especially poly(*N*-vinyl-2-pyrrolidone) (PVP),^[12] are often used to stabilize metal nanoparticles through coordination to the metal cluster and for protection against aggregation.^[13] Recently, a fast reduction process was developed, in which a solution of H₂PdCl₄ in ethanol in the presence of PVP was refluxed to produce monodispersed Pd nanoparticles with a mean diameter ranging from 17 to 21 Å and with a narrow distribution ($\sigma < 5$ Å) [Eq. (1)].^[10a]



We therefore decided to evaluate the Pd nanoparticles as catalysts for the hydrogenolysis of benzyl ether groups on solid supports.

Before attempting solid-phase hydrogenolysis, we tested a reaction of 2,3,4,6-tetra-*O*-benzyl- β -D-glucopyranose^[14] with a colloidal Pd nanoparticle solution and, for comparison, with 10% Pd on charcoal. The colloidal Pd solution was prepared according to the procedure reported previously.^[10a, 15] The solution was concentrated and redissolved in EtOH for hydrogenolysis. The reaction with perbenzyl glucose using 0.1 atom equivalent of the nanoparticle solution proceeded much faster (approximately 10 times faster) than the corresponding reaction of Pd on charcoal, and was completed in 2 h to give D-glucose. Having found that the Pd nanoparticles could be used as hydrogenolysis catalysts, we turned our attention to the solid-phase reaction.

The benzyl-protected glycosides of glucose (1), fucose (4), and lactose (7) attached to a solid support were chosen as



representatives for the study. The syntheses of these materials were conducted as follows. Perbenzyl thioglycosides of D-glucose, L-fucose, and lactose^[16–18] were coupled with the linker methyl 4-[(6-hydroxyhexyl)amino]carbonyl benzoate

[*] Dr. O. Kanie,^[+] Prof. C.-H. Wong,^[++] G. Grotenbreg
 Frontier Research Program
 The Institute of Physical and Chemical Research (RIKEN)
 2-1 Hirosawa, Wako-shi, Saitama 351-0198 (Japan)
 E-mail: kokanee@libra.ls.m-kagaku.co.jp, wong@scripps.edu

[+] Current address:
 Glycoscience Laboratory
 Mitsubishi Kasei Institute of Life Sciences
 Minamiooya 11, Machida-shi, Tokyo 194-8511 (Japan)
 Fax: (+81)42-724-6317

[++] Permanent address:
 Department of Chemistry
 The Scripps Research Institute
 10550 N. Torrey Pines Road, La Jolla, CA 92037 (USA)
 Fax: (+1) 858-784-2409

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in a mixture of dichloromethane and diethyl ether in the presence of *N*-iodosuccinimide (NIS) and a catalytic amount of trifluoromethanesulfonic acid (TfOH)^[19] at 0 °C. The linker was designed to serve as a chromophore for the HPLC analysis of the reaction after cleavage of the products from the solid support. Anomeric mixtures (ca. 1:1) were used to test if there was, for example, any difference in their reactivity or accessibility to the catalyst particle. After saponification of each methyl ester, the acids (**2**, **5**, and **8**) were converted into the corresponding acid chloride using Vilsmeier's reagent and treated with the OH group of a solid support (**Y**) to give **1**, **4**, and **7**, respectively. Two types of solid support, TentaGel and PEGA resin, were used to investigate the effect of pore size.

When Pd nanoparticles were used for the reaction with polystyrene beads it was observed that palladium particles were slowly precipitated in the bead. This undesirable precipitation was perhaps a consequence of the steric stabilization of Pd nanoparticles followed by aggregation inside the pore. This problem could, however, be overcome by using an excess amount of PVP (100 monomer equivalents) to further stabilize the nanoparticles.

Thus, the hydrogenolysis of the benzyl protecting groups of solid-supported compounds was examined at 40 °C and was shown to be successful, in all cases, on the basis of HPLC analysis^[20] of the materials released from the support. Precipitation of Pd nanoparticles was not observed in the case of the reaction using TentaGel. The HPLC profile of the glucose derivative showed that the peaks corresponding to the partially debenzylated compounds (17–30 min) appeared in addition to the starting material **2** (37.8 and 41.1 min) and the fully deprotected compound **3** (11.9 min) was formed gradually. Formation of the product was confirmed by co-injection with the authentic sample in the HPLC and mass spectrometry analyses after isolation of each compound. Although the reaction was not complete after 60 h (85 % yield),^[21] peaks of **2** (α , β) were still present, it seems that no further consumption of **2** would occur. Interestingly, very small amounts of partially debenzylated intermediates remained at this stage, probably because of the presence of sites in TentaGel that were inaccessible to the Pd nanoparticles. In addition, it was found from the HPLC profile that compound **3** exists as an anomeric mixture with the same ratio as that of **2**. Furthermore, the color, clarity, and catalytic activity of the Pd nanoparticles remained unchanged.

Since the reaction could not be completed using the Merrifield-type resin, the reactions using a PEGA resin were next examined to see the effect of pore size. Absorption of almost all Pd nanoparticles into the pores of the resin was observed (from the color of the reaction mixture and the resin); however, the hydrogenolysis proceeded smoothly and reached completion within 36 h to give the glucoside **3** and fucoside **6**, respectively. In the case of lactoside **7**, formation of product **9** was confirmed, but the reaction was still incomplete (yield ca. 95 %). The possible reason for the observed adsorption of Pd particles to the support is perhaps because of the presence of amide functions on the solid support; removal of the Pd nanoparticles from the product mixture of solution-phase hydrogenolysis is a difficult problem and these amide functions may be useful.

In conclusion, we have shown that Pd nanoparticles can be used in the hydrogenolysis of benzyl protecting groups on carbohydrates attached to solid supports such as TentaGel and PEGA resins. It was shown for the first time that hydrogenolysis is possible on solid and in solution phase using Pd nanoparticles, although there is still room for improvement. As this method is as mild and effective as the widely accepted solution-phase hydrogenolysis, it may have great potential in solid-phase synthesis, especially when biological assays are to be carried out with compounds on a resin for high-throughput screening. Work is in progress to optimize the reactions and to prepare a carbohydrate library for screening.

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[20] HPLC conditions: column: Millipore NovaPak C18, 4.6 × 150 mm; flow: 1.00 mL min⁻¹; detection: 254 nm; RT; solvents A: 10% CH₃CN in H₂O with 0.1% TFA, B: 10% H₂O in CH₃CN with 0.1% TFA, solvent gradient: 0 min (100% A), 5 min (50:50 A:B), 35 min (30:70 A:B), 45–55 min (100% B). Retention time: **2** (37.8 and 41.1 min), **3** (11.9 min), **5** (30.5 and 31.8 min), **6** (16.2 min), **8** (56.3 and 58.1 min), **9** (7.6 min).

[21] The yield was estimated from the peak area of the HPLC chromatogram and the molar absorbance of the benzyl and terephthalate groups.

Tuning the Semiconducting Properties of Sexithiophene by α,ω -Substitution— α,ω -Diperfluorohexylsexithiophene: The First n-Type Sexithiophene for Thin-Film Transistors**

Antonio Facchetti, Yvonne Deng, Anchuan Wang, Yoshihiro Koide, Henning Sirringhaus, Tobin J. Marks,* and Richard H. Friend*

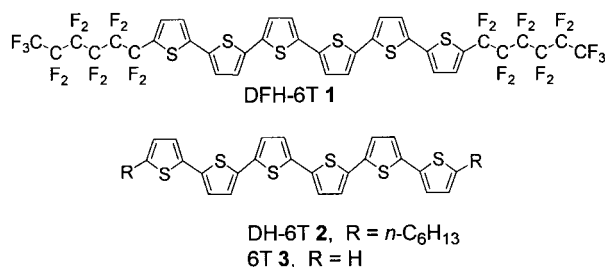
$\alpha\alpha'$ -Conjugated thiophene oligomers (nTs) and polymers (PTs) have attracted great interest as semiconducting elements in organic thin-film transistors (TFTs).^[1, 2] To be useful



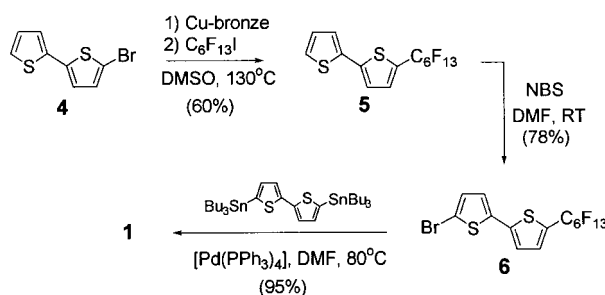
in such structures, the organic material must support a channel of holes or electrons (p- or n-type semiconductor, respectively) created by the gate electrode bias, which switches the device “on”. Furthermore, the charge mobility of the material must be sufficiently large to increase the source–drain on-conduction by many orders of magnitude over the “off” state. The most important examples of this family of compounds are unsubstituted, α,ω -, and β,β' -

dialkylsubstituted nTs ($x = 1, 2$), and β -alkyl-substituted PTs, where optimized carrier mobilities (0.1–0.6 cm² V⁻¹ s⁻¹) and on/off ratios (>10⁶) approach those of amorphous silicon.^[1e, 2a,c,e, 3] Without exception, these systems behave as p-type semiconductors, presumably because the electron-richness of the thiophene renders negative carriers susceptible to trapping by more electronegative residual impurities such as oxygen. On the other hand, developing and understanding new n-type materials would enable applications,^[4] such as bipolar transistors, p-n junction diodes, and complementary circuits, as well as to afford a better fundamental understanding of charge transport in molecular solids. The major barrier to progress, however, is that most n-type organics are either environmentally sensitive, have relatively low field mobilities, lack volatility for efficient film growth, and/or are difficult to synthesize.^[4, 5, 6]

We report here the facile, efficient synthesis of the first n-type sexithiophene conductor, α,ω -diperfluorohexylsexithiophene (DFH-6T; **1**), for TFTs. DFH-6T was synthesized



by a Pd⁰-catalyzed Stille coupling of 5-bromo-5'-perfluorohexyl-dithiophene (**6**) with 5,5'-di(tributylstannyl)dithiophene (Scheme 1). This route affords DFH-6T (**1**) in high purity and yield after multiple gradient sublimation. Although monosubstituted perfluoroalkyl thiophene oligomers with up to three units have been reported, synthetic yields were typically about 10%.^[7]



Scheme 1.

The comparative thermal properties of DFH-6T (**1**) and DH-6T (**2**) were investigated by differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA). The DSC trace of compound **1** exhibits a distinct crystal-to-liquid crystal (LC) transition at 292 °C and a LC-to-isotropic transition at 309 °C, while the crystal-to-LC transition of **2** (300 °C) falls just below the melting point, 308–313 °C.^[8] These mesophase-formation events are not surprising in view

[*] Prof. T. J. Marks, Dr. A. Facchetti, Dr. A. Wang, Dr. Y. Koide
Department of Chemistry and the Materials Research Center
Northwestern University
2145 Sheridan Road, Evanston, IL, 60208-3113 (USA)
Fax: (+1) 847-491-2290
E-mail: tjmarks@casbah.acns.nwu.edu

Prof. R. H. Friend, Y. Deng, Dr. H. Sirringhaus
Cavendish Laboratory, Department of Physics
University of Cambridge
Madingley Road, Cambridge CB3 0HE (UK)
Fax: (+44) 1223-353297
E-mail: rhf10@cam.ac.uk

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of the molecular architectures. However, the most interesting feature is that the two systems exhibit such similar thermal behavior and almost identical melting points, which suggests that the strong $\pi-\pi$ interactions of DH-6T^[1a, 2b] are preserved upon substitution with fluoroalkyl groups. In contrast, the α,ω -fluorocarbon chains have a dramatic effect on the volatility of **1** (Figure 1A). The graph of the percentage

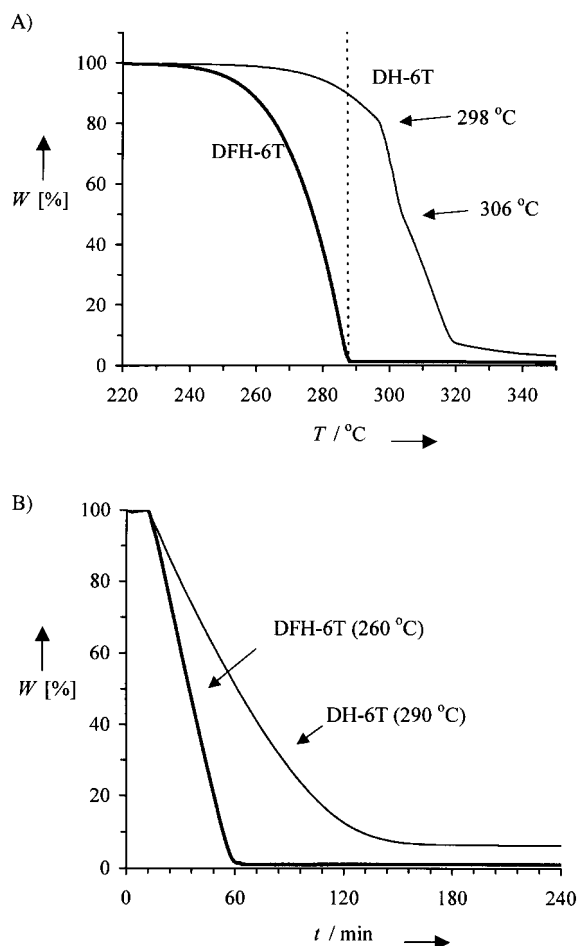


Figure 1. Comparison of the volatility characteristics of DHF-6T and DH-6T by TGA at reduced pressure (0.01 torr N_2). A) Comparison plots of the weight retention (w) for DH-6T and DFH-6T recorded at a ramp rate of $1.5^\circ C min^{-1}$. B) Isothermal data collected at 260 and 290 °C for DFH-6T and DH-6T, respectively.

weight retention versus the temperature for **2** demonstrates that a smooth, clean, quantitative sublimation occurs well below the melting point with negligible residue. In contrast, DH-6T (**1**) exhibits significant volatility only above the melting point. The TGA data reveal inflections at about 298 and 306 °C, which suggests decomposition processes occur. In addition, a significant decomposition-related residue (ca. 6 %) is observed at 320 °C. The enhanced volatility and stability of DFH-6T relative to DH-6T is further demonstrated by isothermal TGA (Figure 1B), which shows that sublimation of DH-6T at the lowest possible temperature leaves about 8 % of a decomposition residue.

Figure 2 compares the optical absorption and emission (PL) spectra of DFH-6T and DH-6T in solution (Figure 2A) and as

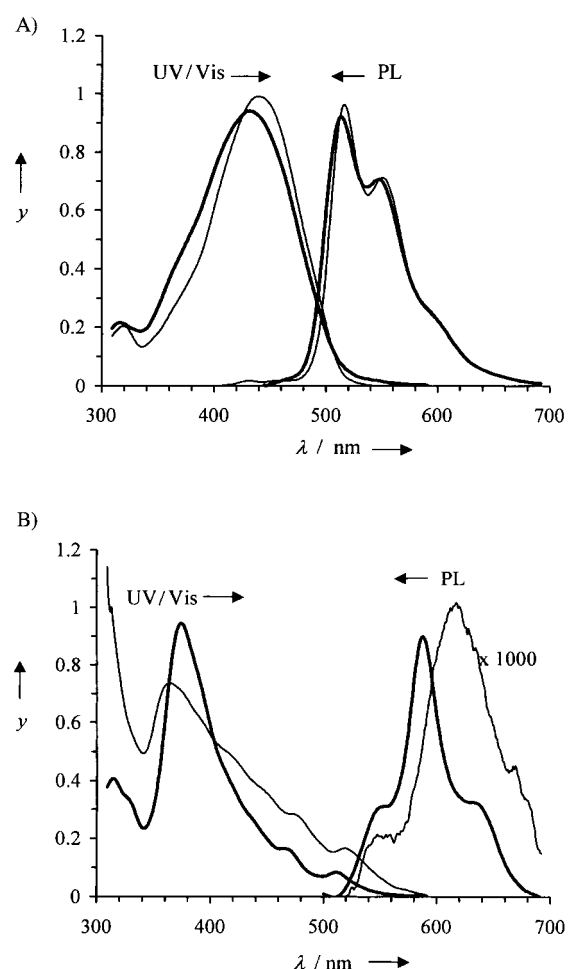


Figure 2. Optical absorption (UV/Vis) and emission (PL) spectra of DFH-6T (bold line) and DH-6T (fine line). A) As dilute solutions in toluene at approximately 80 °C. The PL spectra were obtained by exciting the solutions at 444 nm (DFH-6T) and 436 nm (DH-6T). B) As thin films (ca. 1.8 μm) on glass substrates. The PL spectra were recorded with excitation at 377 nm (DFH-6T) and 363 nm (DH-6T). Intensities (y) are in arbitrary units.

thin vapor-deposited films (Figure 2B). Remarkably, the solution spectra are nearly superimposable, which suggests that the energy differences between the ground and excited states of the 6T core are only marginally sensitive to α,ω -substitution and that the HOMO–LUMO gaps are nearly identical at approximately 2.4 eV. The spectra of the films exhibit both differences and similarities. First, both PL spectra have a central peak and two weaker shoulders. The high-energy shoulders fall, for both systems, at the same wavelength near the tail of the absorption, which yields estimated HOMO–LUMO gaps of about 2.2 eV in the films. However, while DFH-6T exhibits strong green fluorescence ($\lambda_{max} \approx 588$ nm), the DH-6T emission intensity is several orders of magnitude weaker and red-shifted by about 30 nm. The low PL yields of thin films of DH-6T (**2**) and 6T (**3**) as well as the blue-shifted absorption maximum vs. that in solution were previously ascribed to Davydov splitting.^[9–11] The origin of the blue-shifted absorption band in DFH-6T and enhanced PL efficiency is unknown and warrants further study. While both DH-6T and DFH-6T exhibit strong intermolecular $\pi-\pi$ interactions, they are not isostructural (see below).

The electronic consequences of substituting of DH-6T with fluoroalkyl groups were also investigated by cyclic voltammetry (one-compartment cell with a Pt electrode, an Ag/0.1M AgNO₃ (CH₃CN) reference, and an Ag counter electrode).^[11] Voltammograms of DFH-6T (**1**) and DH-6T (**2**) in CHCl₃/0.10M TBABF₄ (TBA = tetrabutylammonium) exhibit two chemically irreversible oxidative waves at +0.68/+0.88 V and +0.41/+0.61 V,^[12] respectively, and negligible reductive features, which are indicative of chemically irreversible oxidative processes likely related to polaron and bipolaron formation.^[13] The first and second anodic peaks of DFH-6T exhibit a uniform anodic shift (ca. +0.27 V) relative to those of DH-6T, which indicates that introduction of perfluoroalkyl chains on the 6T core substantially increases the ionization potential. The similar HOMO–LUMO gaps of **1** and **2** (see above) indicate that the LUMO of **1** is also approximately 0.27 V below that of **2**, which is confirmed by electrochemical studies on thin films.^[14] Low solubility limits reductive studies by conventional cyclic voltammetry. Recently, Katz et al. reported that substitution with fluorinated R^FCH₂ groups improves the air stability and solubility of a well-known n-type naphthalenetetracarboxylic acid diimide while affording mobilities of up to 0.1 cm²V^{−1}s^{−1}.^[15] The perfluoroalkyl chain is not in direct communication with the π system, and electronic effects should be substantially attenuated.

Scanning electron microscopy (SEM) studies reveal that the growth of the DFH-6T film depends significantly on the substrate temperature and surface pretreatment. Films deposited at 25 °C consist of small crystalline grains (ca. 80 × 50 nm²) resulting from rapid condensation on the cold substrate (Figure 3 A), while growth at 85 °C affords elongated grains with larger dimensions (ca. 500 × 60 nm²) and

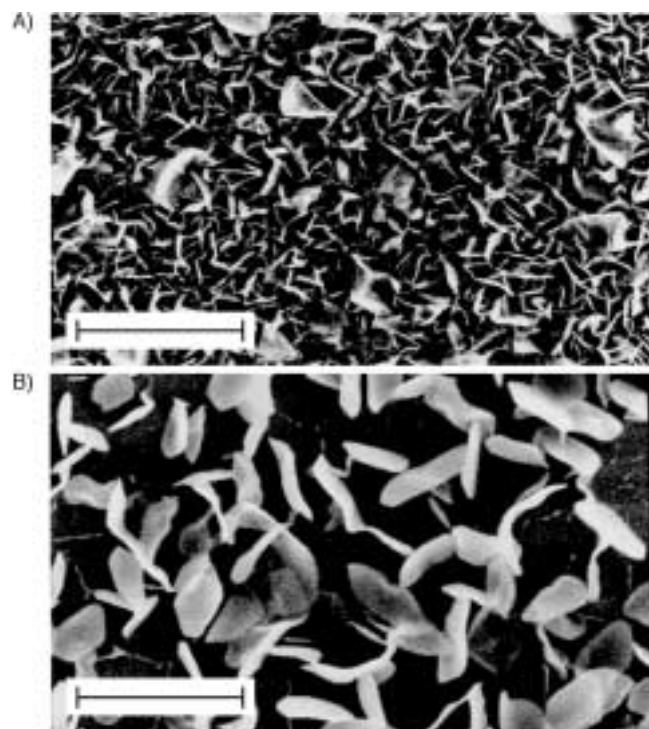


Figure 3. SEM images (2 kV × 15.0 K) of 100-nm thick DFH-6T films deposited on silicon at substrate temperatures of: A) 25 °C, B) 145 °C. Bars represent 2 µm.

random orientation. Growth at 145 °C yields larger crystallites (Figure 3 B; ca. 1000 × 350 nm²), however, film morphology is less smooth, probably because the volatility of DFH-6T prevents its uniform adsorption on the hot surface. The influence of the substrate temperature on crystallite size and film morphology for other thiophene semiconductors such as 6T (**3**)^[13] is known to be related to nucleation site density, growth rate, and surface free energy phenomena during growth.^[16] Since Si/SiO₂ has a hydrophilic native oxide layer, the surface is not ideal for the deposition of a hydrophobic adsorbate. Thus, substrates were also pretreated with CF₃(CF₂)₅CH₂CH₂SiCl₃ to produce a fluorocarbon monolayer. The crystallite dimensions are increased by approximately 10% and the grain-to-grain and grain-to-substrate interconnections are visibly enhanced.

X-ray diffraction (CuK α) scans of DFH-6T films grown at various temperatures (Figure 4) reveal that higher crystalline order is achieved as the substrate temperature is increased to 200 °C. Annealing the films at 240 °C further enhances

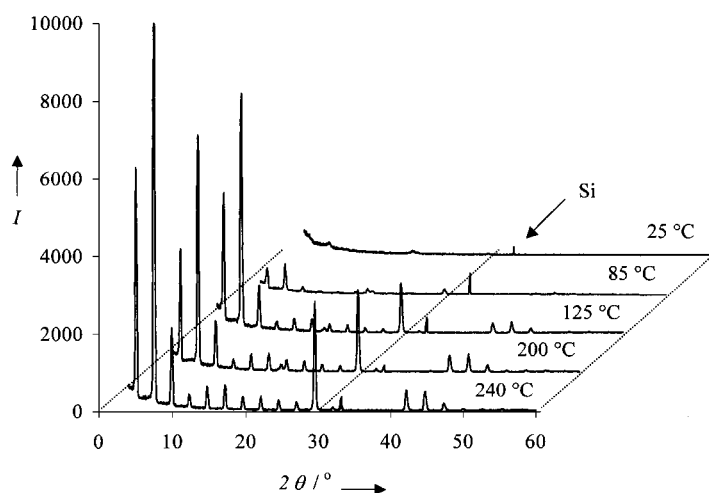


Figure 4. X-ray θ – 2θ diffractograms of approximately 2 µm-thick vacuum-evaporated DFH-6T films grown on Si substrates at various substrate temperatures. The spectrum recorded at 240 °C corresponds to a film grown at 200 °C and annealed at 240 °C for 30 min.

microstructural alignment, with the dominant Bragg features assignable to a family of (*n*00) reflections corresponding to a real-space periodicity of 36.1 ± 0.2 Å. This value is close to the reported DFH-6T monolayer thickness (35.5 Å). Although the crystal structure of DFH-6T has not been determined, the XRD data suggest a preferential molecular orientation with the long axes along the substrate normal, where transport in the plane of the film would benefit from close π – π stacking. The DFH-6T films grown on Si substrates treated with CF₃(CF₂)₅CH₂CH₂SiCl₃ exhibit sharper XRD reflections, which is indicative of more highly ordered films. Finally, powder diffraction reveals that DFH-6T and DH-6T are not isostructural; different unit cell arrangements are expected in view of the different packing characteristics of hydrocarbon versus perfluorocarbon chains.^[17]

Field-effect measurements were carried out on top-contact TFTs consisting of 30–100 nm films of DFH-6T deposited at less than 10^{−6} mbar and at approximately 0.5–1.0 Å s^{−1} onto

n^+ -Si field-effect transistor (FET) substrates with a 230 nm dry thermal SiO₂ insulating layer. The substrate temperature was varied between 50 and 120 °C. The SiO₂ surface was silylated with hexamethyldisilazane^[18] or CF₃(CF₂)₅-CH₂CH₂SiCl₃ prior to DFH-6T deposition; both yielded similar TFT results. The devices were completed by evaporating Au or Al source–drain contacts onto the DFH-6T layer. The device characteristics were measured under N₂ with an HP parameter analyzer. Figure 5 A shows the characteristics

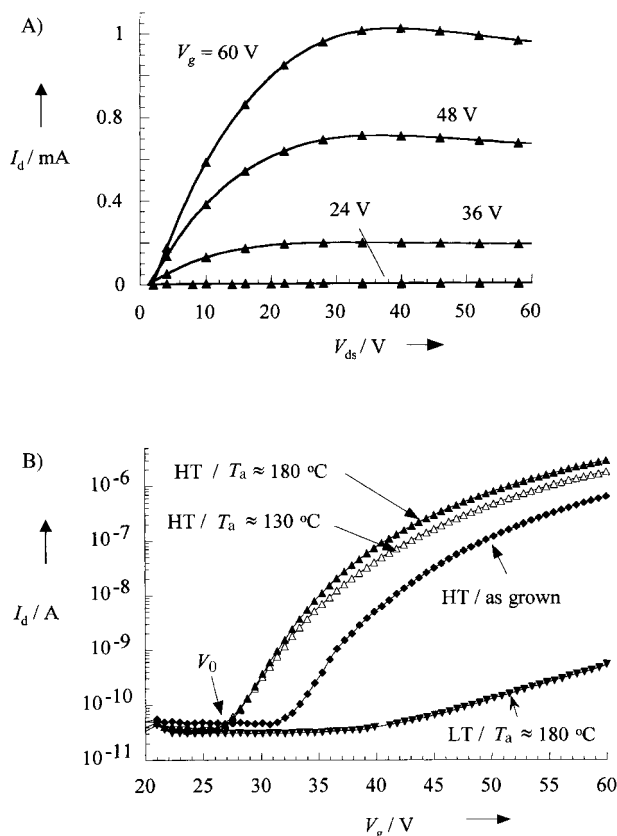


Figure 5. A) Output characteristics of a DFH-6T TFT with Au source–drain contacts deposited at a temperature of about 90 °C (channel length $L = 75 \mu\text{m}$, $W = 1.5 \text{ mm}$). B) Transfer characteristics of devices grown at a low temperature of 50 °C (LT: ∇), and a high temperature of approximately 90 °C (HT). The device characteristics shown at high temperature were measured directly after film growth (as-grown, $\blacklozenge$), and after post-growth annealing in vacuum at 130 °C ($\triangle$), and 180 °C ($\blacktriangle$).

of a typical DFH-6T FET with Au source–drain contacts. Transistor action is observed only for positive gate voltages ($V_g > 0$), which indicates that a layer of mobile electrons accumulates at the interface between DFH-6T and SiO₂. No transistor action is induced by applying a negative gate voltage. Only p-type accumulation ($V_g < 0$) has previously been observed in TFTs of alkyl-substituted and unsubstituted oligo- and polythiophene derivatives. The present contrasting n-type conduction doubtless reflects the greater electron affinity of the fluoroalkyl-substituted sexithiophene. It is remarkable that this relatively minor change in the substituent is sufficient to dramatically alter the thin-film semi-conducting properties from p- to n-type. To our knowledge, the only other example of such a sign inversion in the majority

carrier is the phthalocyanine $\rightarrow$ perfluorophthalocyanine (F16Pc) system.^[19] However, DFH-6T and M(F16Pc) differ markedly in the nature of their substituent effects. In the latter, extensive C–H $\rightarrow$ C–F substitution leads to large electronic perturbations of the core structure. Furthermore, the nature of the substituents differs. Whereas perfluoroalkyl chains exhibit only strong σ -inductive electron-withdrawing effects ($-I$), F atoms attached directly to π systems exhibit both donating-resonance ($+M$) and attracting-inductive ($-I$) characteristics.^[20]

The device of Figure 5 was fabricated with Au source–drain contacts. Devices with Al contacts exhibit similar characteristics, which is surprising considering the significantly higher work function of Au (5.1 eV) compared to that of Al (4.3 eV), which implies, a priori, there is a higher barrier for electron injection from Au. Similar injection effects have recently been reported for other n-type systems, and are not understood at present.^[21] Values for the field-effect mobility were determined from the transfer characteristics in the saturation regime (Figure 5 B).^[22] The highest mobilities of $0.02 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ were obtained for film growth at 80–100 °C [Equation (1)].

$$\frac{dI_d}{dV_g} = \frac{C_i W}{L} \mu_{\text{FET}}^{\text{sat}} (V_g - V_T) \quad (1)$$

Significantly lower mobilities ($< 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) are observed for growth temperatures of approximately 50 °C, and a reduced mobility is also obtained for growth temperatures of 120 °C. This optimum substrate temperature doubtless reflects, among other factors, the dependence of the grain size distribution in the polycrystalline film on the temperature.^[23] The on:off ratio in these DFH-6T devices is as high as $10^5:1$. The devices exhibit relatively high turn-on voltages V_0 of 25–35 V, which indicates a certain fraction of the induced electron carriers occupies localized trap states. The turn-on voltage increases further during operation. This increase results in a decrease of the TFT on-current as a function of time which manifests itself in a nonideal negative slope of the output characteristics in the saturation regime (Figure 5 A). The turn-on voltage can be somewhat reduced and stabilized by post-growth annealing (Figure 5 B).

These results demonstrate that functionalization of the 6T core with fluoroalkyl groups to give DFH-6T (**1**) substantially enhances thermal stability, volatility, and electron affinity relative to DH-6T (**2**) and affords the first n-type oligothiophene TFT. The morphology of the DFH-6T film is sensitive to substrate temperature and surface pretreatment, with crystallite size increasing with increasing growth temperature. UV/Vis, PL, and XRD studies indicate that while DFH-6T has close intermolecular π -stacking interactions, it is not isostructural with DH-6T. Since thiophene oligomers are typically p-type, the present work may provide a pathway by which all-oligothiophene complementary circuits can be realized.

Experimental Section

The reagents 5-bromo-2,2'-dithiophene (**4**),^[24] and 5,5'-bis(tri-*n*-butylstannyl)-2,2'-dithiophene^[25] were prepared according to known procedures. Solvents and chemicals were purchased from Aldrich or Lancaster and purified as required.

5: A suspension of Cu bronze (3.38 g, 53.16 mmol) in dry DMSO (30 mL) was heated at 125 °C for 15 min under nitrogen. Perfluorohexyl iodide (11.34 g, 25.43 mmol) was added dropwise in such a way as to keep the temperature below 135 °C. After 45 min, a solution of 5-bromodithiophene (2.50 g, 10.17 mmol) in the same solvent (7.0 mL) was added dropwise, and the reaction mixture was stirred at 125 °C for 16 h. The reaction was next quenched with cold water (100 mL) and diethyl ether was added (200 mL). After shaking the mixture, it was filtered through celite and the organic phase collected. The aqueous layer was extracted once more with diethyl ether (100 mL) and the two organic phases were combined, washed with water, and dried over MgSO₄. After filtration, the solvent was evaporated to yield a solid (5.18 g), which was chromatographed on silica gel (hexane) to afford pure product as a yellow solid (2.90 g, 5.99 mmol, 58.9 % yield). m.p. 51–53 °C (MeOH); ¹H NMR (CDCl₃): δ = 7.31–7.37 (m, 2H), 7.28 (d, ²J(H,H) = 3.6 Hz, 1H), 7.18 (d, ²J(H,H) = 3.7 Hz, 1H), 7.06 (dd, ²J(H,H) = 4.1 Hz, 1H); ¹⁹F NMR (CDCl₃): δ = –81.07 (3F), –101.64 (2F), –121.83 (br, 4F), –123.11 (2F), –126.43 (2F). Elemental analysis calcd for C₁₄H₅F₁₃S₂ (%): C 34.72, H 1.04, F 51.00; found: C 34.96, H 0.99, F 49.95; HR-MS (EI, 70 eV) for C₁₄H₅F₁₃S₂ [*M*⁺]: calcd: 483.9625, found: 483.9626.

6: With exclusion of light, *N*-bromosuccinimide (1.02 g, 5.73 mmol) was added to a solution of 5-perfluorohexyldithiophene (2.32 g, 4.79 mmol) in DMF (32 mL). After stirring the reaction mixture overnight at 25 °C, it was poured into brine (120 mL), and the solution then extracted with diethyl ether (3 × 100 mL). The organic phase was next washed with water, dried over MgSO₄, filtered, and the solvent evaporated to afford 2.60 g of crude product. Flash chromatography on silica gel (hexane) afforded **6** as a light yellow solid (2.10 g, 3.73 mmol, 77.8 % yield). Sublimation (50 °C/10^{–3} Torr) afforded a purer sample. m.p. 63–65 °C; ¹H NMR (CDCl₃): δ = 7.34 (d, ²J(H,H) = 3.1 Hz, 1H), 7.12 (d, ²J(H,H) = 3.1 Hz, 1H), 7.03 (d, ²J(H,H) = 3.3 Hz, 1H), 7.01 (d, ²J(H,H) = 3.3 Hz, 1H); ¹⁹F NMR (CDCl₃): δ = –81.21 (3F), –101.87 (2F), –122.02 (br, 4F), –123.27 (2F), –126.56 (2F). Elemental analysis calcd for C₁₄H₄BrF₁₃S₂ (%): C 29.86, H 0.72, F 43.86; found: C 29.83, H 0.69, F 44.01; HR-MS (EI, 70 eV) for C₁₄H₄BrF₁₃S₂ [*M*⁺]: calcd: 563.8709, found: 563.8721.

1: A mixture of 5,5'-bis(tri-*n*-butylstannyl)-2,2'-dithiophene (1.40 g, 1.88 mmol), 5-perfluorohexyl-5'-bromo-2,2'-dithiophene (2.00 g, 3.55 mmol), and [Pd(PPh₃)₄] (0.050 g, 0.040 mmol) in dry DMF (40 mL) was deaerated twice with nitrogen. The reaction mixture was then heated at 80 °C for 7 h during which time a precipitate formed. After cooling, the bright orange solid was collected and washed several times with hexane, methanol, and acetone to afford the product (1.91 g, 1.69 mmol, 95.2 % yield), m.p. 309 °C. Additional purification can be achieved by multiple gradient sublimation. Elemental analysis calcd for C₃₆H₁₂F₂₆S₆ (%): C 38.24, H 1.07, F 43.69; found: C 38.16, H 1.15, F 43.42; MS (EI, 70 eV): *m/z*: 1127.4 ([*M*⁺], 70 %).

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Crystallization of a Large-Pore Three-Dimensional Gallium Fluorophosphate under Mild Conditions**

Richard I. Walton, Franck Millange, Thierry Loiseau, Dermot O'Hare,* and Gérard Férey

Microporous solids continue to be the focus of much attention as we enter the 21st century because of their catalytic and shape-selective absorptive properties which find widespread application in diverse areas such as cracking of crude oil, gas separation, water softening in detergents, radioactive waste remediation, and as drying agents.^[1] Although aluminosilicate zeolites (both naturally occurring and synthetic) are perhaps the best known microporous materials,^[2] in the last twenty years a huge number of new inorganic framework materials has been synthesized containing elements from virtually all groups in the Periodic Table, and almost all exhibiting structures not seen in zeolite chemistry.^[3] One widely studied family is the open-framework phosphates which have been the subject of intense research since 1982 when Wilson et al. reported aluminum phosphates with zeolite-like structures.^[4] Much discussion of the formation mechanism of microporous phosphates (usually prepared under hydrothermal conditions) has taken place recently,^[5–7] but still there remains little experimental evidence on which to base a general formation mechanism. An understanding of the formation mechanism of microporous materials would allow the rational synthesis of new materials with properties tailored for a specific application.^[8]

We have recently studied the synthesis of open-framework aluminum and gallium fluorophosphates, a large family of materials that exhibit chain and layered structures as well as three-dimensional zeolite-like structures,^[5] with the aim of collecting data to formulate a reaction mechanism. We have performed a systematic study of the influence of experimental conditions on the outcome of a reaction (choice of reagents, temperature, pH, time etc.),^[5, 9] and in situ studies of the hydrothermal reactions using X-ray diffraction^[10–13] and NMR spectroscopy.^[14] A third approach we have very recently adopted is the stabilization of materials which might have the role of precursors to microporous solids.^[15] We have recently isolated a new chain gallium fluorophosphate at room

temperature from a reaction mixture which if heated under hydrothermal conditions yields a three-dimensional (3D) phase.^[15] We have also discussed its transformation into an open-framework material.^[16] Herein we describe the crystallization under very moderate conditions of a three-dimensional open-framework gallium fluorophosphate, with large pores constructed from 20-ring windows.

The new material $\text{Ga}_4(\text{HPO}_4)_2(\text{PO}_4)_3(\text{OH})_2\text{F} \cdot [\text{NH}_3(\text{CH}_2)_4\text{NH}_3]_2 \cdot 6\text{H}_2\text{O}$, designated ICL-1, was isolated from an aqueous reaction mixture (containing Ga_2O_3 , H_3PO_4 , HF, and 1,4-diaminobutane)^[17] which if heated under hydrothermal conditions in a sealed autoclave at 180 °C yields the known small-pore 3D phase ULM-3.^[18] The same mixture was held at 50 °C for 20 days and powder X-ray diffraction showed that a novel phase had been produced. After 20 days at room temperature the same phase is produced, albeit with lower crystallinity. The sample prepared at 50 °C was selected for detailed characterization. Transmission electron microscopy revealed the presence of well-formed square-tile-shaped crystallites of maximum dimension 4 μm . Microanalysis was performed on the crystallites by measuring the fluorescence spectrum and converting the observed ratio of the intensities of the gallium L and phosphorus K emissions into relative atomic concentrations using a calibration constant calculated by similar study of the crystalline material GaPO_4 (quartz structure type). This revealed that the new phase contains Ga and P in the ratio 1:1.3(1), and that O, F, C, H, and N are also present.

Although a phase-pure sample of ICL-1 could be readily obtained, we found no means of producing crystals suitable for single-crystal X-ray diffraction (for example by seeding the reaction mixture with previously made crystallites, or by performing the reaction for extended periods), and so we turned to powder X-ray diffraction for structural characterization. The CRYSFIRE software^[19] was used to index the diffraction data and the unit cell with the highest figures of merit was found to be tetragonal with $a = 15.25$ and $c = 28.88$ Å. Evaluation of the systematic absences using the program CHECKCELL^[20] restricted the space-group to $I4_1/a$. A search of the literature revealed that three phosphates have similar cell parameters, and the same space group; one iron phosphate ($[\text{Fe}_4(\text{OH})_3(\text{HPO}_4)_2(\text{PO}_4)_3][\text{H}_3\text{N}(\text{CH}_2)_3\text{NH}_3]_2 \cdot x\text{H}_2\text{O}$),^[21] and one gallium phosphate ($[\text{Ga}_4(\text{OH})_3(\text{HPO}_4)_2(\text{PO}_4)_3][\text{H}_3\text{N}(\text{CH}_2)_3\text{NH}_3]_2 \cdot 5.4\text{H}_2\text{O}$) with a vanadium-substituted analogue ($[\text{Ga}_{3.6}\text{V}_{0.4}(\text{OH})_3(\text{HPO}_4)_2(\text{PO}_4)_3][\text{H}_3\text{N}(\text{CH}_2)_3\text{NH}_3]_2 \cdot 6\text{H}_2\text{O}$).^[22] None of these materials contains fluorine, but given the similarity in cell parameters the structures were used as a starting point to model the diffraction data from the new phase. Elemental microanalysis allowed the formula of the material to be deduced, and location of the fluorine in the material was aided by comparison to the large number of already known gallium fluorophosphates^[23] Figure 1 shows the final Rietveld fit to the data.

The structure of ICL-1 is constructed from gallium in six-coordinate $\{\text{GaO}_4(\text{OH})_2\}$ and $\{\text{GaO}_5(\text{OH})\text{F}\}$ polyhedra and $\{\text{PO}_4\}$ and $\{\text{HPO}_4\}$ tetrahedra. It is very similar to previously characterized iron and gallium phosphates, whose structures have already been discussed.^[21, 22] The constituent polyhedra are linked (edge- and corner-sharing gallium units and corner-sharing phosphorus units) to produce a three-dimensional,

[*] Prof. D. O'Hare, Dr. R. I. Walton, Dr. F. Millange
Inorganic Chemistry Laboratory
South Parks Road, Oxford, OX1 3QR (UK)
Fax: (+44) 1865-272690
E-mail: dermot.ohare@chem.ox.ac.uk

Dr. T. Loiseau, Prof. G. Férey
Institut Lavoisier, UMR CNRS 8637
Université de Versailles-St-Quentin en Yvelines
45, Avenue des Etats-Unis, 78035 Versailles Cedex (France)

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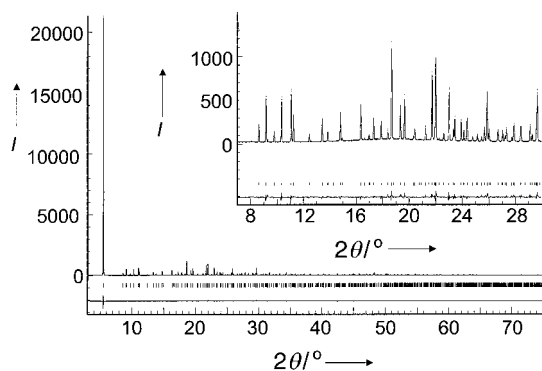


Figure 1. Observed (points) and calculated (line) powder X-ray data for the title compound ($\lambda = 1.3005 \text{ \AA}$). The lower line is the difference curve. The inset shows the detailed pattern between 2θ values of 8 and 30° . I is the X-ray intensity (arbitrary units).

open-framework structure. The most important feature of the structure is the presence of channels, running in a zigzag manner along the a and b axes (Figure 2). These channels are

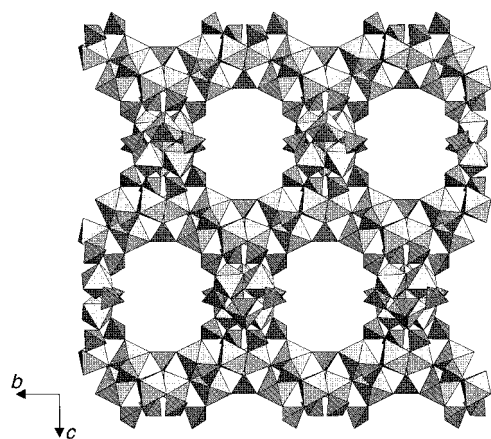


Figure 2. The structure of the title compound viewed along the a axis showing channels constructed from 20-ring windows. Dark gray tetrahedra are $[\text{PO}_4]$ units and pale gray polyhedra are $[\text{GaO}_5\text{F}]$ units. Occluded amine molecules and water molecules are not shown for clarity.

bounded by rings constructed from 20 $[\text{GaO}_4(\text{OH})_2]$, $[\text{GaO}_5(\text{OH})\text{F}]$, and $[\text{PO}_4]$ polyhedra. These rings are among the largest known for gallium and aluminum phosphates (only found elsewhere in the gallium fluorophosphate cloverite^[24] and the aluminum phosphate JDF-20^[25]). The overall negative charge of the gallium fluorophosphate framework is balanced by 1,4-butyldiammonium cations which reside in the voids, along with water molecules.

In order to exploit the potential for microporosity in ICL-1, the occluded species must be removed. Thermogravimetric analysis (TGA) experiments were performed to test whether the open-framework was maintained on heating. On heating to 200°C in a dry helium atmosphere the diffraction data showed that the largest d spacing (101) reflection, characteristic of the pore system, remains unchanged, although more subtle changes in the powder diffraction pattern at higher angle were apparent. Thermogravimetric analysis (TGA) shows that at 180°C the occluded water is totally removed. Figure 3 shows a simulation of the X-ray powder pattern of the structure excluding

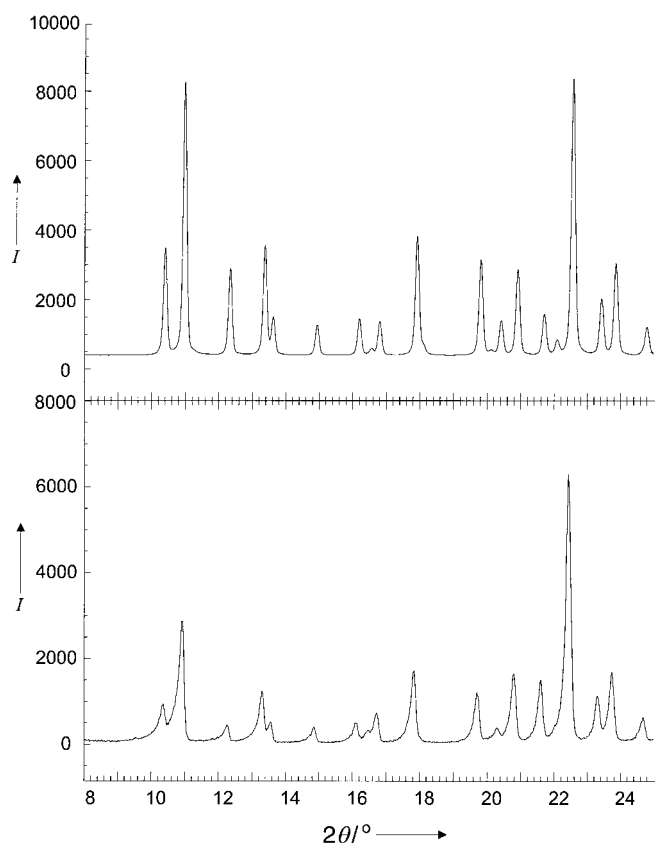


Figure 3. Simulated X-ray powder pattern ($\text{CuK}\alpha$) for the dehydrated form of the title compound (top) and diffraction data measured from the material at 180°C (bottom).

all water molecules compared with the data measured at 180°C . The excellent agreement between these two diffractograms (it should be noted that the relative intensities of the experimental data are subject to preferred orientation effects) shows that the open-framework is retained on dehydration; this is a defining characteristic of a zeolitic phase. Figure 4 shows a view of a single channel of the dehydrated material, which illustrates that although the charge-balancing amine remains in the structure it lines the pore wall, leaving considerable free space in the center of the channels. The dimensions of the pores (maximum $10 \text{ \AA}$ (N–N contact) $\times$ $7.8 \text{ \AA}$ (C–C contact)) of the inorganic–organic hybrid are similar to many commercially important zeolites, for example faujasite (zeolites X and Y) and mordenite.^[26] Nitrogen adsorption experiments on a sample degassed at 120°C under vacuum showed a Type I isotherm typical of a microporous material, and a BET surface area of $146 \text{ m}^2\text{g}^{-1}$ was determined. Further TGA experiments were performed to confirm the microporous character of the material; it was found that on heating to 180°C , and subsequent cooling to room temperature in air, the mass of the sample returned to about 75% of its original value after only 2 h. This shows the reversibility of the dehydration. Dehydration to leave a truly microporous material is very unusual in phosphate chemistry, although such behavior has recently been reported for a Ni^[27] and a Zn^[28] phosphate, both having channels constructed from 24-ring windows and both prepared under hydrothermal conditions.

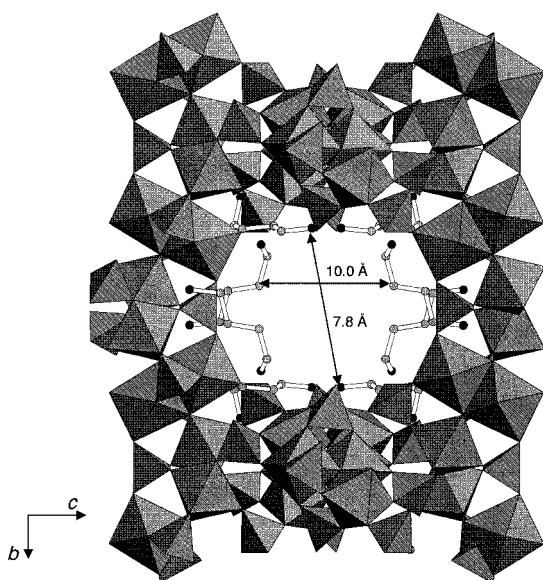


Figure 4. A view of one of the pores of the dehydrated phase, with selected interatomic distances between amine molecules marked to illustrate the void in the center of the channels. Black circles are nitrogen atoms and gray circles are carbon atoms.

All other aluminum or gallium phosphates with three-dimensional open-framework structures have been prepared by using hydrothermal conditions. The two previous studies of the low-temperature ($<50^{\circ}\text{C}$) crystallization of Group 13 phosphates have described the synthesis of materials with chain^[15] or layered^[29] structures. Interestingly, one silicate system described by Oberhagemann et al. exhibits similar behavior: at low temperature low-dimensional phases (1D and 2D) are produced, whereas under hydrothermal conditions the same reaction mixtures produce zeolitic (3D) phases.^[30] At first glance, the formation of metastable low-dimensional structures followed by transformation into structures of higher dimensionality might appear to be the basis for a general reaction mechanism for the formation of open-framework structures. Indeed, Ozin and co-workers postulated that three-dimensional aluminum phosphates might be formed via metastable chain and layered phases,^[6] and other groups since have assumed the same model.^[31, 32] However, there is little direct experimental evidence for this crystallization model and our current work shows that metastable three-dimensional phosphates may crystallize at close to room temperature.

A further model for crystallization of phosphates was recently postulated by Rao and co-workers who suggested that certain open-framework materials may form via amine phosphate intermediates and by using this hypothesis they synthesized a huge number of new materials.^[7] The same workers also showed that a monomeric zinc phosphate cluster would transform into an open-framework 3D material on heating at 50°C .^[33] We have found very different behavior for the system we have studied; we have never observed the formation of amine phosphates and we find that metal fluorophosphates encapsulating amines form equally as readily at very moderate temperatures. In terms of reaction mechanism for our particular system our work on the

formation of gallium fluorophosphates under mild conditions has shown that at low temperature materials containing solely six-coordinate Ga are produced, whereas it is well known that under hydrothermal conditions materials containing four-, five-, or six-coordinate Ga can be formed.^[5] This is consistent with recent *in situ* NMR studies on a closely related aluminum fluorophosphate system which showed a modification of metal coordination number in solution from six to five once hydrothermal conditions are reached.^[14] Perhaps an equally important result from our work is that new potentially zeolitic materials can be formed at low temperature, phases which are unstable under hydrothermal conditions. This offers new possibilities in the synthesis of new microporous phases.

Experimental Section

Powder X-ray diffraction data were recorded on Station 2.3 of the Daresbury synchrotron radiation source, UK, from a sample contained in a 0.7 mm diameter quartz capillary. Station 2.3 receives X-rays from the synchrotron source (which operates with an average energy of 2 GeV and a beam current of typically 200 mA) from a dipole insertion device. The incident X-ray wavelength was $1.3005\text{ \AA}$ using a Si (111) monochromator and data collection over the angular range 3 to $75^{\circ} 2\theta$, with a step 0.01° . Whole pattern fitting using the program FULLPROF^[34] led to $R_{\text{wp}} = 0.0868$, $\chi^2 = 5.54$ and $R_i = 0.0479$. Further details of the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, 76344 Eggenstein-Leopoldshafen, Germany (Fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de) on quoting the depository number CSD-411407. Variable-temperature powder X-ray diffraction measurements were performed by using a Siemens D5000 diffractometer ($\text{Cu}_{K\alpha}$ radiation) equipped with an Anton Paar furnace (HTK1200) under a flow of dry He. The diffractometer was used in reflection mode and data measured over the angular range $2\theta = 5$ – 25° in order to observe the most intense low-angle Bragg reflections. Temperature steps of 10°C were typically used from room temperature to 600°C , and the temperature was stabilized for 5 min before beginning data collection.

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- [23] Bulk fluorine analysis showed the new phase to contain 1.13% F by mass, suggesting the formula $\text{Ga}_4(\text{HPO}_4)_2(\text{PO}_4)_3(\text{OH})_2\text{F} \cdot [\text{NH}_3(\text{CH}_2)_4\text{NH}_3]_2 \cdot x\text{H}_2\text{O}$ by analogy with the previously characterized phases (expected 1.73%). Thermogravimetric analysis showed a distinct mass loss from 40 to 180°C of 10.5%; this suggests six water molecules per formula unit (expected mass loss of 9.8%). In all known gallium fluorophosphates, fluorine is located either in a position bridging two gallium atoms, or at the center of an octameric unit constructed from $[\text{GaO}_4]$ and $[\text{PO}_4]$ tetrahedra. In the previously studied $\text{Ga}_4(\text{HPO}_4)_2(\text{PO}_4)_3(\text{OH})_3 \cdot [\text{NH}_3(\text{CH}_2)_4\text{NH}_3]_2 \cdot \approx 5.4\text{H}_2\text{O}$ one of the hydroxide groups bridges two gallium atoms, and the others bridge three gallium atoms. Choosing the former hydroxide site as the most chemically sensible position for the fluorine atom in the new phase allowed an excellent fit to the diffraction data to be achieved.
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A One-Dimensional Metallocyclophane with Columnar Aromatic Stacking: The Silver(I) η^2 -Coordination Complex of 1,2-Benztriphenylene**

Megumu Munakata,* Gui Ling Ning, Yusaku Suenaga, Takayoshi Kuroda-Sowa, Masahiko Maekawa, and Tetsuhiro Ohta

Cation– π interactions between metal ions and polycyclic aromatic hydrocarbons (PAHs) provide a powerful tool for building novel molecular architectures and allow the introduction of a wide variety of useful electrical and electrochemical properties.^[1–5] With the aim of designing new solids with structural diversity and desirable physical and chemical properties, we synthesized a number of organosilver(I) complexes of PAHs with remarkable features, such as W-type sandwiches,^[2, 6] helical structures,^[7] and double-decker,^[5] triple-decker,^[7] and multidecker frameworks.^[5] In spite of these achievements, complexes with high conductivity are very rare. The dependence of the electrical conductivity of the complexes on the manner in which their aromatic rings are stacked has not been investigated. Here we present the unprecedented supramolecular architecture of the *para*-metallocyclophane polymer $[\text{Ag}_{0.5}(\text{btp})_{0.5}(\text{ClO}_4)_{0.5}]$ (**1**; btp = 1,2-benztriphenylene), which shows an interesting cyclophane structure,^[8] unusual aromatic interactions, and high electrical conductivity.

Cyclophanes form an important class of organic molecules. Since the first report on the synthesis of [2.2]paracyclophane (**2**), in which two benzene rings are held face to face by methylene bridges,^[8a,b] the attractively simple naming of **2** led to the development of the cyclophane nomenclature for bridged aromatic compounds.^[9] Complex **1** shows interesting structural similarities to cyclophanes such as **2**, [2.2](2.7)pyrenophane (**3**),^[10] and the like,^[8, 10–15] and hence can be regarded as a metallocyclophane, in which metal ions form the bridges. The silver(I) ions and the T-shaped planar btp ligand are assembled into a stacked structure with high electrical conductivity.

The reaction of btp with AgClO_4 in *p*-xylene leads to formation of **1**. The X-ray structural determination^[16] revealed a one-dimensional framework held together by cation– π interactions between Ag^+ and btp and perchlorate

[*] Prof. Dr. M. Munakata, Dr. Y. Suenaga, Dr. T. Kuroda-Sowa, Dr. M. Maekawa, Dr. T. Ohta
Department of Chemistry
Kinki University
Kowakae, Higashi-Osaka, Osaka 557 (Japan)
Fax: (+81)6-67232721
E-mail: munakata@chem.kindai.ac.jp
Prof. Dr. G. L. Ning
School of Chemical Engineering
Dalian University of Technology (China)
Fax: (+86)411-363-3080
E-mail: ninggl@dlut.edu.cn

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anions. The coordination sphere around the silver(I) ion is tetrahedral (taking the C=C group as one ligand) and is made up of two C=C π bonds and two oxygen atoms from two symmetry-related btp ligands and perchlorate anions, respectively (Figure 1). The Ag–O and Ag–C distances are almost

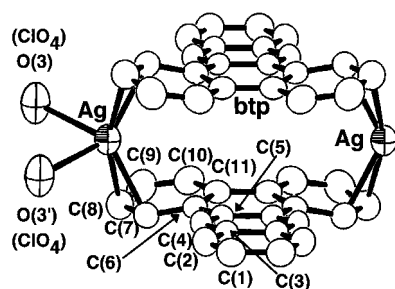


Figure 1. Structure of **1**.

equal, being 2.487(2) Å for O(3/3'), 2.423(3) Å for C(8), and 2.436(3) Å for C(7). Each btp moiety exhibits μ -di- η^2 coordination, and two such moieties are held face to face by two bridging silver(I) ions to generate a metallocyclophane motif, which is further linked to its adjacent counterpart by bridging O atoms of the perchlorate ions (Ag–O(3/3')–Ag) to form a polymeric chain structure. Between chains, aromatic π – π stacking occurs with an interplanar separation of 3.48–3.64 Å. The overall structure thus formed is a two-dimensional polymeric metallocyclophane framework involving columnar aromatic stacks (Figure 2a).

Although the cyclophane theory is well established,^[9, 17] most reported cyclophane species have C, O, S, or N atoms in the bridges. In the few reported metal-bridged aromatic compounds,^[18, 19] no polymeric framework was found, and the metal ions interact with aromatic rings in an η^6 fashion^[18] or form O–M–O linkages.^[19] In **1**, the btp molecules participate in di- η^2 bonding to the silver(I) ions to form a polymeric metallocyclophane structure which, to the best of our knowledge, is unprecedented.^[2, 5–7, 20] Interestingly, apart from structural similarities to organic cyclophanes, compound **1** also displays noticeable differences in its configuration. For organic cyclophanes **2** and **3**, the methylene-bridged aromatic moieties exhibit an out-of-plane deformation, and the intraplanar separations vary from 2.53 and 2.79 Å at the sides to 3.09 and 3.80 Å at the center, respectively (Figure 3a, b). This indicates strong intraplanar repulsion between the overlapping aromatic rings caused by the conjugation of the bridging carbon atoms with aromatic rings.^[6, 9] In contrast, the btp molecules in **1** are bent inward by 0.35 Å, and the intraplanar distances vary from side to center from 4.33 to 3.64 Å (Figures 2a and 3c). This indicates the existence of interplanar attraction. Such an attractive force may be attributable to the tetrahedral coordination environment of the silver(I) ions, which leads to Ag–btp bond angles and lengths suitable for the intraplanar aromatic π – π interaction. In addition, complex **1** also exhibits interchain aromatic π – π interactions with interchain distances of 3.48–3.64 Å in the columnar aromatic stacking along the *c* axis (Figure 2a). This is in sharp contrast to the crystal structures of **2** and **3**, in which no intermolecular

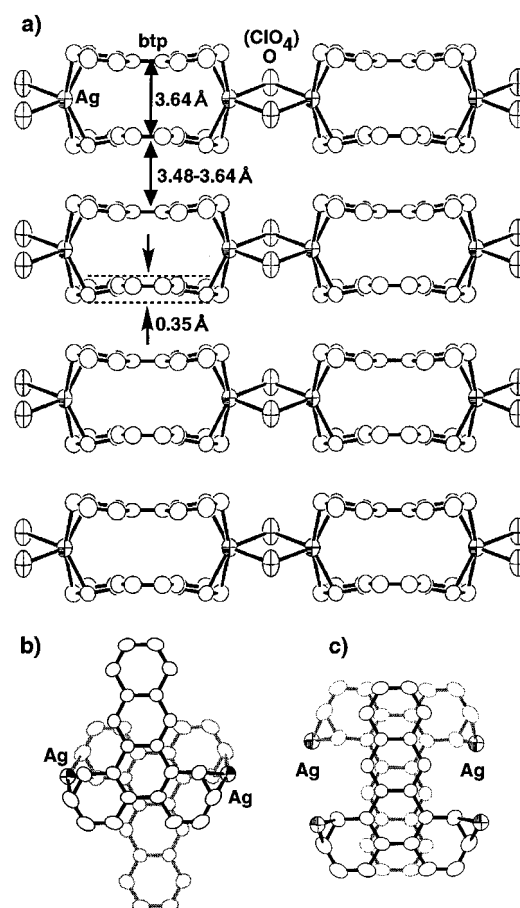


Figure 2. Columnar aromatic stacking in **1** with π – π interactions within and between polymeric chains (a) and intra- (b) and intermolecular overlap (c) of btp ligands (black and gray lines represent the upper and lower btp molecules, respectively).

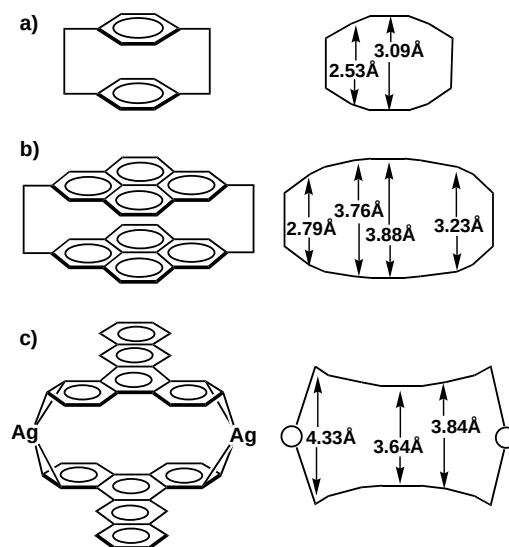


Figure 3. Deformations of the aromatic planes in [2.2]paracyclophane (a), [2.2](2.7)pyrenophane (b), and **1** (c).

contact shorter than the sum of the van der Waals radii was found.

The most remarkable feature is the recognition of silver(I) ions and the interesting assembly pattern of btp molecules to

form a unique supramolecular argentophane architecture, which gives rise to a high conductivity. The silver(I) ions in **1** are symmetrically coordinated to C(7/7') and C(8/8') on both sides of the head of the T-shaped btp ligand to form a stable cyclophane structure (Figures 1 and 3c). Such coupling brings about a head-to-head connection of the pair of btp ligands in each argentophane moiety (Figure 3c) and results in an alternating tail-up and tail-down arrangement of the aromatic rings along the columnar aromatic stacks. The complex thus assembled exhibits alternating overlap of intra- and interaromatic moieties (Figure 2b, c), which contribute to the strong aromatic $\pi-\pi$ interactions.

As a result of this a columnar aromatic stacking of the metallocyclophane framework, **1** shows unusual conductivity. The electrical conductivity of a compacted pellet, measured by a two-probe technique with silver-paste-coated probes, showed the highest reported conductivity of all organosilver(I) complexes of PAH ligands with $\sigma = 1.32 \text{ Scm}^{-1}$ at room temperature, although btp itself is nonconducting. We found that the conductivity of silver(I) π complexes of PAH ligands is strongly dependent on inter- and/or intramolecular aromatic stacking. Complexes with weak or no aromatic stacking, such as those with discrete monomeric, W-type sandwich, or helical structures,^[6,7] have very low conductivity ($\sigma = 0-1 \times 10^{-5} \text{ Scm}^{-1}$). In multilayer complexes with triple- or multi-decker frameworks^[5-7] and strong inter- and/or intramolecular aromatic $\pi-\pi$ interactions, higher σ values of 1.6×10^{-3} to $6.9 \times 10^{-2} \text{ Scm}^{-1}$ can be obtained, depending on the coordination or stacking mode. It is noteworthy that complex **1** exhibits ESR signals with a g value of about 2.002, attributable to the aromatic radicals^[21].

Further studies directed toward the synthesis of other metallocyclophanes to establish the relationship between the structure and the electrical properties are underway.

Experimental Section

$\text{AgClO}_4 \cdot \text{H}_2\text{O}$ (45 mg, 0.2 mmol) was added to a solution of btp (11.1 mg, 0.04 mmol) in *p*-xylene (4 mL). The solution was stirred for about 15 min and then transferred to a 7-mm glass tube and layered with *n*-hexane. The tube was sealed under Ar and wrapped in aluminum foil. After standing at room temperature for 1 d yellow prismatic crystals were obtained.

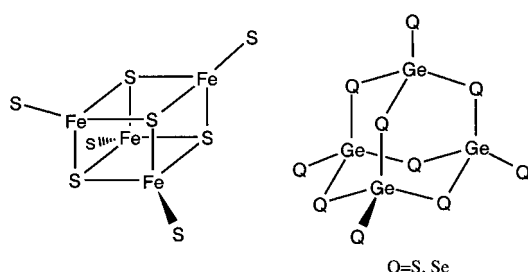
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Supramolecular Assembly of Hexagonal Mesosubstructured Germanium Sulfide and Selenide Nanocomposites Incorporating the Biologically Relevant Fe₄S₄ Cluster**

Pantelis N. Trikalitis, Thomas Bakas, Vasilios Papaefthymiou, and Mercouri G. Kanatzidis*

The design and construction of open-framework materials with mesoscale characteristics (20–200 Å) has been profoundly influenced by the use of organized surfactant assemblies as templating media.^[1] Whereas the overwhelming majority of systems are oxides, only a few reports exist that describe nonoxidic mesosubstructured nanocomposites^[2–4] which are now attracting great interest.^[5–7] Recently, we^[3, 8] and others^[4, 9] demonstrated that nonoxidic mesosubstructured networks form by the binding of divalent metal ions with tetrahedral [Ge₄Q₁₀]^{4–} (Q = S, Se) clusters in the presence of appropriate surfactants. These clusters (see Scheme 1) bind



Scheme 1.

metal ions in tetrahedral arrangements that can give rise to diamondoid frameworks. On the other hand the functional implications of solid-state materials with biological attributes are not yet explored, but they are many and far-reaching. In this context the introduction of metallo-bioinorganic active sites to nonbiological inorganic matrices has not been previously described. Fe/S clusters are exceptional and ubiquitous in biological systems as they provide a major part of the foundation for electron transfer, catalytic, and enzymatic processes in living organisms.^[10, 11] These clusters are some of the most studied entities in chemistry and biology. They can be readily synthesized as pure discrete species in high yield.^[12, 13] Many proteins such as ferredoxins and hydrogenases contain Fe/S clusters and exploit their redox properties. Arguably, the cubane geometry is the most pervasive structural arrangement in biological cluster chemistry, and consists of a core of four iron and four sulfur atoms

distributed over alternating vertices of a cube (see Scheme 1). Thus, it would be interesting to incorporate it as a functional unit into materials.

Here we report a rational approach, using the supramolecular assembly of adamantane [Ge₄Q₁₀]^{4–} (Q = S, Se) clusters with substitutionally labile [Fe₄S₄Cl₄]^{2–} clusters templated by cationic surfactant molecules to produce novel hexagonal mesophases built with the biologically relevant [Fe₄S₄] core (Fe4S4-MSU-1 for [Ge₄S₁₀]^{4–}) and (Fe4S4-MSU-2 for [Ge₄Se₁₀]^{4–}). The preferred coordination geometry of the [Fe₄S₄] core is tetrahedral and is well suited to form three-dimensional frameworks when combined with other linking anions which also prefer tetrahedral binding, for example the [Ge₄Q₁₀]^{4–} (Q = S, Se) clusters. These structures have parallel pore tunnels with separations of ~35 Å and could provide the architectural and conceptual framework for exploring and understanding a new class of materials at the interface of materials science and bioinorganic chemistry. The inclusion of well-studied protein active sites in extended solid-state frameworks represents an important step towards achieving useful and functional interfaces between biological and materials chemistry.

Supramolecular assembly of adamantane [Ge₄S₁₀]^{4–} and [Ge₄Se₁₀]^{4–} clusters, in a nonaqueous medium, with substitutionally labile [Fe₄S₄Cl₄]^{2–} clusters^[14, 15] in the presence of a cationic surfactant resulted in the formation of novel mesophases containing [Fe₄S₄]²⁺ cores. Metathesis reactions between [Ge₄S₁₀]^{4–} or [Ge₄Se₁₀]^{4–} and [Fe₄S₄Cl₄]^{2–} in the presence of cetylpyridinium (CP) bromide, in formamide/dimethylformamide (DMF) mixtures gave ordered hexagonal mesosubstructured CP-[Fe₄S₄]/(Ge₄S₁₀) and CP-[Fe₄S₄]/(Ge₄Se₁₀) materials. These materials, referred to as Fe4S4-MSU-1 and Fe4S4-MSU-2, respectively, are dark brown and have a reproducible and consistent composition. The samples were analyzed with energy-dispersive spectroscopy (EDS) in a scanning electron microscope and showed only Fe, S, Ge, and Se (Figure 1a). Combined with C, H, N analyses we obtain the empirical formulae (CP)₃Fe₄S₄Ge₄S_{10+δ} for Fe4S4-MSU-1 and (CP)_{3.4}Fe₄S₄Ge₄Se_{10+δ} for Fe4S4-MSU-2.^[16]

The mesosubstructured nature of Fe4S4-MSU-1 and Fe4S4-MSU-2 is evident in the powder X-ray diffraction patterns that show strong diffraction peaks at very low scattering angles (Figure 1b). These are analogous to those of the hexagonal mesoporous silica phases.^[17] We index the three reflections for Fe4S4-MSU-1 at 34.2, 19.5, and 17.3 Å as (100), (110), and (200), respectively, with a hexagonal lattice parameter of *a*_H = 39.5 Å. The corresponding values for Fe4S4-MSU-2 are 34.9, 20.6, and 18.5 Å with *a*_H = 40.3 Å. As with the conventional mesosubstructured oxides,^[18] we do not observe Bragg diffraction peaks at wide angles, suggesting that the inorganic walls of Fe4S4-MSU-1 and Fe4S4-MSU-2 lack long-range order. Nevertheless, these materials exhibit well-defined diffuse scattering at 2θ > 10° consistent with the presence of short-range local order in the wall structure.

Thermal gravimetric analyses (TGA) of Fe4S4-MSU-X materials show that they lose all their surfactant molecules between 200 and 500 °C (Figure 1c). The first weight loss step occurs at ~250 °C which corresponds to the removal of the pyridine head group (identified by mass spectrometry). This is

[*] Prof. M. G. Kanatzidis, Dr. P. N. Trikalitis
Department of Chemistry, Michigan State University
East Lansing, MI 48824-1322 (USA)
Fax: (+1) 517-353-1793
E-mail: kanatzid@cem.msu.edu
Dr. T. Bakas, Prof. V. Papaefthymiou
Department of Physics
University of Ioannina (Greece)

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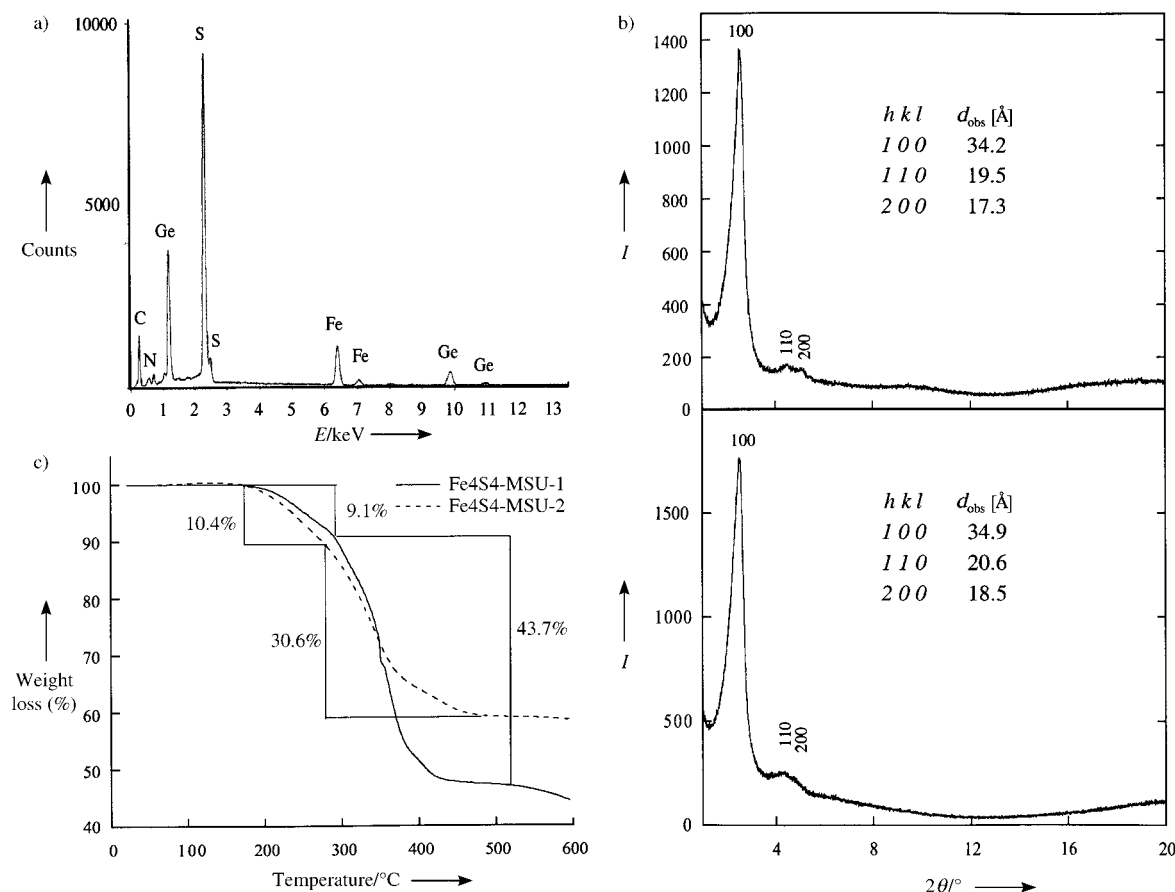


Figure 1. a) Typical energy-dispersive X-ray fluorescence spectrum showing the presence of C, N, Fe, Ge, and S elements in Fe4S4-MSU-1. The absence of elements associated with the counterions of the starting reagents (e. g. Br, Cl, and P of Ph_4P^+) is evident. b) X-ray scattering ($\text{CuK}\alpha$ radiation) from Fe4S4-MSU-1 (top) and Fe4S4-MSU-2 (bottom). Peaks are indexed to a hexagonal cell. I = intensity. c) Corresponding thermal gravimetric analysis (TGA) data. Experiments were performed under nitrogen flow with a heating rate of 10 deg min^{-1} .

followed by a second step from 250 to 500°C , which is associated with the loss of the long hydrocarbon chain. The weight loss values are in good agreement with the elemental C, H, N analyses. At 500°C the materials decompose to amorphous GeS_2 and FeS_x .

High-resolution transmission electron micrographs (HRTEM) of Fe4S4-MSU-1 and Fe4S4-MSU-2 reveal a strikingly regular hexagonal organization of the inorganic framework pores (Figure 2), which resemble those obtained for hexagonal mesoporous oxides (MCM-41)^[17] and cetyltrimethylammonium (CTA)/ $\text{M}_2\text{Ge}_4\text{S}_{10}$.^[9] Overall, the samples appear homogeneous. The dimensions of the channels and the presence of hexagonal order are consistent with the X-ray diffraction results. The width of the channels in Figure 2 is roughly $24\text{--}26 \text{ \AA}$. The hexagonal domains are very large ($>400 \text{ nm}$) in both Fe4S4-MSU-1/2 and often span the length of entire particles.

The existence of the $[\text{Fe}_4\text{S}_4]$ core in these mesostructures was confirmed with Mössbauer spectroscopy. The Mössbauer spectrum of Fe4S4-MSU-1 at 85 K (Figure 3a) shows two closely spaced quadrupole doublets (equal

areas) with an isomer shift δ of 0.47 and 0.45 mm s^{-1} and a quadrupole splitting ΔE_q of 1.14 and 0.84 mm s^{-1} , respectively. The parameters for Fe4S4-MSU-2 are $\delta = 0.50/0.47 \text{ mm s}^{-1}$ and $\Delta E_q = 0.66/1.14 \text{ mm s}^{-1}$, respectively. These are typical values for $[\text{Fe}_4\text{S}_4]^{2+}$ cores in asymmetric environments both in ferredoxins^[19, 20] and synthetic model compounds.^[21, 22]

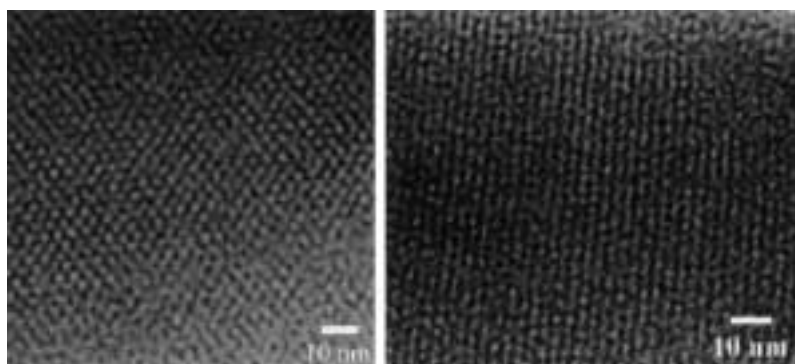


Figure 2. TEM images of Fe4S4-MSU-1; view down the tunnels (left; $[001]$ direction) and view perpendicular to the tunnel axis (right; $[100]$ direction), showing the parallel organization and straight character of the tunnels. TEM samples were prepared by suspending the precipitate in diethyl ether, then casting on a holey carbon grid. High-resolution transmission electron micrographs were acquired with a JEOL 120CX instrument equipped with a CeB_6 filament.

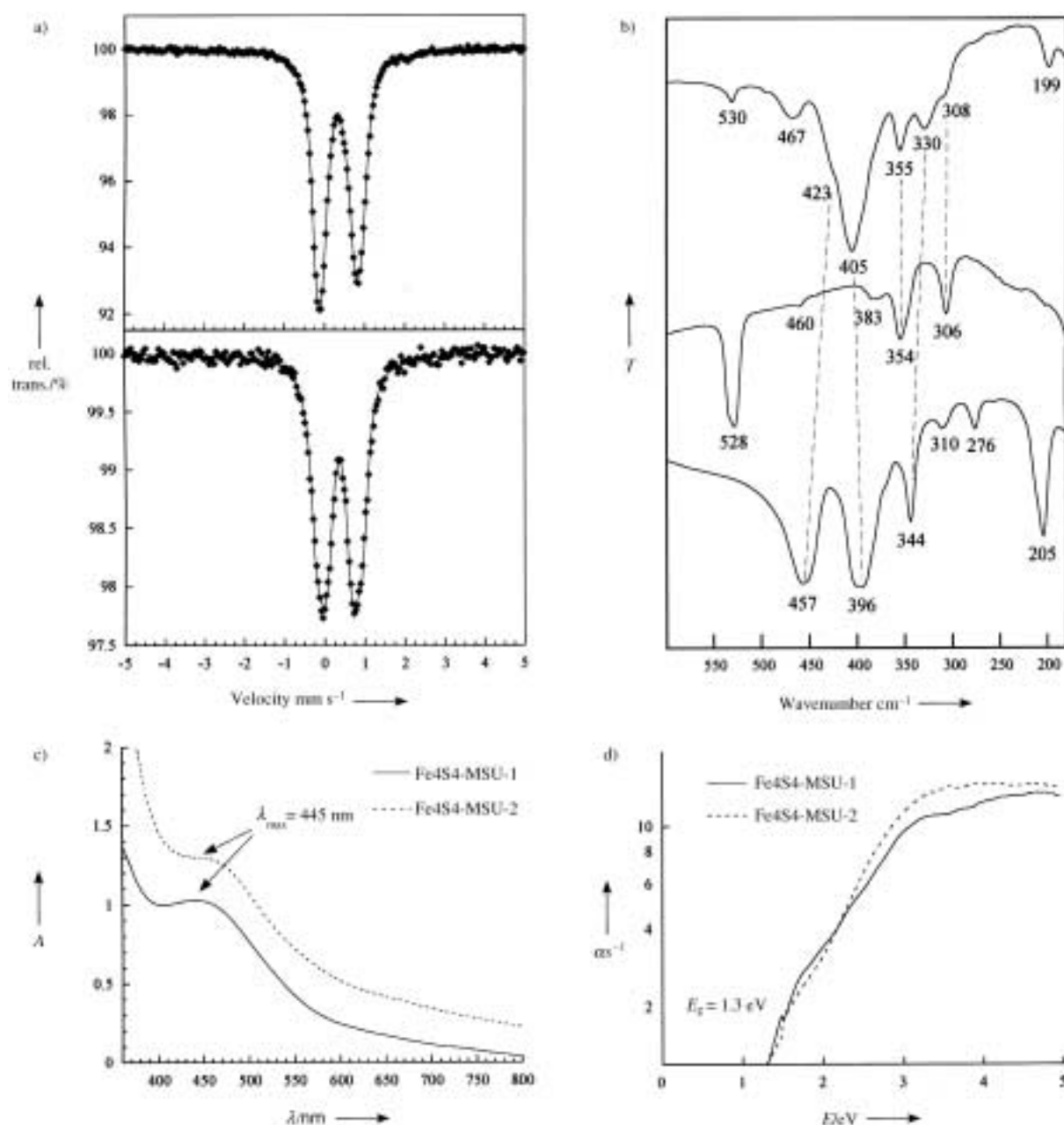


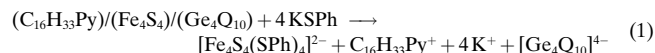
Figure 3. a) ^{57}Fe Mössbauer spectra of Fe4S4-MSU-1 (top) and Fe4S4-MSU-2 (bottom) at 85 K. The solid lines are least-squares fits for the δ and ΔE_{q} parameters quoted in the text. rel. trans. = relative transmission. b) Infrared spectra of Fe4S4-MSU-1 (top), $(\text{Ph}_4\text{P})_2\text{Fe}_4\text{S}_4\text{Cl}_4$ (center); the strong peak at 528 cm^{-1} is from the Ph_4P^+ cation, and $(\text{Me}_4\text{N})_4\text{Ge}_4\text{S}_{10}$ (bottom). The spectra were taken as pressed CsI pellets. T = transmittance. c) UV/Vis spectrum in DMF of the $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ cluster extracted from Fe4S4-MSU-1 and Fe4S4-MSU-2 upon reaction with excess PhS^- or PhSH showing the characteristic λ_{max} at $\sim 445\text{ nm}$ (13, 20). A = absorbance. d) Typical electronic absorption spectra of Fe4S4-MSU-1 and Fe4S4-MSU-2. E = energy.

The far-IR spectrum of Fe4S4-MSU-1 shows characteristic bands at 467 (m), 423 (m,sh), 405 (vs), 355 (s), 330 (m), 308 (m), and 199(m) cm^{-1} , respectively (Figure 3b). Those at 467, 423, 405, 330, and 199 cm^{-1} correspond to the modes of the adamantane $[\text{Ge}_4\text{S}_{10}]^{4-}$ cluster, whereas those at 355 and 308 cm^{-1} are due to the internal modes of the Fe_4S_4 cluster. The terminal Ge–S stretching modes in the free $[\text{Ge}_4\text{S}_{10}]^{4-}$ occurring at 457 and 344 cm^{-1} (Figure 3b top) shift to lower frequencies at 423 and 330 cm^{-1} in Fe4S4-MSU-1 due to Fe ligation. The strong peak at 396 cm^{-1} in the free $[\text{Ge}_4\text{S}_{10}]^{4-}$ is due to modes internal to the Ge_4S_6 core and is not significantly affected; it occurs at 405 cm^{-1} in Fe4S4-MSU-1. Fe4S4-MSU-

2 shows bands at 386 (m), 352 (vs), 321 (m, sh), 307 (vs), 284 (s), 255 (w), 227 (w), and 202 (w) cm^{-1} , respectively. The bands at 321, 284, originate from the $[\text{Ge}_4\text{Se}_{10}]^{4-}$ cluster,^[23] whereas those at 352 and 307 cm^{-1} are due to Fe_4S_4 cluster.

The presence of Fe_4S_4 clusters in these materials was independently determined by the excision method used to identify them in proteins.^[24, 25] This involves cluster extraction from the protein by ligand exchange with excess thiol (e.g. PhSH). When Fe4S4-MSU-1 and Fe4S4-MSU-2 were treated with excess PhSH (or PhS^- ; 4–8 equiv) in DMF they dissolved promptly, indicating ligation and subsequent excision of the $[\text{Fe}_4\text{S}_4]^{2+}$ core from the framework according to

Equation (1). The brown solution obtained displayed a UV/Vis spectrum with an absorption maximum at 445 nm (Figure 3c). This is characteristic of the presence of the molecular $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ cluster.^[15, 26] The latter species was also identified by its NMR spectrum from the characteristic *ortho*-, *meta*-, and *para*-H resonances of the SPh ligands which occur at $\delta = 5.84$, 8.25, and 5.15, respectively.^[27]



The optical absorption properties of these materials suggest that they are medium band-gap semiconductors. The electronic spectra show clearly defined energy gaps at 1.3 eV for both Fe4S4-MSU-1 and Fe4S4-MSU-2 (see Figure 3d). These gaps are in the same range as those of many important semiconductors such as GaAs (1.54 eV) and CdTe (1.45 eV).

The construction of mesostructured chalcogenide phases that embody the biological Fe_4S_4 center has been demonstrated by using a templated assembly process. Based on the above results we surmise that the inorganic frameworks (Figure 4) of Fe4S4-MSU-1 and Fe4S4-MSU-2 resemble those

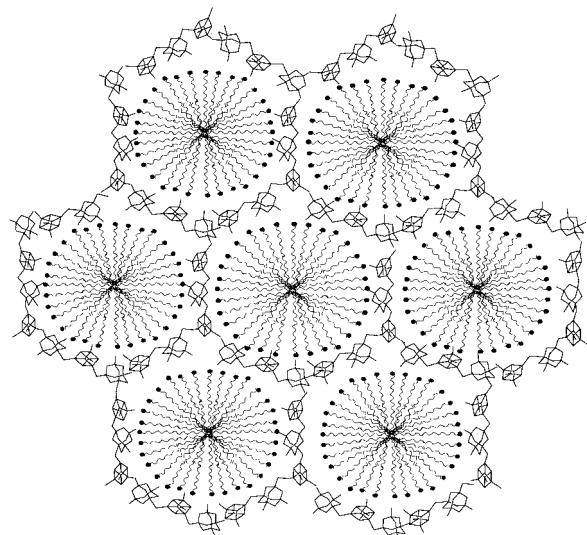


Figure 4. Schematic representation of the constituents of the hexagonal framework in which Fe_4S_4 cores are linked with $[\text{Ge}_4\text{Q}_{10}]^{4-}$ clusters around the surfactant counterions.

of MCM-41 and $\text{CTA}/\text{M}_2\text{Ge}_4\text{S}_{10}$. The approach described here clearly points to a pathway for generating stable hybrid systems with combined biological and abiological characteristics. Such hybrid structures may find applications in shape-selective electro- and photocatalysis, artificial photosynthesis, and biosensors, particularly those based on enzymatic processes related to Fe_4S_4 centers.^[28] As part of a rigid framework, Fe_4S_4 clusters are expected to be stable in more reactive oxidation states, for example $[\text{Fe}_4\text{S}_4]^{1+/3+}$. These states are readily found in proteins but are difficult to stabilize in molecules in solution. The combination of elegant, naturally refined catalytic sites with the robustness and diversity of extended solid-state systems could engender a new class of biologically inspired materials with unique or multiple

functionality. Particularly intriguing, for example, would be materials incorporating the MoFe_7S_9 -cofactor^[29] of nitrogenase, an important nitrogen-fixing enzyme, that may be rendered capable of similar activity.

Experimental Section

Methods: ^{57}Fe Mössbauer spectra were obtained at 85 K. A constant acceleration spectrometer was used to move a ^{57}Co (Rh) source kept at 300 K. The spectrometer was calibrated with α -Fe, and isomer shift values are given relative to α -Fe at 300 K. Optical absorption spectra were obtained at ~ 300 K with a Shimadzu UV-3101PC double beam, double monochromator spectrophotometer equipped with an integrating sphere. For details see T. J. McCarthy, S.-P. Ngeyi, J.-H. Liao, D. DeGroot, T. Hogan, C. R. Kannewurf, M. G. Kanatzidis, *Chem. Mater.* **1993**, *5*, 331–340.

Typical synthetic procedure: Fe4S4-MSU-1: Cetylpyridinium bromide monohydrate (4.00 g, 10 mmol) and $\text{K}_4\text{Ge}_4\text{S}_{10}$ (0.767 g, 1 mmol) were dissolved in formamide (20 mL) at 75 °C to form a colorless solution. To this $(\text{Ph}_4\text{P})_2\text{Fe}_4\text{S}_4\text{Cl}_4$ (0.883 g, 1 mmol) dissolved in DMF (10 mL) was added dropwise under stirring over a period of 2 min. The deep brown precipitate formed immediately and was aged for 24 h. It was then filtered, washed with warm water, and dried under vacuum. Approximately 1.5 g of product was obtained (yield > 80% based on $\text{K}_4\text{Ge}_4\text{S}_{10}$). The procedure is similar in the case of Fe4S4-MSU.

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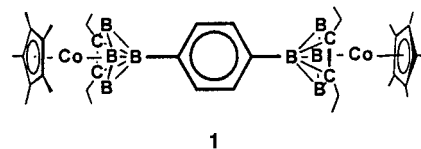
Benzene-Centered Tri- and Tetrametallacarborane Sandwich Complexes**

Martin Bluhm, Hans Pritzkow, Walter Siebert,* and Russell N. Grimes*

The special steric and electronic properties of polyhedral borane and carborane clusters—notably their three-dimensional geometry and electron delocalization (aromatic character)—are being employed to advantage in the construction of novel molecular architectures for use in a variety of projected applications in materials synthesis, microelectronics, optics, and medicine.^[1] In recent work some remarkable compound types have been produced in which boron clusters serve as scaffolds or templates for attachment of organic moieties; examples include $(1,7-C_2B_{10}H_{10})_nHg_n$ “anti-crowns” ($n = 3, 4$),^[1d, 2] peralkylated carboranes that mimic spherical hydrocarbons,^[3] and benzene-*m*-carboranyl macrocycles.^[4]

This paradigm can be reversed: Hydrocarbons may serve as frameworks for attachment of multiple boron clusters. If an aromatic system such as benzene is employed as a linchpin connecting several metallaboron units, one might prepare polynuclear, electron-delocalized, mixed-valence systems that are not only interesting from a fundamental perspective, but may also have practical potential as precursors or models for new kinds of electronically tailorable organometallic com-

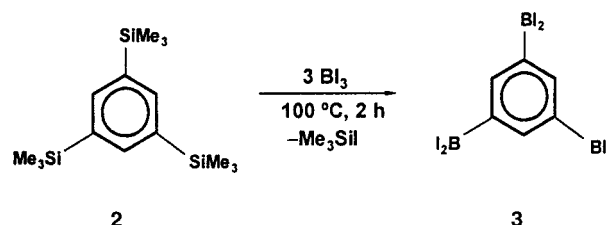
pounds. Although 1,3,5-tris(carboranyl)benzene derivatives incorporating icosahedral 1,2- or 1,12- C_2B_{10} polyhedra have been reported,^[5] no corresponding metallaborane or metallacarborane species (i.e., having metal atoms in the polyhedral framework) have been described; to our knowledge, the closest known metal system is the dimetallic 1,4-bis-(7-cobaltacarboranyl)benzene complex **1** ($B = BH, B$), which



1

was recently prepared in one of our laboratories.^[6] In contrast, a number of benzene-centered trinuclear organometallic compounds of the type $1,3,5-C_6H_3(XML_n)_3$ have been synthesized,^[7] in which transition metals *M* are linked to benzene through groups *X* (e.g. alkynyl). Here we report the designed synthesis of the first metallacarborane systems of this class.

The approach utilized boron-recapping (“recapitation”) of *nido*-[1,2,3- $Cp^*Co(2,3-Et_2C_2B_3H_3)$]²⁻ ($Cp^* = C_5Me_5$), a method recently developed in our groups for preparing mono- or dimetallic species bearing substituents at the apex [B(7)] boron atom.^[6, 8] In the present work, we required a suitable trifunctional benzene derivative as a precursor to the desired metal complexes. Accordingly, 1,3,5-tris(diiodoboryl)benzene (**3**), previously prepared but not isolated,^[9] was generated from the corresponding tris(trimethylsilyl)benzene as shown in Scheme 1 and isolated in 30% yield as a colorless, air-



Scheme 1. Synthesis of **3**.

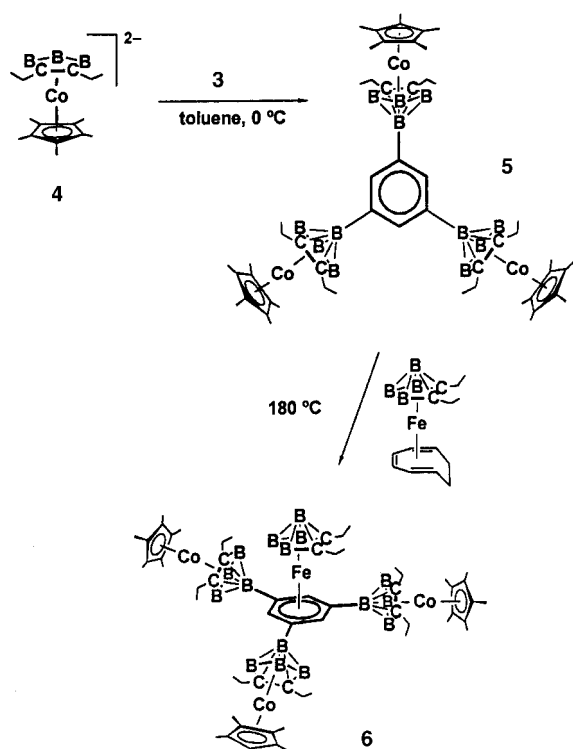
sensitive solid. Treatment of $Li_2[Cp^*Co(2,3-Et_2C_2B_3H_3)]$ (**4**) with one-third molar equivalent of **3** in toluene at 0 °C gave the target compound $[Cp^*Co(2,3-Et_2C_2B_4H_3-7)]_3C_6H_3$ (**5**) as a moderately air-stable yellow complex in 46% yield (Scheme 2).

Characterization of **5** by multinuclear NMR and IR spectroscopy as well as mass spectrometry supports the trigonally symmetric geometry shown (the rotamer depicted is arbitrary), and the solid-state structure has been confirmed by X-ray crystallography (Figure 1).^[10] With the exception of the benzene ring (see the figure caption), the bond lengths and angles are normal, and the metal atoms and apical boron atoms are coplanar with the benzene ring. A notable feature of the ¹H NMR spectrum of **5** is the strong upfield shift of the aromatic benzene resonances ($\delta = 5.92$ in $CDCl_3$ versus 7.51 for benzene itself), reflecting the electron-donating character of the carborane ligand (for comparison, the corresponding

[*] Prof. Dr. R. N. Grimes, Dr. M. Bluhm
 Department of Chemistry
 University of Virginia
 Charlottesville, VA 22901 (USA)
 Fax: (+1) 804-924-3710
 E-mail: rng@virginia.edu

Prof. Dr. W. Siebert, Dr. H. Pritzkow
 Anorganisch-chemisches Institut der Universität
 Im Neuenheimer Feld 276
 69120 Heidelberg (Germany)
 Fax: (+49) 6221-54-5609
 E-mail: ci5@ix.urz.uni-heidelberg.de

[**] Organotransition-Metal Metallacarboranes, part 56. This work was supported in part by the National Science Foundation (grant CHE 9980708 to R.N.G.) and the Deutsche Forschungsgemeinschaft (SFB 247, to W.S. for M.B.). Part 55: T. Dodge, M. A. Curtis, J. M. Russell, M. Sabat, M. G. Finn, R. N. Grimes, *J. Am. Chem. Soc.* **2000**, 122, 10573.



Scheme 2. Synthesis of **5** and **6**. B = BH, B.

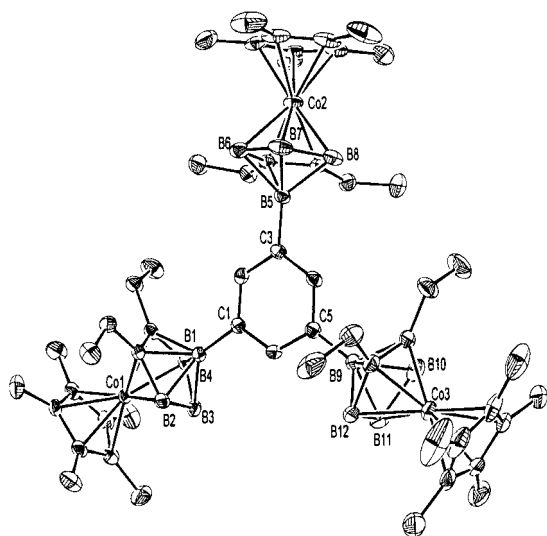


Figure 1. Molecular structure of **5**. The mean benzene ring C–C distance is 1.400 Å (range 1.399–1.402 Å). The mean benzene C–C–C angle centered on C1, C3, and C5 is 117.1°; that centered on C2, C4, and C6 is 122.9°.

signal in **1** appears at $\delta = 6.38$, while those in the monocobalt species $[\text{Cp}^*\text{Co}(2,3\text{-Et}_2\text{C}_2\text{B}_4\text{H}_3\text{-7-C}_6\text{H}_5)]$ are found at $\delta = 6.75\text{--}7.08$.^[6]

A fourth metallocarborane unit was added to **5** by thermal displacement of cyclooctatriene from $[(\eta^6\text{-C}_8\text{H}_{10})\text{Fe}(\text{Et}_2\text{C}_2\text{B}_4\text{H}_4)]^{[11]}$ to give the heterotetranuclear complex $[\text{Cp}^*\text{Co}(\text{Et}_2\text{C}_2\text{B}_4\text{H}_3\text{-7})]_3[\text{Fe}(\text{Et}_2\text{C}_2\text{B}_4\text{H}_4)]\text{C}_6\text{H}_5$ (**6**, Scheme 2). This species was isolated as a yellow solid in 30% yield and characterized by spectroscopy and X-ray crystallography (Figure 2).^[10]

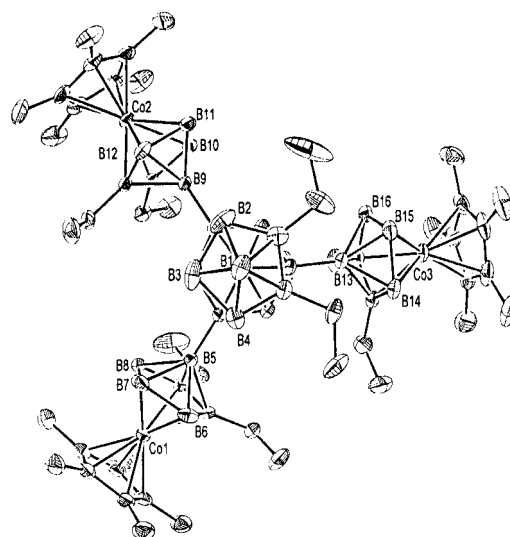
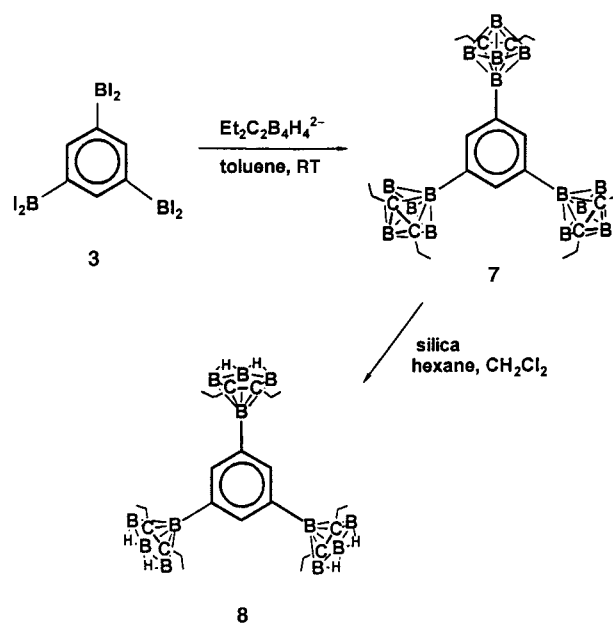


Figure 2. Molecular structure of **6**. The mean benzene ring C–C distance is 1.422 Å (range 1.421–1.425 Å). The mean benzene C–C–C angle centered on C1, C3, and C5 is 117.4°; that centered on C2, C4, and C6 is 122.6° (the numbering of the benzene ring is analogous to that in **5**, Figure 1).

Tris(diiodoboryl)benzene (**3**) can also serve as a precursor to other trigonally substituted complexes. Scheme 3 shows the preparation of the tris(*nido*-carboranyl)benzene species **8**, a potential building block for metal-connected trigonal sheet



Scheme 3. Synthesis of **8**. B = BH, B.

polymers. The structure of **8** is assigned from NMR and mass spectra. Full details on this work, including electrochemical and other data bearing on the electronic properties of the polymetallocarborane complexes, will be presented in a later publication.

Experimental Section

3: BI_3 (3.000 g, 7.66 mmol) and $(\text{Me}_3\text{Si})_3\text{C}_6\text{H}_3$ ^[12] (0.752 g, 2.55 mmol) were heated at 100 °C for 1.5 h, following which the Me_3SiI was removed by

vacuum distillation. The solid residue was taken up in pentane (30 mL), and the suspension was cooled to -78°C and filtered under N_2 to obtain colorless, air-sensitive **3** (0.670 g, 0.77 mmol, 30%). ^1H NMR (300 MHz, C_6D_6): $\delta = 9.27$ (s, 3 H_{ar}); ^{13}C NMR (75.5 MHz, C_6D_6): $\delta = 152.9$ (C_6H_3), 142.6 (*ipso*- C_6H_3); ^{11}B NMR (115.8 MHz, CDCl_3): $\delta = 47.7$ (s); UV/Vis (CH_2Cl_2): λ (rel. intens.) = 229 nm (100); $\epsilon = 7014 \text{ cm}^{-1}\text{M}^{-1}$.

5: $[\text{Cp}^*\text{Co}(\text{Et}_2\text{C}_2\text{B}_3\text{H}_5)]$ (200 mg, 0.64 mmol) in toluene (30 mL) at 0°C was treated with 1.6 M *n*-butyllithium (0.8 mL, 1.27 mmol). The solution turned red-orange during warming to room temperature (RT) over a 6 h period. It was cooled to 0°C , and **3** (185 mg, 0.21 mmol) was slowly added under nitrogen, causing the solution to become cloudy and turn orange-brown as the flask was warmed to RT. The mixture was stirred for 16 h, after which the toluene was removed in vacuo. The residue was taken up in hexane and washed through silica (3 cm), first with hexane and then with CH_2Cl_2 ; the hexane wash contained only $[\text{Cp}^*\text{Co}(\text{Et}_2\text{C}_2\text{B}_3\text{H}_5)]$. Column chromatography of the CH_2Cl_2 wash on silica with hexane/ CH_2Cl_2 (1/4) afforded **5** as a major yellow band (100 mg, 0.10 mmol, 46%). ^1H NMR (300 MHz, CDCl_3): $\delta = 5.92$ (s, 3 H_{ar}), 2.18 (m, ethyl CH_2), 1.77 (C_5Me_5), 1.27 (t, ethyl CH_3); ^{13}C NMR (75.5 MHz, in C_6D_6): $\delta = 134.8$ (C_6H_3), 94.3 (*ipso*- C_6H_3), 90.4 (C_5Me_5), 22.1 (CH_2), 15.1 (ethyl CH_3), 10.1 (C_5Me_5); ^{11}B NMR (115.8 MHz, C_6D_6): $\delta = 5.0$, 15.0 (B–H coupling not resolved); $\text{CI}^+\text{-MS}$: m/z (%): 1044.7 ($[\text{M}^+]$, 100), 721.8 ($[\text{M}^+ - \text{Cp}^*\text{Co}(\text{Et}_2\text{C}_2\text{B}_4\text{H}_5)]$, 0.9); UV/Vis (CH_2Cl_2): λ (rel. intens.) = 229 (41), 304 nm (100); $\epsilon = 11435 \text{ cm}^{-1}\text{M}^{-1}$.

6: Compound **5** (20 mg, 0.020 mmol) and $[(\eta^6\text{-C}_6\text{H}_{10})\text{Fe}(\text{Et}_2\text{C}_2\text{B}_4\text{H}_4)]$ (56 mg, 0.19 mmol) were heated in a Pyrex tube at 185°C for 20 min, after which the black solid was taken up in CH_2Cl_2 and washed through silica (3 cm), first with CH_2Cl_2 and then with ethyl acetate; the CH_2Cl_2 wash contained only unchanged starting materials. The ethyl acetate wash was purified by chromatography on a TLC plate with hexane/ethyl acetate (3/1), affording a major yellow band that was characterized as **6** (7 mg, 30%). ^1H NMR (300 MHz, CDCl_3): $\delta = 3.75$ (s, 3 H_{ar}), 2.44 (m, ethyl CH_2), 2.22 (m, ethyl CH_2), 2.07 (m, ethyl CH_2), 1.78 (C_5Me_5), 1.19 (t, ethyl CH_3), 1.07 (t, ethyl CH_3); ^{13}C NMR (75.5 MHz, C_6D_6): $\delta = 93.8$ (*ipso*- C_6H_3), 90.6 (C_5Me_5), 87.6 (C_6H_3), 25.5 (CH_2), 22.4 (CH_2), 15.7 (ethyl CH_3), 15.3 (ethyl CH_3), 9.9 (C_5Me_5); ^{11}B NMR (115.8 MHz, C_6D_6): $\delta = 3.8$ (B–H coupling not resolved); $\text{CI}^+\text{-MS}$: m/z (%): 1229.9 ($[\text{M}^+]$, 100), 1044.6 ($[\text{M}^+ - \text{Fe}(\text{Et}_2\text{C}_2\text{B}_4\text{H}_4)]$, 7); UV/Vis (CH_2Cl_2): λ (rel. intens.) = 230 (57), 296 nm (100); $\epsilon = 91138 \text{ cm}^{-1}\text{M}^{-1}$.

8: $2,3\text{-Et}_2\text{C}_2\text{B}_4\text{H}_6$ (118 mg, 0.9 mmol) in absolute diethyl ether (30 mL) at -78°C was treated with 1.6 M *n*-butyllithium (1.1 mL, 1.8 mmol). The solution was stirred for 4 h at RT. The Et_2O was removed, the residue taken up in absolute toluene (30 mL), and the mixture cooled to -14°C . Compound **3** (260 mg, 0.3 mmol) was slowly added under nitrogen while warming up to RT. The resulting solution became cloudy. The mixture was stirred for 16 h, after which the toluene was removed in vacuo. The residue was taken up in hexane and washed through silica (3 cm), first with hexane and then with CH_2Cl_2 . The complete wash was purified by chromatography on a TLC plate with CH_2Cl_2 /hexane (1/4), affording a colorless band that was characterized as **8** in low yield. ^1H NMR (300 MHz, CDCl_3): $\delta = 6.51$ (s, 3 H_{ar}), ethyl proton resonances from **8** and unchanged $\text{Et}_2\text{C}_2\text{B}_4\text{H}_6$; $\text{CI}^+\text{-MS}$: m/z (%): 468.4 ($[\text{M}^+]$, 100)

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- [10] Crystal structure determinations: Data were collected on a Bruker AXS SMART 1000 diffractometer (MoK_α radiation, $\lambda = 0.71073 \text{ \AA}$, ω scans), at 173 K, up to $\theta_{\text{max}} = 26.4^{\circ}$. Structures were solved with direct methods and refined by least-squares against F^2 (SHELXTL V5.10); non-hydrogen atoms were anisotropic, and hydrogen atoms were located and refined isotropically. **5** ($\text{C}_{61}\text{H}_{95}\text{B}_{12}\text{Co}_3$, crystal contains one toluene molecule per complex): $M_r = 1134.9$, orthorhombic, space group $Pbca$, $a = 14.6958(3)$, $b = 24.7128(5)$, $c = 34.4880(8) \text{ \AA}$, $V = 12525.2(5) \text{ \AA}^3$, $Z = 8$, $\rho_{\text{calcd}} = 1.204 \text{ g cm}^{-3}$. Of 81401 measured reflections, 12821 were independent ($R_{\text{int}} = 0.071$), 1033 parameters, $R1 = 0.041$ (for reflections with $I > 2\sigma(I)$), $wR2 = 0.111$ (for all reflections), max./min. residual electron density $0.72/-0.51 \text{ e \AA}^{-3}$. **6** ($\text{C}_{63}\text{H}_{104}\text{B}_{16}\text{FeCo}_3$, crystal contains 0.5 benzene molecules per complex): $M_r = 1267.1$, triclinic, space group $P\bar{1}$, $a = 11.1196(3)$, $b = 13.9542(4)$, $c = 23.2691(6) \text{ \AA}$, $\alpha = 85.959(2)^{\circ}$, $\beta = 79.768(2)^{\circ}$, $\gamma = 77.633(2)^{\circ}$, $V = 3468.7(2) \text{ \AA}^3$, $Z = 2$, $\rho_{\text{calcd}} = 1.213 \text{ g cm}^{-3}$. Of 46579 measured reflections, 16911 were independent ($R_{\text{int}} = 0.037$), 1153 parameters, $R1 = 0.036$ (for reflections with $I > 2\sigma(I)$), $wR2 = 0.096$ (for all reflections), max./min. residual electron density $0.61/-0.38 \text{ e \AA}^{-3}$. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-147113 (**5**) and CCDC-147114 (**6**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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Ground-State Reversal by Matrix Interaction: Electronic States and Vibrational Frequencies of CUO in Solid Argon and Neon**

Lester Andrews,* Binyong Liang, Jun Li, and Bruce E. Bursten*

The reactions of laser-ablated metal atoms with small molecules during their condensation in frozen noble gas matrices have led to a remarkable number of fundamental small molecules that provide new insights into the structures of and bonding in metal complexes. The coupling of these experiments with state-of-the-art electronic structure calculations provides a combined experimental/theoretical approach to identifying and characterizing transient species in the matrix.^[1] We have recently combined frozen matrix experiments with relativistic calculations using density functional theory (DFT) to study the reactions of actinide atoms with small molecules, such as N₂, CO, and CO₂. The relativistic DFT calculations have been powerful tools in the identification of new small molecules, such as NUN, CanO, An(CO)_n, and OAnCO (An = Th, U).^[2–5]

It is generally assumed that the noble gas matrix that confines the reaction products is electronically innocent, that is, the reaction products can be treated as isolated molecules. This assumption has been key in using electronic structure theory to characterize the products; it is assumed that the products can be modeled as isolated “gas-phase” molecules when their molecular and electronic properties are calculated. This approach has worked remarkably well for most of the small actinide-containing products that we have studied. For example, the reaction of U atoms with CO during condensation with Ne at 4 K leads to the formation of CUO, among other products.^[3] The CUO molecule is of considerable interest as it is isoelectronic to the important uranyl dication UO₂²⁺, and CUO contains the strongest uranium–carbon bond characterized to date. A key to identifying this molecule within the Ne matrix was the observation that our DFT-calculated stretching frequencies for the isolated ground-state CUO molecule are within 2 cm^{−1} of the experimentally observed frequencies, and the isotopic shifts upon using ¹³CO or C¹⁸O are predicted with high accuracy.

Herein we report surprising new experimental results on the CUO molecule when it is formed during condensation with Ar at 7 K. The properties of CUO in the Ar matrix are apparently very different from those in the Ne matrix, which suggests that the molecule is being influenced by the more polarizable Ar atoms. Relativistic DFT calculations provide preliminary evidence that the form of CUO in the Ar matrix can be ascribed to a low-lying electronic excited state of the isolated CUO molecule that corresponds to a change in the electron configuration of the molecule relative to the ground state.

Previous Hartree–Fock pseudopotential calculations found a stable linear CUO species,^[6] and DFT calculations characterized low-lying linear and bent singlet states with frequencies near the 1047.3 and 872.2 cm^{−1} values observed for CUO in solid neon.^[3] By using refined DFT calculations in the form of the Amsterdam Density Functional (ADF 2.3) code,^[7] which includes scalar relativistic corrections and high-precision numerical integration, we find a ¹Σ⁺ ground state for the CUO molecule with calculated ¹²C¹⁶O bond-stretching frequencies at 1049 and 874 cm^{−1}, which are in excellent agreement with the values obtained in a neon matrix (Table 1). Of equal significance, the calculated ¹³C¹⁶O and ¹²C¹⁸O frequencies match the observed values extremely well, as shown by the frequency ratios in Table 1. On the basis of the observed isotopic shifts, the bands at 1047.3 and 872.2 cm^{−1} are assigned to essentially pure U–C and U–O stretching modes, respectively, with the values being in excellent agreement with simple mechanical calculations for “U–C” and “U–O” diatomic molecules. Thus, the ¹Σ⁺ state seems well described by the relativistic DFT calculation, and no other state mixes significantly with the ground state.

The formation of CUO in solid argon leads to a species with very different vibrational frequencies. The CUO molecule is

Table 1. Calculated and experimental data for the ¹Σ⁺ and ³Φ states of linear CUO.

	¹ Σ ⁺	³ Φ ^[a]
	calculated values	
U–C [Å]	1.747	1.846
U–O [Å]	1.802	1.815
<i>E</i> _{total} [eV]	−20.8714	−20.8039
zero point energy [eV]	0.1270	0.1115
<i>ν</i> _{bend} [cm ^{−1} (kmol ^{−1})] ^[b]	63 (381)	54 (159)
<i>ν</i> _{U–O} [cm ^{−1} (kmol ^{−1})] ^[b]	874 (199)	843 (115)
<i>ν</i> _{U–C} [cm ^{−1} (kmol ^{−1})] ^[b]	1049 (120)	902 (311)
	experimental values	
noble gas matrix	Ne	Ar
<i>ν</i> _{U–O} [cm ^{−1}]	872.2	804.3
<i>ν</i> _{U–C} [cm ^{−1}]	1047.3	852.5
¹⁶ O/ ¹⁸ O (U–O) ^[c]	1.0549 (1.0554)	1.0514 (1.0469)
¹² C/ ¹³ C (U–O) ^[c]	1.0023 (1.0020)	1.0127 (1.0196)
¹² C/ ¹³ C (U–C) ^[c]	1.0357 (1.0361)	1.0260 (1.0188)
¹⁶ O/ ¹⁸ O (U–C) ^[c]	1.0019 (1.0010)	1.0052 (1.0092)

[a] The reported geometry and energy for the ³Φ state are those for the ³A' component under C_s symmetry, while the frequencies and intensities of the vibrations are averages of the ³A' and ³A'' components. [b] Calculated wavenumber; the intensity is given in parenthesis. [c] Isotopic ratios for the stretching frequencies of CUO, presented as calculated (*experimental*). The calculated values are from DFT frequency calculations in which the mass of the appropriate atom has been changed. The values are ratios and of the form *n*:1.

[*] Prof. L. Andrews, B. Liang
Department of Chemistry
University of Virginia
Charlottesville, VA 22904-4319 (USA)
Fax: (+1) 804-924-3710
E-mail: lsa@virginia.edu
Prof. B. E. Bursten, Dr. J. Li
Department of Chemistry
The Ohio State University
Columbus, OH 43210 (USA)
Fax: (+1) 614-688-3306
E-mail: bursten.1@osu.edu

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prepared by treating laser-ablated U atoms with CO in excess argon during condensation at 7 K. The Nd:YAG fundamental (1064 nm, 10 Hz, 10 ns) focused on a rotating uranium metal target using low energy (1–5 mJ pulse) evaporates U atoms as described previously.^[2, 3, 8–10] Infrared spectra of the co-deposited samples conclusively enable two absorptions at 852.5 and 804.3 cm^{−1} to be identified as CUO (Figure 1). These bands

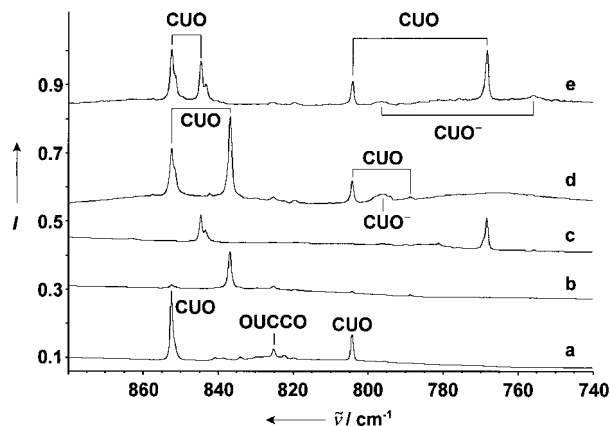


Figure 1. Infrared spectra in the 880–740 cm^{−1} region for laser-ablated U atoms co-deposited at 7 K with different CO isotopes diluted in argon: a) 0.3% ¹²CO after deposition and photolysis at $\lambda > 240$ nm, b) 0.2% ¹³CO after deposition and photolysis at $\lambda > 240$ nm, c) 0.3% ¹⁸O after deposition and photolysis at $\lambda > 240$ nm, d) 0.2% ¹²CO + 0.2% ¹³CO after deposition, and e) 0.2% ¹⁶O + 0.2% ¹⁸O after deposition.

shift to 836.8 and 788.8 cm^{−1} with ¹³CO and to 844.7 and 768.3 cm^{−1} with ¹⁸O, which shows that both vibrations involve substantial motions of the carbon and oxygen atoms. In addition, no new absorptions are observed with ¹²CO+¹³CO and ¹⁶O+¹⁸O mixtures, which indicates that a single CO molecule is involved in the primary reaction. The bands increase in intensity upon ultraviolet photolysis, which indicates that the insertion of uranium into CO requires activation. A similar energy requirement was found for the U+N₂ insertion reaction.^[2] Extensive studies with laser-ablated actinide metals shows that single metal atom reaction products dominate the spectrum at these relatively low metal concentrations.^[2–5]

The large red-shift of 194.8 and 67.9 cm^{−1} in the stretching frequencies of CUO in solid argon relative to the values in solid neon is very unusual. In contrast, the stretching frequencies of the CUO[−] ion exhibit red-shifts of only 5.1 and 5.2 cm^{−1} from neon to argon, and the secondary reaction product OUCCO, formed by the addition of another molecule of CO to CUO, gives frequencies 23.7 and 15.8 cm^{−1} lower in solid argon than in solid neon.^[3] These small shifts are attributed to normal matrix effects on the ground states of CUO[−] and OUCCO owing to the change in polarizability of the host environment.^[11]

We believe that the frequencies at 852.5 and 804.3 cm^{−1} for CUO in solid argon arise from a different electronic state of CUO than that observed in solid neon, and that this state corresponds to a low-lying excited state of the isolated CUO molecule. The isotopic shifts and intensities show that the bond-stretching modes are strong mixtures of U–C and U–O

internal coordinates as both modes exhibit substantial shifts upon the use of ¹³CO and ¹⁸O. Our DFT calculations on linear CUO reveal a ³ Φ state^[12] that is only 1.2 kcal mol^{−1} (420 cm^{−1}) above the ¹ Σ^+ ground state, with ¹²CU¹⁶O stretching frequencies predicted at 902 and 843 cm^{−1}. The calculated intensity ratio of these two bands (2.7:1) is in good accord with the experimentally observed intensity ratio (3:1). Further, the calculations on this state with the isotopomers ¹³CU¹⁶O and ¹²CU¹⁸O demonstrate that the observed frequencies involve significant mixing of both the U–C and U–O internal coordinates, which is in contrast to the ¹ Σ^+ ground state. The mode mixing is more extensive than predicted by our calculations, as shown by the isotopic frequency ratios in Table 1. We propose that the species observed in the Ar matrix corresponds to the ³ Φ excited state of the isolated CUO molecule, and that the disagreement between the calculated and observed frequencies and isotopic shifts is an indication of a non-negligible interaction between the CUO molecule and the Ar host. Apparently the interaction of the argon matrix makes ³ Φ CUO(Ar)_n the minimum energy species in solid argon whereas ¹ Σ^+ CUO is lower in energy in a solid neon matrix, as in the gas phase. Other possible isomers of CUO, such as a triangular form of the molecule, are much too high in energy to be feasible.

The presence of a low-lying excited state for linear CUO is consistent with our previously advanced molecular orbital descriptions of CUO and UO₂²⁺ (see Figure 8 of ref. [3]). The lower electronegativity of C compared to O leads to a much smaller HOMO–LUMO gap in CUO (0.37 eV) than in UO₂²⁺ (2.36 eV). The ¹ Σ^+ ground state of CUO corresponds to a U(VI) f⁰ molecule with a (1 π)⁴(3 σ)²(2 π)⁴(4 σ)² electron configuration; the 1 π and 3 σ molecular orbitals (MOs) are strongly O-localized, U–O bonding orbitals while the 2 π and 4 σ MOs are C-localized, U–C bonding orbitals. The ³ Φ excited state corresponds to a (1 π)⁴(3 σ)²(2 π)⁴(4 σ)¹(1 ϕ)¹ electron configuration, in which one of the predominantly U–C bonding 4 σ electrons has been excited to the non-bonding U 5f ϕ orbital, which leads to a formal U(V) f¹ molecule with a much longer (ca. 0.1 Å) calculated U–C bond length. The 19% reduction in the experimental U–C stretching frequency for CUO in going from Ne to Ar (as compared to an 8% reduction in the U–O stretching frequency) is consistent with the transfer of an electron from a predominantly U–C bonding orbital to a nonbonding orbital.

It is perhaps surprising that we are proposing that the excited-state triplet form of the isolated CUO molecule is actually the ground state of CUO in an Ar host. We believe that the formation of a p σ “hole” on the carbon atom or the presence of a U-localized electron in the excited state of CUO may facilitate a weak but favorable interaction between ³ Φ CUO and its argon host that is not possible in the harder neon cage. The lower quality of the agreement between the calculated and observed vibrational frequencies in the Ar matrix may be a consequence of interactions between the molecule and the Ar host. Preliminary DFT calculations on model cage molecules CUO(Ng)_n (Ng = Ne, Ar; n = 13, 17, 20, 23) provide support for this proposal inasmuch as there appears to be a significant attractive interaction between the

CUO molecule and the Ar atoms of the model cages. We are examining these interactions carefully, especially in light of the recent report of the new stable argon molecule HArF.^[13]

The potential energy surfaces (PES) along the bending coordinate are quite complicated for both the $^1\Sigma^+$ ground state and the $^3\Phi$ excited state of linear CUO. Although CUO is isoelectronic to UO_2^{2+} , the U–O and U–C interactions are much more localized in CUO than are the symmetry-equivalent U–O interactions in UO_2^{2+} . As such, the bending PES for both states are quite shallow, which leads to very small predicted bending frequencies ($< 75 \text{ cm}^{-1}$) in both the ground and excited state. Upon bending, the $^1\Sigma^+$ and $^3\Phi$ states correlate to $^1A'$ and $^3A' + ^3A''$ states under C_s symmetry, and interaction of the $^1A'$ with both the $^3A'$ and $^3A''$ states will be allowed by spin–orbit (SO) coupling. We are currently undertaking higher level calculations on CUO, including SO effects, to better chart the relative energetics of this fascinating molecule.

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Visualization of Single Multivalent Receptor–Ligand Complexes by Transmission Electron Microscopy**

Jason E. Gestwicki, Laura E. Strong, and
Laura L. Kiessling*

Multivalent ligands have the capacity to interact simultaneously with multiple receptors. The binding of a divalent ligand, for example, can bring two receptors together.^[1, 2] Many natural and synthetic ligands are not divalent, however, but rather contain many possible receptor binding sites.^[3] The complexation of such multivalent ligands with multiple receptors may be important for their biological activities.^[4, 5] Unfortunately, whether multiple receptors bind to a multivalent ligand and how many associate in a complex often can only be inferred. The difficulties associated with investigating such molecular details are exacerbated by the lack of tools available to characterize these binding events.

Previous efforts to study multivalent receptor–ligand complexes have used light scattering,^[6] fluorescence resonance energy transfer,^[7–9] capillary electrophoresis,^[10] or analytical ultracentrifugation. Transmission electron microscopy (TEM) methods are complementary to these indirect measurements as they allow direct visualization of complexes.^[11–13] Another advantage of TEM experiments is that since single molecules or complexes can be viewed the amount of material needed is typically less than that required for other techniques. Prior applications of electron microscopy to examine multiple receptors interacting with a ligand have focused on large complexes that can be imaged directly.^[14, 15] Many important receptor–ligand complexes, however, are too small or of insufficient density to be directly imaged in this way. Here we report a strategy that extends the range of receptor–ligand complexes that can be imaged by TEM.

We reasoned that a method that increased the contrast of receptors would allow visualization of receptors bound to a given ligand and facilitate the characterization of individual small receptor–ligand complexes. Our solution employs colloidal gold particles as labels to monitor the receptor position in the presence of a ligand. Because of its density, colloidal gold allows high-contrast imaging by electron microscopy. This approach is routinely taken in immunohistochemical applications, in which cellular proteins are located by colloidal gold particles attached to antibodies. Proteins other than antibodies can be attached to colloidal gold, and streptavidin-conjugated gold particles of different sizes are readily available. Such particles are used commonly because

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- [12] Strictly speaking, spin is no longer a good quantum number because of spin–orbit coupling and it is not rigorously correct to speak of a “triplet” state. Under spin–orbit coupling, the 4σ HOMO and 1ϕ LUMO transform into the $7e_{1/2}$ and $1e_{5/2} + 1e_{7/2}$ spinors, respectively, with the $1e_{7/2}$ spinor greatly destabilized (0.65 eV) relative to the $1e_{5/2}$ spinor. Thus, under spin–orbit coupling, the ground state of the isolated molecule corresponds to the $(7e_{1/2})^2$ configuration, and the first excited state corresponds to the $(7e_{1/2})^1(1e_{5/2})^1$ configuration. Because spin-polarization energy is an important factor in actinide complexes, these two states will be dominantly singlet and triplet in character, respectively, and we will use these descriptors for clarity.
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[*] Prof. L. L. Kiessling, J. E. Gestwicki, L. E. Strong
Departments of Chemistry and Biochemistry
University of Wisconsin-Madison
Madison, WI 53706 (USA)
Fax: (+1) 608-262-3453
E-mail: kiessling@chem.wisc.edu

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of the high affinity of the streptavidin for biotin ($K_d = 10^{-15}$ M). By attaching a single biotin group to a target receptor the number of receptors complexed to a multivalent ligand can be visualized through detection of the streptavidin-conjugated gold particles. This approach provides a general method for visualizing a wide range of complexes formed between receptors and multivalent ligands.

To illustrate the utility of TEM for characterizing multivalent binding events we explored the binding of the lectin concanavalin A (Con A) to a series of multivalent ligands that vary in average length and valency. Con A is a tetrameric mannose-binding plant lectin that has been used as a model for the study of multivalent interactions.^[16–18] We have synthesized mannose-substituted ligands by ring-opening metathesis polymerization (ROMP)^[19] that are potent inhibitors of Con A function.^[20–22] Ligands of different lengths and valencies have been generated by this strategy; these have unique properties relative to their monovalent counterparts.^[23, 24] Significantly, we found that as the average ligand valency increased from 1 to 50, the activities of the mannose-substituted materials increased dramatically (10^2 – 10^5 -fold) in assays with Con A.^[21, 22, 25] Thus, ligand valency is an important determinant of ligand potency in this system. The influence of ligand valency may stem from the number of receptors that can assemble on a particular ligand. TEM was used to characterize complexes between Con A and synthetic mannose ligands **1–4** to determine whether this mode of complexation occurs (Figure 1).

The complexation experiments employed Con A tetramers that were labeled with biotin using conditions that favored attachment of a low number of biotin residues (namely, 1–2 copies per tetramer). The resulting tetramers were allowed to associate with a ligand of interest (**1–4**) in solution. Complexes of biotinylated Con A and ligand were then treated with an excess of streptavidin-bound 10-nm gold particles. These solutions were spread on grids and viewed by TEM. In some experiments samples were treated with 2% phosphotungstic acid (pH 7.0 for 30 seconds) to enhance the contrast. Images of random fields (Figure 2) were acquired for each treatment and analyzed for formation of Con A complexes. Gold particles within 25 nm or less of each other were considered to be part of a complexes. This distance was based on the modeled length of the synthetic, multivalent ligands^[21] and the structure of tetrameric Con A determined by X-ray crystallographic analysis.^[26] The number of gold particles employed was chosen to minimize their coincidental adjacent placement while maximizing the relevant data that could be obtained from each experiment.

The number of gold particles incorporated into complexes was determined to assess the ability of **1–4** to bind multiple copies of Con A (Figure 3). Only the multivalent ligands **2–4** were able to interact simultaneously with multiple Con A tetramers. Importantly, the number of Con A tetramers bound depended on the valency of the ligand. In the presence of compound **2** complexes that contain two copies of Con A predominate (referred to as “dimers” in Figure 3). With compounds **3** and **4** complexes possessing three Con A tetramers (referred to as “trimers” in Figure 3) were observed in addition to those with two. When **4** is the multivalent ligand

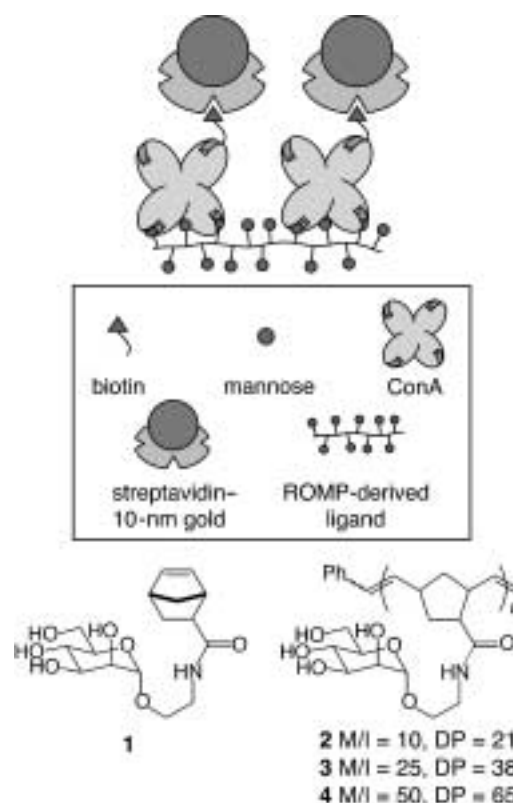


Figure 1. General strategy for the visualization of multivalent ligand complexes with receptors using transmission electron microscopy (TEM). In this example, biotinylated Con A tetramers are added to multivalent mannose-bearing ligands synthesized by ROMP. After complexation, streptavidin-conjugated gold particles are added. The resulting clusters are visualized by TEM as described in the text. The synthesis of ligands **1–4** is described elsewhere.^[20] The value n is determined by the monomer to initiator ratio (M/I) used in the synthesis. The average polymer length (degree of polymerization, DP) was determined by comparison of the integration of the phenyl proton signal in the ^1H NMR spectrum with that of the signal corresponding to the backbone protons.

the higher order complex (trimer) is favored. As ligand valency increases, therefore, the average number of Con A receptors bound to each ligand also increases. These results suggest that the previously observed increases in activities for ligands of higher valency may be derived from their abilities to bind and/or cluster multiple copies of Con A.^[21]

Multivalent receptor–ligand interactions are an integral component of many biological systems. The ability of a multivalent ligand to cluster receptors may be an important determinant of its biological activity.^[27, 28] We have shown that TEM can be used to illuminate the features of individual multivalent complexes and obtain information on populations of these complexes. Our data reveal that this method is effective even for relatively weak receptor–ligand interactions (Con A binds mannose with a $K_d \approx 1$ mM). Higher affinity interactions, therefore, are expected to yield similar or better results. Additionally, the TEM experiments disclosed here can be used to relate the number of receptors associated with a ligand to its functional affinity and biological activity, a critical step in elucidating the mechanism(s) by which multivalent ligands function.

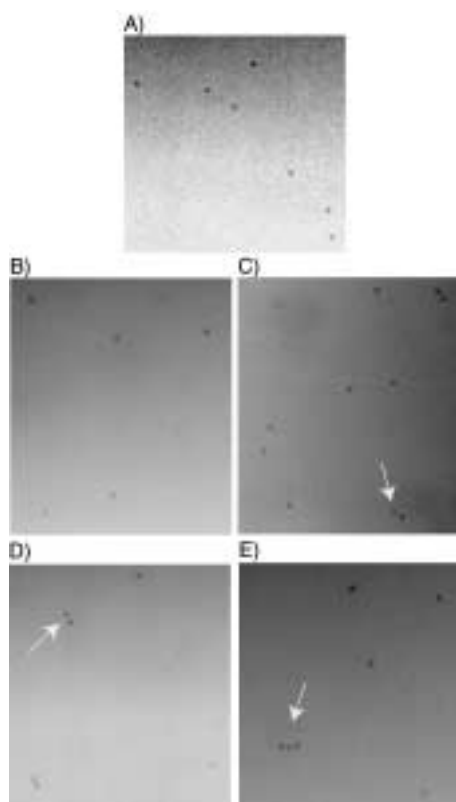


Figure 2. Images of sample fields from electron microscopy experiments using streptavidin-conjugated gold particles of biotinylated Con A: A) without ligand; B) with **1**; C) with **2**; D) with **3**; E) with **4**. Fields are representative segments of those collected for three experiments. Approximately 10–20 fields (80–150 gold particles) were collected during each independent experiment and a total of 250–650 gold particles were counted for each treatment. The arrows point to dimers in (C) and (D) and a trimer in (E). Magnification is $12500\times$.

Experimental Section

Biotinylation reactions: These were carried out in 0.1M sodium borate at pH 8.8 for 12 h at room temperature using a Con A concentration of 1 mg mL^{-1} and a final biotinylating reagent concentration ranging from 0 to $500\text{ }\mu\text{g mL}^{-1}$ in a final volume of 1 mL. Each reaction was quenched with a 1M aqueous solution of NH_4Cl . The biotinylating reagent was sulfo-succinimidyl-6-(biotinamido) hexanoate (EZ-link Sulfo-NHS-LC-Biotin, Pierce, Rockford, IL). Biotinylated Con A was dialyzed extensively against 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) pH 7.0 at 4°C to remove excess biotin. The protein concentration after dialysis was determined by a Bradford assay using bovine serum albumin (BSA) as a standard. Molar ratios of biotin to Con A were determined using 2-(4'-hydroxyazobenzene)-benzoic acid (HABA, Pierce) according to the manufacturers specifications. The Con A used in the subsequent electron microscopy experiment had a biotin to Con A tetramer ratio of 1.9:1, and was derived from a reaction with a starting biotinylating reagent concentration of $5\text{ }\mu\text{g mL}^{-1}$.

Electron microscopy: Biotinylated Con A ($2.3\text{ }\mu\text{M}$ tetramers) and ligand ($0.75\text{ }\mu\text{M}$ mannose) in phosphate-buffered saline (PBS) pH 7.2 were mixed. These solutions ($5\text{ }\mu\text{L}$) were incubated for 15 min at room temperature before streptavidin-bound 10-nm gold particles ($3.0\text{ }\mu\text{M}$) were added. The molar ratio of ligand:Con A:streptavidin in the optimized assay was 1:3:4. No insoluble biotinylated Con A complexes were observed. The streptavidin concentration was determined by the bicinchoninic acid (BCA) method using BSA as a standard. TEM was performed using Formvar-treated grids on a LEO Omega 912 EFTEM. Images were collected on a ProScan Slow Scan CCD camera and processed in Adobe Photoshop 5.0. Images were analyzed for complex formation manually, using the 10-nm gold particles as a convenient internal size standard. Occasionally, large

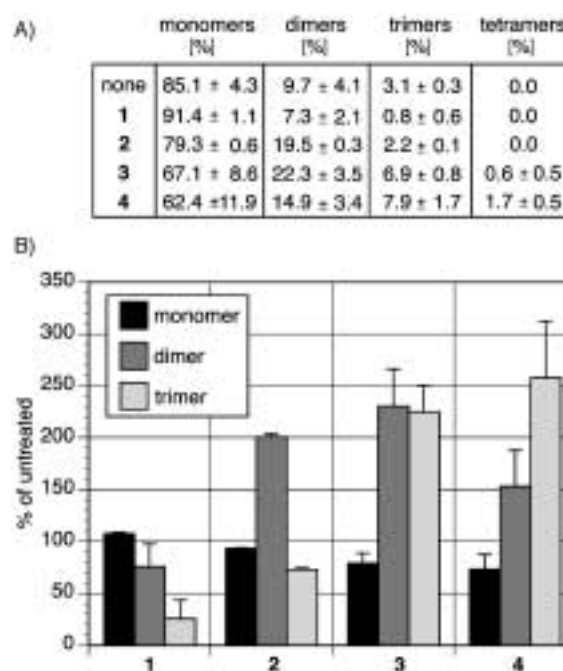


Figure 3. The number of biotinylated Con A tetramers bound by the ligand depends on the valency of the ligand partner. A) The number of gold particles incorporated into complexes (complexes are termed monomers, dimers, trimers, and tetramers as described in the text) are shown for each treatment as a percentage of the total number of gold particles counted. Dimers were defined as two gold particles within 25 nm. Trimers and tetramers were defined as three and four gold particles within this distance, respectively. Three experiments were performed on separate days using freshly prepared grids. On each day, 80–150 gold particles were counted from approximately 10–20 images of random fields for each treatment. The percentage of each complex type (monomer–tetramer) was determined on each day and the final reported percentages are averages of the three experiments. The errors represent single standard deviations. B) Graphical representation of data. Results are expressed as a percentage of untreated (biotinylated Con A and streptavidin-bound 10-nm gold particles alone). The number of monomers decreases with increasing ligand valency, while the number of clusters (dimers and trimers) increases. Error bars represent single standard deviations.

aggregates of gold were observed in the preparations, these particles were discounted from the analysis and could be removed by centrifugation prior to sample preparation.

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A Remarkable, Stable Radical–Molecule Complex: $\text{HO}_2 \cdot \text{CF}_3\text{C}(\text{O})\text{OH}$

Joseph S. Francisco

The hydrogen bond is of considerable interest in understanding the structure and energetics in a wide range of chemical systems.^[1–3] Many studies have examined hydrogen bonding in neutral molecule–molecule and ion–molecule complexes,^[4] but few have focused on radical–molecule complexes. A vigorous research effort is just beginning to examine fundamental questions of hydrogen bonding in open-shell complexes. Molecular complexes are generally weakly bound^[5, 6] and hence relatively short-lived. Such complexes are reported to play key roles in the Earth's atmosphere.^[7] Strongly bound complexes of atmospheric species suggest long atmospheric lifetimes, and this leads to the possibility of their being transported to remote regions of the atmosphere, where their concentration profiles are not expected to be large. The atmospheric oxidation of alternative fluoro- and chlorofluorocarbons, such as HFC-134a, HCFC-123, and HCFC-124, is a major atmospheric source of trifluoroacetic acid (TFA).^[8, 9] Because of the increased use and production of alternative chlorofluorocarbons, TFA concentrations are

projected to increase.^[10] Trifluoroacetic acid is chemically and biologically stable and highly resistant to photochemical breakdown. To date, little evidence is available for the degradation of TFA; consequently, little is known about the consequences of trifluoroacetic acid for atmospheric chemistry. Here we report on density functional calculations that suggest the existence of a complex between trifluoroacetic acid and $\text{HO}_2 \cdot$ radicals. The structural and energetic data of the complex suggest novel bonding and stability that have important ramifications for the atmospheric role of trifluoroacetic acid.

Unrestricted density functional calculations with Becke's three parameters and Lee–Yang–Parr functionals (UB3LYP)^[11, 12] with the 6-311++G(3df,3pd) basis set were carried out. The fully optimized geometries with minimum energies were confirmed to be stable minima by vibrational frequency analysis.^[13] Only the global minimum energy structure is reported in this study. The structure of $\text{HO}_2 \cdot \text{CF}_3\text{C}(\text{O})\text{OH}$ (Figure 1) is characterized by two hydrogen

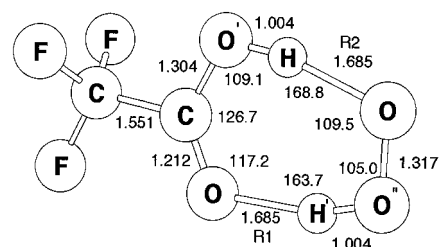


Figure 1. Structure and geometry of $\text{HO}_2 \cdot \text{CF}_3\text{C}(\text{O})\text{OH}$.

bonds, labeled R1 and R2. The hydrogen bond between the hydrogen atom of the hydroperoxyl radical and the carbonyl oxygen atom of the trifluoroacetic acid has a length of 1.685 Å, as does that between the hydroxyl hydrogen atom of trifluoroacetic acid and the terminal oxygen atom of the hydroperoxyl radical. These bonds are both significantly shorter than the typical hydrogen-bond lengths in the water dimer (ca. 1.95 Å) and in the well-studied formic acid/ H_2O complex (1.786 Å). Unlike weakly bound complexes, in which the structures of the components are virtually unaffected by hydrogen bonding, there is a significant degree of structural perturbation of the geometry of the two components as a result of the bonding in the $\text{HO}_2 \cdot \text{CF}_3\text{C}(\text{O})\text{OH}$ complex. The C–O' bond in trifluoroacetic acid is shortened by 0.032 Å (i.e., 2.4%), and the O–H bond is lengthened by 0.037 Å (i.e., 3.8%). The O–H bond length in the $\text{HO}_2 \cdot$ radical in the complex is lengthened by 0.029 Å (i.e., 3.0%) relative to isolated $\text{HO}_2 \cdot$. These structural changes indicate strong interactions in the $\text{HO}_2 - \text{CF}_3\text{C}(\text{O})\text{OH}$ complex.

The bonding energies at various levels of theory are presented in Table 1. At the highest level of theory, the binding energy D_0 and well depth D_e of $\text{HO}_2 \cdot \text{CF}_3\text{C}(\text{O})\text{OH}$ are 15.1 and 17.1 kcal mol^{−1}, respectively. Considering that the water dimer^[14–16] has a well depth of about 4.8 kcal mol^{−1}, this is tremendous. Typically, hydrogen bonds have energies of 2–7 kcal mol^{−1} and are thought to involve essentially linear arrangements of donor and proton acceptors.^[1, 16, 17] In this case, each component acts as a hydrogen donor and acceptor,

[*] Prof. Dr. J. S. Francisco
Department of Chemistry and Department of Earth and Atmospheric Sciences
Purdue University
West Lafayette, IN 47907–1393 (USA)
Fax: (+1) 765-494-0239
E-mail: jfrancis@purdue.edu

Table 1. Calculated binding energies for $\text{HO}_2 \cdot \text{CF}_3\text{C}(\text{O})\text{OH}$.

Level of theory	D_e	D_o
B3LYP/6-31G(d)	21.3	19.3
B3LYP/6-311++G(d,p)	17.1	15.1
B3LYP/6-311++G(2d,2p)	16.8	14.8
B3LYP/6-311++G(2df,2p)	16.8	14.8
B3LYP/6-311++G(3df,3pd)	17.1	15.1

and a cyclic arrangement is created by the formation of two hydrogen bonds. This also occurs in the dimers of formic and acetic acids,^[2] for which binding energies of 12–15 kcal mol⁻¹ were measured. Note that these interactions are between two closed-shell molecules, while the interaction between $\text{HO}_2 \cdot$ and $\text{CF}_3\text{C}(\text{O})\text{OH}$ is between open- and closed-shell molecules.

The chlorine oxide dimer ClOOCl plays important roles in the chemistry of polar ozone depletion. This species is bound by about 17.0 ± 2 kcal mol⁻¹,^[18, 19] and is held together by weak covalent bonding. The dimer of NO_2 (N_2O_4) is another important atmospheric species that is held together by weak covalent bonding (ca. 13.6 kcal mol⁻¹).^[19] The strong hydrogen bonding in the $\text{HO}_2 \cdot \text{CF}_3\text{C}(\text{O})\text{OH}$ complex is on the order of magnitude of weak covalent bonds.

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Stochastic Resonance in a Bistable Chemical System: The Oxidation of Ascorbic Acid by Oxygen Catalyzed by Copper(II) Ions**

Peter E. Strizhak, Igor Demjanchyk, Frank Fecher, Friedemann W. Schneider, and Arno F. Münster*

The term “stochastic resonance” (SR) refers to the enhancement of a weak periodic signal by statistical perturbations (noise) in dynamic systems with a threshold value.^[1] The threshold displayed by an autocatalytic (nonlinear) chemical reaction can be a complex function of several parameters that separate qualitatively different types of dynamic behavior (e.g., two stable steady states). If the threshold value of a suitable parameter is crossed by the combined amplitudes of the subthreshold periodic signal and the noise, the response of the weak signal is enhanced.^[2] Despite an increasing number of publications dedicated to stochastic resonance in various fields of science,^[1–3] there are only five publications concerning the experimental study of SR in chemical reactions in a continuous flow stirred tank reactor (CSTR).^[4–8] Particularly, SR occurs in various nonlinear reactions if the unperturbed system is in a steady state close to a Hopf bifurcation, i.e. a control parameter is set close to the value at which oscillations appear. This has been revealed in CSTR studies for the Belousov–Zhabotinsky (BZ) reaction,^[4] the minimal bromide oscillator,^[5] the enzymatic peroxidase-oxidase reaction,^[6,7] and autooxidation of NADH catalyzed by copper(II) ions.^[8] A positive effect of statistical fluctuations (noise) on wave propagation in a photosensitive BZ reaction has also been reported.^[9]

The classical case of stochastic resonance concerns a perturbation of bistable systems, that is, systems that exhibit hysteresis as a result of the coexistence of two stable steady states.^[1–3,10] Herein we report the first experimental observation of SR in a bistable chemical system—the autooxidation of ascorbic acid by oxygen as catalyzed by copper(II) ions in a CSTR. The appearance of SR is confirmed by an analysis of probability distribution functions and power spectra; it is also supported by the singular-value decomposition method.

We have found that autooxidation of ascorbic acid by oxygen catalyzed by copper(II) ions in a CSTR exhibits hysteresis in response to a cyclic variation of the flow rate (k_f). (Figure 1). At low flow rates the system is in a steady state characterized by a low potential at the platinum electrode (Pt-

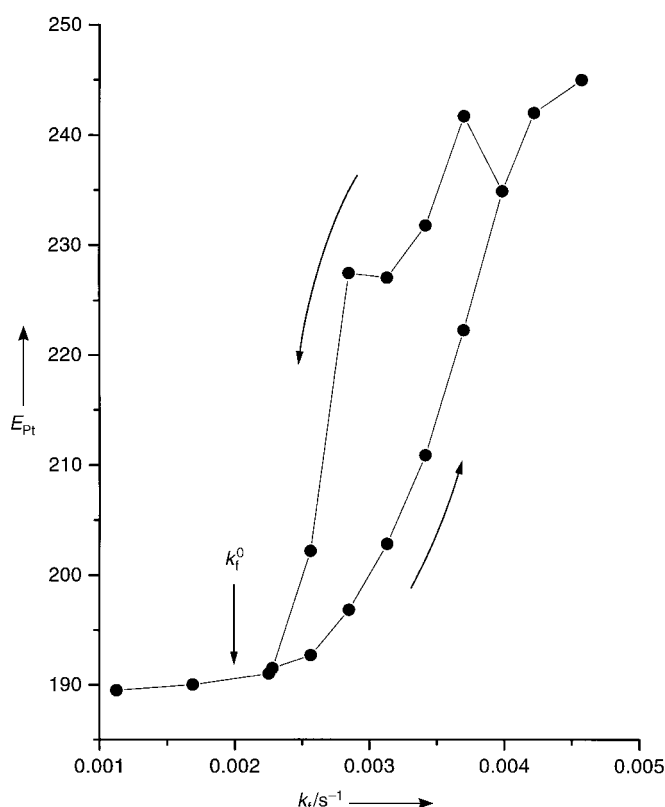


Figure 1. Dependence of the reaction on the flow rate k_f monitored by the Pt-electrode potential E_{Pt} (in arbitrary units) for the following reactor concentrations: $[H_2Asc]_0 = 5.0 \times 10^{-4} M$; $[Cu^{2+}]_0 = 2.0 \times 10^{-6} M$; $[H_2SO_4]_0 = 1.2 \times 10^{-4} M$; $[Na_2SO_4]_0 = 0.08 M$.

potential). If we increase the flow rate the system response follows the low branch and finally reaches a second steady state characterized by high values of the Pt-potential. The transition from the lower to upper branch occurs at $k_f = 0.0037 s^{-1}$. If k_f is reduced the system response follows the upper branch, and the reverse transition occurs at $k_f = 0.0023 s^{-1}$. Therefore the system exhibits hysteresis in the range of $k_f = 0.0023–0.0037 s^{-1}$, that is, a coexistence of two stable steady states is observed.

Periodic and stochastic perturbations of the lower branch have been studied by perturbing the flow rate. A sinusoidal modulation is imposed on the flow rate at a given frequency and amplitude together with a statistical perturbation of variable amplitude as given in Equation 1:

$$k_f = k_f^0 (1 + \alpha \sin(\omega t) + \beta R(\delta)) \quad (1)$$

where k_f^0 is the constant average flow rate in the steady state as marked in Figure 1, ω is the frequency of the sinusoidal perturbation, α is the amplitude of the sinusoidal perturbation, δ is the time interval of applied statistical fluctuations, and β is the amplitude of equally distributed stochastic noise. Typical time courses of the flow rate modulated by a simultaneously applied sinusoidal and stochastic perturbation have been shown in Figure 2 of ref [4] and Figures 3 and 6 of ref [5]. In our experiments we varied the value of β while all the other parameters were fixed: $k_f^0 = 0.0020 s^{-1}$; $\alpha = 0.086$; $\omega = 0.024 s^{-1}$; $\delta = 2.6 s$. The value of α has been chosen in such

[*] Dr. A. F. Münster, F. Fecher, Prof. F. W. Schneider
Institute of Physical Chemistry
University of Würzburg
Am Hubland, 97074 Würzburg (Germany)
Fax: (+49) 931-888-6302
E-mail: phch030@phys-chemie.uni-wuerzburg.de

Prof. P. E. Strizhak, I. Demjanchyk
L. V. Pysarzhevsky Institute of Physical Chemistry
National Academy of Sciences of Ukraine
pr.Nauki 31, Kiev, 03039 (Ukraine)

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a way that a sinusoidal modulation does not force the system into the bistable region, that is, without applied stochastic perturbations ($\beta = 0$) the system always remains in its lower steady state.

Depending on the amplitude β , the system exhibits different dynamic behaviors. Figures 2a,d,g,j give the time series obtained at increasing β . The middle panels represent a probability distribution function for Pt-potentials obtained from the time series. The right panels give the power spectra

of the time series. The power P is given in dimensionless units as is usually done for the Fourier-transformed spectra of finite, discrete time series. At low noise amplitudes β the system remains in the same steady state and no transitions appear from the lower to the upper branch (Figure 2a). The corresponding probability distribution function W (Figure 2b) is a bell-shaped Gaussian distribution with a single maximum located at the position of the lower steady state. This indicates that the system remains in a single steady state.

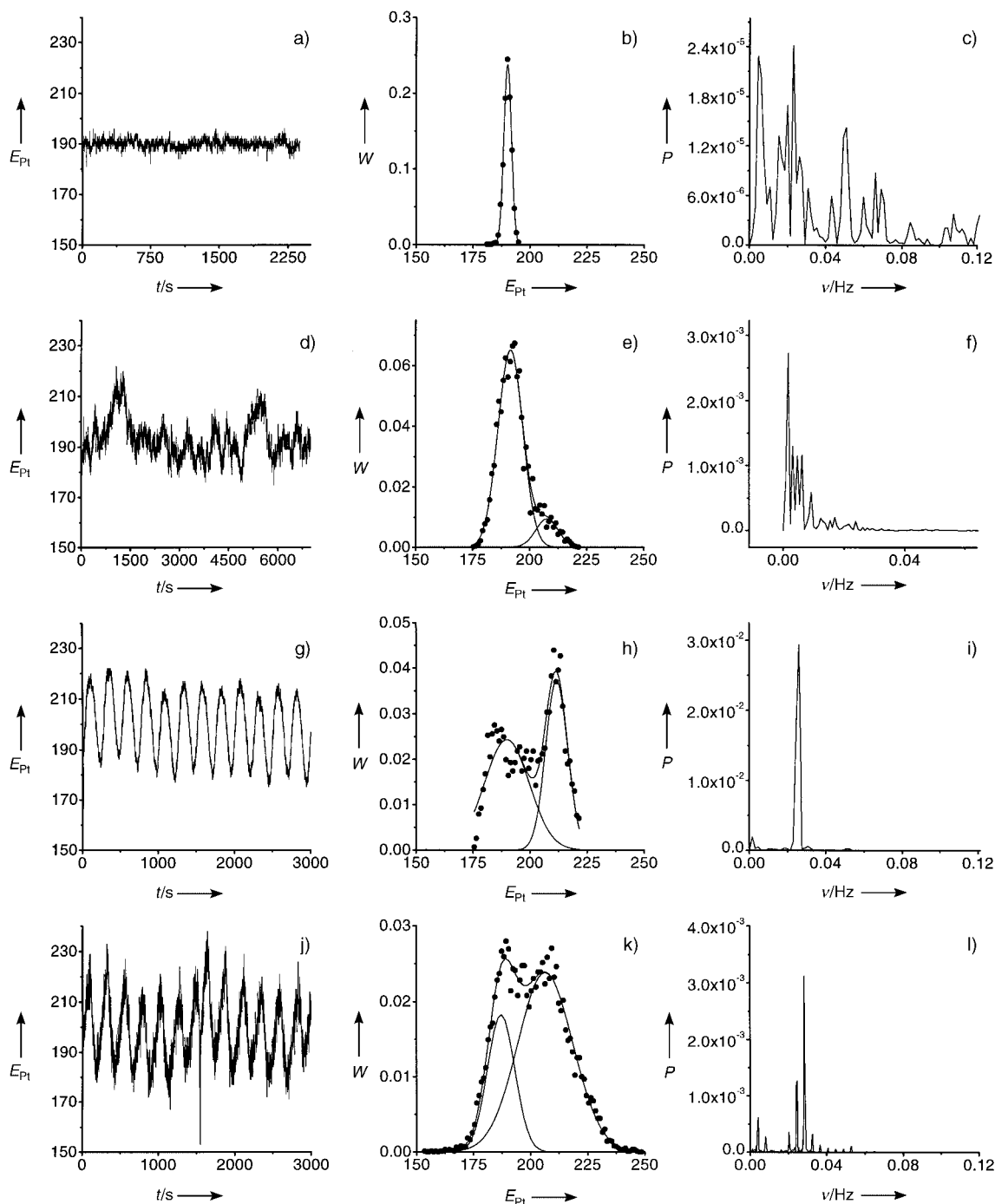


Figure 2. Time series, their probability distribution function and Fourier transformed power spectra at different levels of noise. In the figure E_{Pt} denotes the relative potential, t the time, W the probability and P the relative power. a,b,c) $\beta = 0.028$; d,e,f) $\beta = 0.057$; g,h,i) $\beta = 0.143$; j,k,l) $\beta = 0.257$. The system has been perturbed according to Equation (1) with the following parameters: $k_t^0 = 0.0020 \text{ s}^{-1}$; $\alpha = 0.086$; $\omega = 0.024 \text{ s}^{-1}$; $\delta = 2.6 \text{ s}$. Other conditions are the same as in Figure 1. The solid curves show the fitted total probability function and the probability distribution of the two steady states.

The power spectrum of the time series in Figure 2c shows no significant maximum, that is, the system varies statistically around the steady state. An increase of the noise amplitude β produces bursts into the higher steady state as illustrated in Figure 2d. The corresponding probability distribution function shown in Figure 2e becomes wider and it is characterized by an appearance of a second maximum; the power spectrum of which is shown in Figure 2f. However, for this value of β the system is still governed by statistical noise. This behavior changes at the "optimal" value of β ($= 0.143$) where the stochastic resonance appears. Figure 2g shows a time series of the Pt-potential under the SR conditions. The oscillations are almost sinusoidal and the frequency matches that of the sinusoidal modulation of the flow rate. Two well-resolved maximums appear in the probability distribution function (Figure 2h). The power spectrum is characterized by a single sharp maximum at ω , the frequency of the periodic perturbation. Therefore, the system response is synchronized with the frequency of the small amplitude periodic modulation of the flow rate.

A further increase of the noise amplitude β reduces SR and the response of the reaction becomes increasingly noisy (Figure 2j,k,l). The two maximums in the total probability distribution function become less resolved and overlap, and various harmonics (lines) appear in the power spectrum. At the highest values of β (not shown) the system behavior is totally governed by noise (the sinusoidal perturbation is totally obscured by noise), that is, the probability distribution function has a single maximum located at the position of the upper steady state, and there are no significant peaks in the power spectrum; the noise obscures the weak sinusoidal signal. The data in Figure 2g,h,i give a semiquantitative illustration for the appearance of SR at $\beta = 0.143$. Typically, a description of SR is based on the power spectrum.^[1–10] Under the SR condition a peak appears at the frequency of the weak periodic signal. In Figure 3a the intensity of the first harmonic ($|C_1|^2$) is shown as a function of noise amplitude. The value of $|C_1|^2$ has been calculated as the squared value of the dimensionless coefficient in the Fourier spectrum of the time series at the frequency ω . In Figure 3a the intensity of the first harmonic rises with the amplitude of the noise to a maximum and then decreases for $\beta > 0.2$. Therefore, the stochastic resonance appears in the range $\beta = 0.143–0.20$. The value of β where SR occurs may be located by an analysis of the third harmonic in the Fourier spectrum as well. In Figure 3b the intensity of the third harmonic ($|C_3|^2$) is shown as a function of noise, under SR conditions the third harmonic is depressed^[11] (see arrow). Stochastic resonance appears at $\beta = 0.143$, at this value the third harmonic shows a minimum (Figure 3b). In Figure 3c the number of degrees of freedom (N) computed from the singular value decomposition (SVD)^[12] is shown as a function of β . The dependence is characterized by a minimum at $\beta = 0.143$, that is, at the same noise amplitude where in the Fourier spectrum the amplitude of the first harmonic rises and the amplitude of the third harmonic is depressed.

The data presented in Figure 3 illustrate that stochastic resonance can be quantified by methods other than an analysis of the first harmonic of the power spectrum. It is

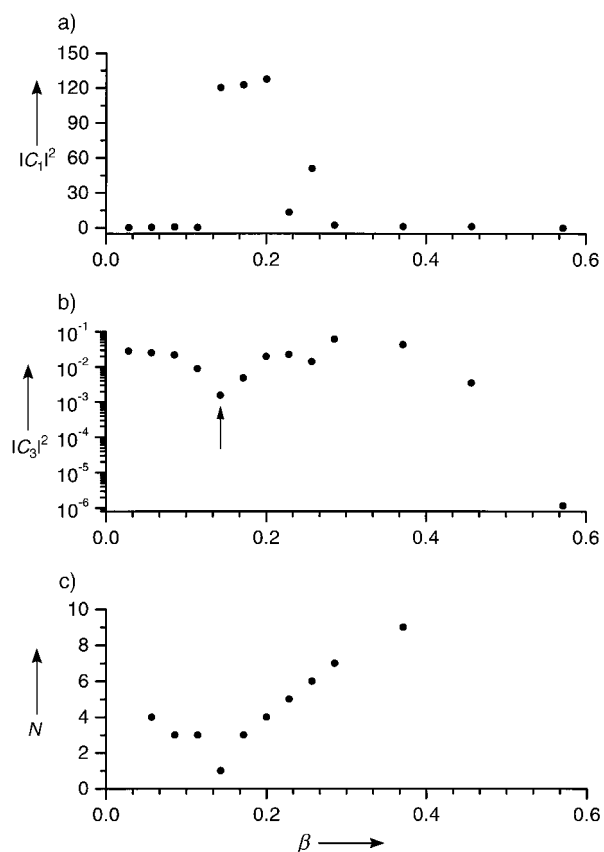


Figure 3. Quantitative characteristics illustrating the appearance of stochastic resonance at $\beta = 0.143$: a) dependence of the amplitude of the first harmonic $\omega = 0.024 \text{ s}^{-1}$ and b) third harmonic in the Fourier analysis, c) dependence of the degrees of freedom of the system on the noise amplitude β .

also worth noting that an analysis of the signal-to-noise ratio is of limited value for the system studied here, since the amplitude of the signal (the measured Pt-potential) grows with an increase of flow rate (see Figure 1). Therefore, it may happen that the signal-to-noise ratio grows even if the system is far from SR. Stochastic resonance is well pronounced in an analysis of the amplitude of the third harmonic (Figure 3b) or by the number of SVD modes which are reduced to a single mode $N = 1$ under SR conditions (Figure 3c).

The system studied is a typical example for the oxidation of an organic substrate by molecular oxygen catalyzed by a coordinated metal ion. Similar reactions are widely used in industrial processes. This allows us to predict that stochastic resonance may be observed in various chemical systems. Chemical reactions may be conducted in batch, semibatch, or in flow reactors and their dynamic behavior is usually affected by various types of interfering noise which can be reduced by an appropriate design of the system but may never be eliminated.^[13] Noise, or stochastic perturbations of a chemical reaction, is usually considered as a disturbing factor that should be eliminated. However, our experimental studies indicate that noise may play a positive role through stochastic resonance. Particularly, to achieve a high signal-to-noise ratio one may *increase* the noise amplitude instead of decreasing it.

Experimental Section

All reagents were analytical grade. Stock solutions of ascorbic acid (H_2Asc , $1.0 \times 10^{-3} \text{ M}$) and copper(II) sulfate ($4.0 \times 10^{-6} \text{ M}$), each containing H_2SO_4 ($1.2 \times 10^{-4} \text{ M}$) and Na_2SO_4 (0.08 M), were prepared using doubly distilled water. Solutions containing ascorbic acid were prepared immediately before use.

All studies were conducted in a CSTR thermostatically controlled at $(298.0 \pm 0.1) \text{ K}$. Details of the experimental setup are presented elsewhere.^[4–7] The state of the system was monitored by measuring the platinum-electrode potential; a Ag/AgCl electrode was used as the reference electrode. Signals were amplified electronically and recorded by a desk computer as a function of time with time steps of 0.1 s. As a consequence of the amplification of the signal the potential is given in arbitrary units.

CSTR experiments have been performed at different flow rates and noise intensities. Solutions of inflow reagents were pumped into the reactor by a precise two-channel piston pump. Syringe 1 contained H_2Asc ($1.0 \times 10^{-3} \text{ M}$), H_2SO_4 ($1.2 \times 10^{-4} \text{ M}$), and Na_2SO_4 (0.08 M); syringe 2 contained copper(II) sulfate ($4.0 \times 10^{-6} \text{ M}$), H_2SO_4 ($1.2 \times 10^{-4} \text{ M}$), and Na_2SO_4 (0.08 M). Correspondingly, the reactor concentrations after mixing but before any reaction were: $[\text{H}_2\text{Asc}]_0 = 5.0 \times 10^{-4} \text{ M}$; $[\text{Cu}^{2+}]_0 = 2.0 \times 10^{-6} \text{ M}$; $[\text{H}_2\text{SO}_4]_0 = 1.2 \times 10^{-4} \text{ M}$; $[\text{Na}_2\text{SO}_4]_0 = 0.08 \text{ M}$.

Perturbation of the reaction has been performed according to Equation (1) by using a 16 bit D/A (digital/analogue) converter.

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Enantioselective Ribozyme Catalysis of a Bimolecular Cycloaddition Reaction**

Burckhard Seelig, Sonja Keiper, Friedrich Stuhlmann, and Andres Jäschke*

There is currently a wide-spread interest in the development of new catalytic methods for regio- and stereoselective syntheses. Various approaches aim at the utilization and improvement of synthetic compounds, surfaces, and biomolecules as stereoselective catalysts or synthetic auxiliaries.^[1–4] Combinatorial strategies have developed into powerful tools in catalysis research, allowing the isolation of potential catalysts from vast libraries by either screening or iterative deconvolution.^[5, 6]

Ribozymes are RNA molecules with catalytic properties. While the chemistry of the naturally occurring ribozymes is restricted to cleavage and joining reactions at internucleotide bonds, artificial ribozymes isolated from synthetic combinatorial libraries have been shown to accelerate the chemical steps in a broad range of reactions, including carbon–carbon and peptide bond formation.^[7–10] These ribozymes, however, behave unlike typical chemical catalysts or protein enzymes in that they require at least one of the reactants to be RNA or to be covalently tethered to RNA. Except for the formation of dinucleotides from activated mononucleotides,^[11] there is no example of ribozymes accelerating bond-forming reactions between two small organic substrates, a central reaction type in organic synthesis and the basis of all anabolic biochemical pathways. Also, although RNA molecules can exquisitely distinguish between enantiomers when binding target molecules,^[12, 13] enantioselective bond formation by ribozymes has not yet been described.

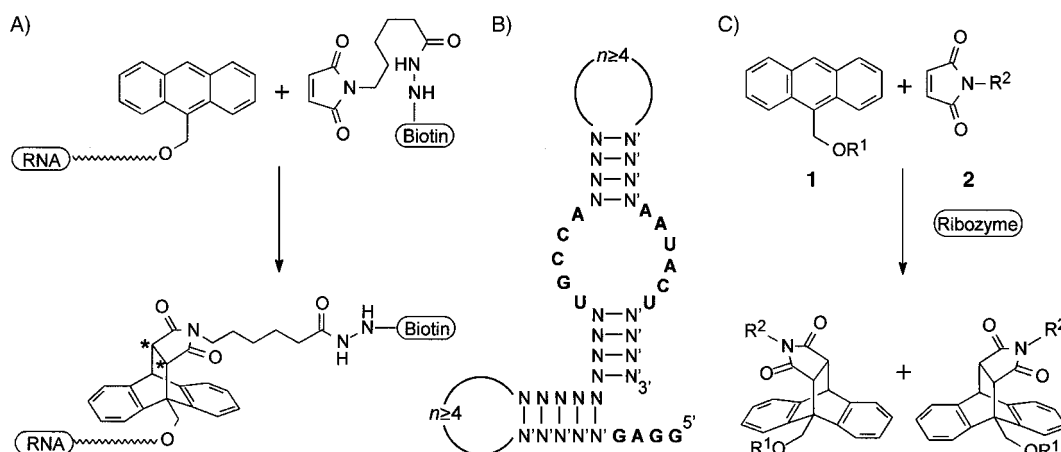
The Diels–Alder reaction is one of the most important carbon–carbon bond-forming processes available to organic chemists. The reaction creates two carbon–carbon bonds and up to four new stereocenters. There is currently much interest in developing catalytic methods for improving its rate and selectivity because, for many applications, the synthetic value of the Diels–Alder reaction relies on the degree of stereocontrol that can be exercised.^[14–16]

We have recently described the isolation of Diels–Alderase ribozymes from a combinatorial RNA library that accelerate up to 20000-fold the carbon–carbon bond formation between anthracene, which is covalently tethered to the ribozyme, and a biotinylated maleimide (Scheme 1 A).^[17] 90% of the active sequences contained a small common structural motif (Scheme 1 B), and a synthetic 49-mer oligoribonucleotide containing all elements of this motif was shown to be catalytically

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[*] Priv.-Doz. Dr. A. Jäschke, Dr. B. Seelig, S. Keiper, Dr. F. Stuhlmann
Freie Universität Berlin
FB Biologie, Chemie und Pharmazie, Institut für Chemie
Thielallee 63, 14195 Berlin (Germany)
Fax: (+49) 30-8385-5060
E-mail: jaschke@chemie.fu-berlin.de

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Scheme 1. RNA-catalyzed Diels–Alder reactions. A) Previously selected ribozymes accelerate the reaction between a tethered anthracene and biotin maleimide.^[17] B) Minimum motif shared by most of the selected Diels-Alderase ribozymes. For the experiments shown in (A), anthracene was tethered through the 5' terminus of the ribozyme, while the experiments in (C) were done without tethering. N = any nucleotide, N' = nucleotide complementary to N. C) True catalysis by ribozymes in the bimolecular Diels–Alder reaction of **1** and **2**. R¹ is either H (**1a**) or (C₂H₄O)₆-H (**1b**); for R², see Table 1 and Figure 2.

active towards a covalently tethered anthracene. We now report the action of Diels-Alderase ribozymes as true catalysts for a reaction involving two small, organic substrate molecules in solution (Scheme 1C).

The reaction of different anthracene derivatives with biotin maleimide was studied by UV spectroscopy in the absence and presence of the 49-mer ribozyme in aqueous buffer. Covalent tethering of anthracene to the RNA was found not to be required. 9-(Hydroxymethyl)anthracene (**1a**, R¹ = H) is the minimum diene which is efficiently converted into the respective Diels–Alder product (Scheme 1C). Analysis of a series of fragments and analogs of biotin maleimide revealed that the biotinyl residue can be removed with little loss of activity (Table 1). The maleimidyl group, in combination with an alkyl chain, appears to be the structural feature required for recognition by the minimum 49-mer ribozyme. Maleimides as small as *N*-ethylmaleimide were accepted as substrates. The highest rate acceleration was measured for *N*-pentylmaleimide, which reacted significantly faster than biotin maleimide. Thus, the 49-mer ribozyme accelerates a bond-forming reaction between two small organic molecules.

The kinetics of the ribozyme-catalyzed reactions were determined by measuring the differences in the initial rates between the catalyzed and the background reactions. For solubility reasons, anthracene hexaethylene glycol **1** (R¹ = (C₂H₄O)₆-H) and maleimidocaproic acid (**2**, R² = (CH₂)₅COOH) were used for these measurements. Initial velocities were determined by monitoring the decrease of the anthracene absorbance at 365 nm over approximately 5% conversion. Catalysis was examined as a completely random bireactant system.

The initial rate of the ribozyme-catalyzed reaction was found to be proportional to the ribozyme concentration. The reaction shows saturation-type kinetics with respect to both substrates (Figure 1A). The secondary double-reciprocal plot shown in Figure 1B gives Michaelis constants of 370 μ M for the diene and 8 mM for the dienophile. The calculated maximum rate $V_{\max}$ is 150 μ M min⁻¹, which corresponds to a

Table 1. Relative rate constants of ribozyme-catalyzed Diels–Alder reactions of **1a** with different maleimides.^[a]

Compound	k_{rel}
	1.0
	0.7
	0.7
	0.8
	1.6
	0.7
	0.1
	< 0.1
	0

[a] Conditions: 100 μ M **1a**, 500 μ M maleimide, and 7 μ M 49-mer ribozyme in buffer (composition: 300 mM NaCl, 80 mM MgCl₂, 30 mM Tris-HCl (pH 7.4), 10% ethanol). The reaction mixture was transferred into a 7 μ L cuvette with a 1 cm light path and the anthracene absorbance at 365 nm was recorded. For the sequence of the 49-mer RNA, see Figure 2.^[24] Tris = tris(hydroxymethyl)aminomethane.

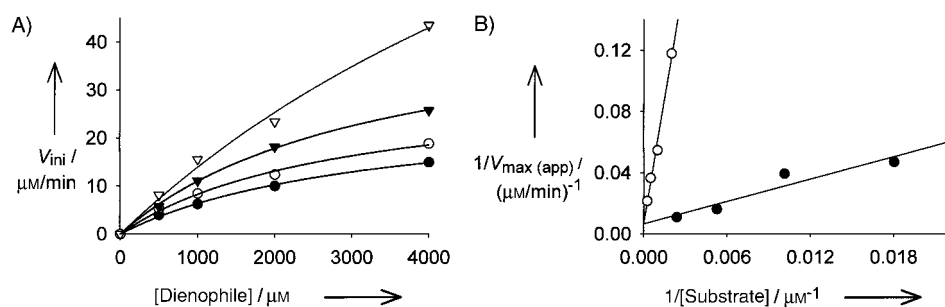


Figure 1. Kinetic characterization of the ribozyme-catalyzed reaction of **1b** with maleimidocaproic acid (**2**, $R^2 = (\text{CH}_2)_5\text{COOH}$). A) Dependence of the reaction rate on the dienophile concentration at fixed diene concentrations of $\bullet$ 55, $\circ$ 99, $\blacktriangledown$ 190, and $\triangledown$ 415 μM . All conditions were as in Table 1, except for the concentrations of ethanol (0%) and DMSO (2%). Initial rates (V_{ini}) were obtained by monitoring the UV absorbance over the first 5% of conversion and calculating the least-square fits of the progress curves. For each diene concentration (at fixed dienophile concentrations), the apparent maximum rate $V_{max(\text{app})}$ was obtained as the intercept with the y axis of the Lineweaver–Burk plot ($1/V_{ini}$ against $1/[\text{dienophile}]$). B) Plot of the apparent maximum rate values against fixed substrate concentrations ($\bullet$ diene, $\circ$ dienophile) to obtain the true V_{max} value as $1/(\text{the y intercept})$ and the Michaelis constant (K_m) values as $-1/(\text{the x intercept})$.^[25]

k_{cat} of 21 min^{-1} ($[\text{ribozyme}] = 7 \mu\text{M}$). The highest initial rate that was directly measured (at subsaturation concentrations of 415 μM diene and 4 mM dienophile) was $43 \mu\text{M min}^{-1}$ (Figure 1A), which corresponds to a catalytic turnover of about six transformations per catalyst molecule per minute. The second-order rate constant for the background reaction, k_{uncat} , was found to be $3.2 \text{ M}^{-1} \text{ min}^{-1}$. A measure of the entropic gain of this ribozyme-catalyzed reaction can be estimated by the ratio $k_{\text{cat}}/k_{\text{uncat}}$. Here, the “effective molarity” was determined to be 6.6 M, and $(k_{\text{cat}}/K_{m,\text{dienophile}})/k_{\text{uncat}}$ gives the lower estimate for the rate acceleration as 1100-fold.^[15]

Thus, the ribozyme acts as a true enzyme with fast multiple turnovers. In agreement with earlier results,^[17] a reverse reaction cannot be detected, which indicates an irreversible chemical reaction step. Addition of Diels–Alder product, however, inhibits the forward reaction; this suggests that the product competes for the same binding sites as the substrates. The mode of inhibition is currently under investigation.

In the course of the reaction, two product enantiomers can be generated (Scheme 1C). RNA is a homochiral polymer and should therefore not only accelerate the reaction but also influence the enantiomeric distribution. To investigate this possibility, the reaction products ($R^1 = (\text{C}_2\text{H}_4\text{O})_6\text{-H}$, $R^2 = (\text{CH}_2)_5\text{COOCH}_3$) were analyzed with HPLC on a chiral stationary phase (Figure 2). The peaks at 94 min and 102 min represent the two product enantiomers, as confirmed using reference compounds synthesized according to standard procedures. The uncatalyzed reaction yields, as expected, a racemic mixture, while the reaction catalyzed by the 49-mer minimum ribozyme produced

the two compounds in a 20:1 ratio according to peak areas, which corresponds to an enantiomeric excess (*ee*) value of 90%. After correction of the peak areas for the uncatalyzed background reaction, an *ee* value of over 95% is obtained. Both compounds have the same molecular weight (by ESI-MS) as that calculated and produce the same UV spectra. The CD spectra, however, are inverted, as is always the case for enantiomers.

If a homochiral catalyst induces enantioselective bond formation, then, by the laws of stereochemistry, the other

enantiomer of the catalyst molecule should show exactly the opposite enantioselectivity. While similar experiments with RNA aptamers showed reciprocal binding specificities of the corresponding D- and L-nucleic acids,^[18, 19] there is no example of a catalytically active L-nucleic acid to date. In agreement with the assumption, a synthetic 49-mer composed of L-nucleotides, a mirror-image ribozyme, not only showed similar rate acceleration but also the opposite product ratio (1:20) compared to the natural 49-mer D-ribozyme (Figure 2).

The substituent in the 9 position of the diene **1** is an important determinant of the enantioselectivity of the ribozyme-catalyzed reaction. If R^1 is shortened to $\text{C}_2\text{H}_4\text{OH}$, the enantioselectivity is reduced to 33% *ee*, and the products of the reaction with 9-(hydroxymethyl)anthracene **1a** are obtained with only 16% *ee*. Variations of the substituent at the R^2 position on the maleimide, however, cause less significant changes in enantioselectivity.

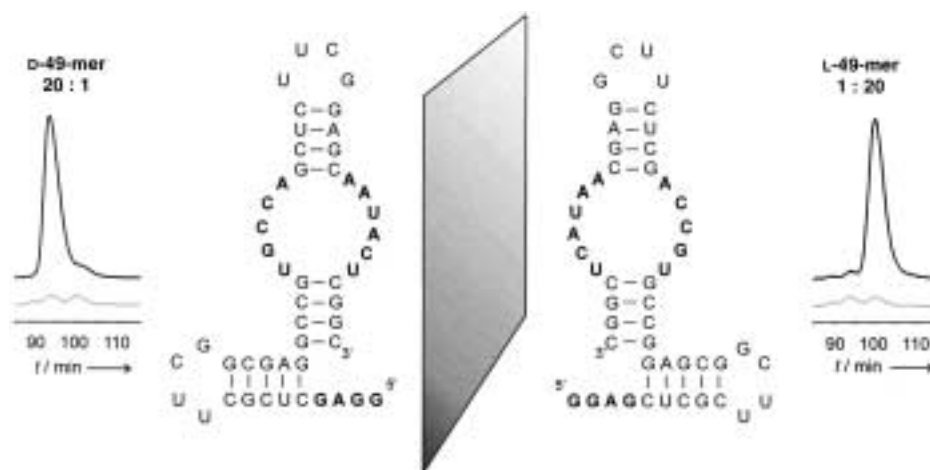


Figure 2. HPLC analysis of Diels–Alder reactions catalyzed by 49-mer D-RNA and the enantiomeric 49-mer L-ribozyme. 1 mM **1b**, 1.5 mM **2** ($R^2 = (\text{CH}_2)_5\text{COOCH}_3$), and 80 μM ribozyme were mixed for 6 minutes under the conditions given in Table 1. Samples were analyzed on a chiral NEA (R) column (YMC Europe, $250 \times 4.6 \text{ mm}$) with water:ethanol (65:35) as the eluent, isocratic at 0.8 mL min^{-1} , with UV detection at 210 nm. The light gray curve in the chromatograms corresponds to the uncatalyzed background reaction that occurs in the absence of ribozyme.

There are currently no data indicating by which mechanism the Diels–Alder reaction is catalyzed by the ribozymes. It is generally agreed that in a Diels–Alder reaction, bond formation occurs by concerted mixing of the diene and dienophile π -orbitals, specifically of the highest occupied molecular orbital (HOMO) of the diene and the lowest unoccupied molecular orbital (LUMO) of the dienophile.^[20] The majority of small-molecule (Lewis acid) catalysts operate by coordination to (and withdrawal of electron density from) the dienophile, thereby lowering the energy of its LUMO.^[14, 16] For antibody catalysis of a Diels–Alder reaction, both binding of the reactants in a reactive conformation and lowering of the dienophile's LUMO through hydrogen bonding to the dienophile carbonyl oxygen have been discussed.^[21, 22] It is conceivable that similar mechanisms are involved in ribozyme catalysis as well. Recent mutation experiments have identified conserved nucleotides in the formally single-stranded regions of the ribozyme (see Scheme 1B); this indicates participation in the formation of the catalytic site. Experiments on the chemical basis of catalysis and on the origin of stereoselectivity using a variety of diene, dienophile, and product analogues are under way.

The results demonstrate that ribozymes can act as catalysts in C–C bond forming reactions. They can recognize two small organic molecules, bring them to reaction, dissociate the product(s), and repeat this cycle several times. Another characteristic of natural enzymes has also been demonstrated for the first time: enantioselective bond formation.

Thus, selective catalysts for synthetically relevant reactions can be generated by in vitro selection from combinatorial nucleic acid libraries. Compared to classical chemical catalysts, they offer the advantages that their development does not require prior knowledge of structural prerequisites and reaction mechanisms or laborious trial and error synthesis. The iterative procedure of in vitro selection, in which active compounds are first enriched and then deconvoluted,^[17] allows the handling of up to 10^{16} different compounds in a single test tube, an order that is far beyond the capacities of high-throughput screening. Selective pressure allows the properties of the catalyst to be fine tuned.

The rate accelerations and enantioselectivities of over 95 % *ee* described here are similar to those of catalytic antibodies for cycloaddition reactions. Compared to these antibodies, the ribozymes described here are much smaller (molecular weight of 16000 Da) and, most importantly, are amenable to preparative chemical or enzymatic synthesis at reasonable costs. Therefore, we envision the use of in vitro selected oligonucleotides as tailor-made catalysts in organic synthesis.^[23]

Enantioselective bond formation is of broad interest beyond synthetic chemistry. Most reactions in biochemistry display a high level of stereocontrol, and the results shown here demonstrate that the level of stereocontrol exercised by a ribozyme can be similar to protein enzymes. This indicates how in a hypothetical “RNA world” ribozymes might have controlled the stereochemistry of chemical reactions.

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- [25] The fitted equations for the secondary plots are $y = 2.4309x + 0.0066$ ($R^2 = 0.903$) for the diene and $y = 54.539x + 0.0066$ ($R^2 = 0.990$) for the dienophile. Despite fairly good regression coefficients, the standard deviations of the intercept with the y axis were in the same range as the mean values (± 0.0060 and ± 0.0045 , respectively), rendering the $V_{\max}$ values (which are $1/(\text{the } y \text{ intercept})$) and the K_m values ($-\text{slope}/(\text{the } y\text{-intercept})$) potentially erroneous. To support the obtained kinetic constants, we additionally carried out direct nonlinear least-square regression of the data shown in Figure 1A according to a completely random bi–uni mechanism and obtained a $V_{\max}$ value of $140 \pm 25 \mu\text{M min}^{-1}$, a $K_{m,\text{diene}}$ value of $200 \pm 35 \mu\text{M}$, and a $K_{m,\text{dienophile}}$ value of $5.2 \pm 1.3 \text{ mM}$ with a correlation coefficient of $R^2 = 0.980$.

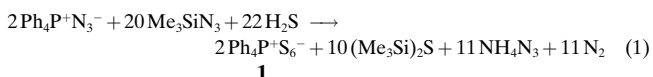
(Ph₄P)S₆—A Compound Containing the Cyclic Radical Anion S₆^{•−}**

Bernhard Neumüller, Fritjof Schmock, Reinhard Kirmse, Andreas Voigt, Axel Diefenbach, F. Matthias Bickelhaupt, and Kurt Dehnicke*

Dedicated to Professor Max Schmidt on the occasion of his 75th birthday

The spectroscopic evidence of the S₃^{•−} radical anion as the pigment of Lapis Lazuli (ultramarine) led to the unravelling of the more than 5000-year-old secret of this beautiful blue dye.^[1] At the same time, the green S₂^{•−} ion in ultramarine green was established. On the other hand, it is still unclear which entity is at the origin of ultramarine red, for which species such as S₄ and S₄^{•−} have been discussed. All these radical anions are also contained in solutions of sulfur in oleum, of polysulfides in organic solvents, and in various salt melts.^[2–4]

Unexpectedly, we now obtained orange-red needles of Ph₄P⁺S₆^{•−} (**1**) through the reaction of sulfane and tetraphenylphosphonium azide in the presence of trimethylsilyl azide at 20 °C. In this process, **1** is formed topochemically at the surface of Ph₄P⁺N₃[−], with the hydrogen diazide,^[5] which is developed at first, acting as the oxidant of sulfane. For the formation of **1** we propose the overall reaction given in Equation (1). This is supported by the identification of all reaction products. The fact that Ph₄P⁺[H(N₃)₂][−] (**2**) is also involved in the formation of **1** follows from the observation that, at first, white needles of **2** are formed which, after a few days, disappear under the simultaneous formation of **1**. The identity of these crystals was established by comparing their X-ray structure with that of **2**,^[5] which has been determined previously.^[6] Solutions of **1** decompose in dichloromethane within a few seconds; crystals of **1** are conserved only slightly longer in moist air.



Compound **1** crystallizes monoclinically in the space group C2/c with four formula units per unit cell.^[7] The P atoms of the

cations adopt twofold site symmetry, whereas the S₆^{•−} ions form centrosymmetric rings of C_{2h} symmetry in the chair conformation (Figure 1). The packing is completely equiva-

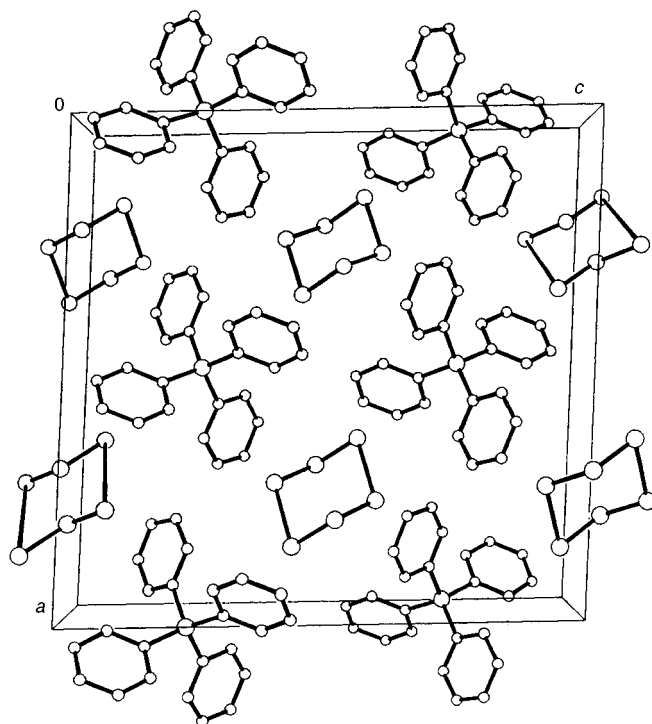


Figure 1. Unit cell of **1** without split positions of the anion.

lent with that in the crystal of Ph₄P⁺NbBr₆[−].^[8] The anions in **1** are separated by the bulky cations, which contributes to their stability in the crystal, similar to the situation for the S_n^{•−} ions in zeolites and sodalites. The S₆^{•−} ions in **1** are disordered in two positions with an occupancy factor 1:1 (Figure 2).^[9]

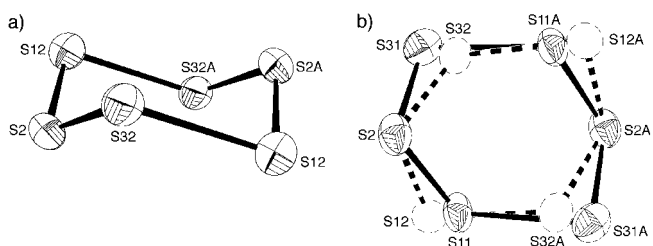


Figure 2. a) Structure of the S₆^{•−} ion and b) the disorder behavior of this ion in the crystal.

The structure of the S₆^{•−} ions is characterized by two long, central S–S bonds with distances of 263.3(4) pm, which connect the S₃ units. The angle between the plane defined by an S₃ unit and the S₄ plane of the S₆^{•−} ions adopts values of 112° and 108° for the two disordered species. The average S–S distance (206.0 pm) in the two S₃ fragments in **1** lies in the range of bond lengths in neutral sulfur rings and is characteristic for a normal S–S single bond.^[10] Note that significantly longer S–S distances have been associated with bonds, for example, 239 pm in Na₂S₂O₄,^[11] 253 pm in [Re^{VII}Cl₄(N₂S₂)][−],^[12] as well as 258 pm for the transannular

[*] Prof. Dr. K. Dehnicke, Prof. Dr. B. Neumüller, F. Schmock, A. Diefenbach
Fachbereich Chemie, Philipps-Universität Marburg
Hans-Meerwein-Strasse, 35043 Marburg (Germany)
Fax: (+49) 6421-2828917

Prof. Dr. R. Kirmse, Dr. A. Voigt
Institut für Anorganische Chemie, Universität Leipzig
Johannisallee 29, 04103 Leipzig (Germany)
Fax: (+49) 341-9736199

Dr. F. M. Bickelhaupt
Afdeling Theoretische Chemie
Scheikundig Laboratorium der Vrije Universiteit
De Boelelaan 1083, 1081 HV Amsterdam (The Netherlands)
Fax: (+31) 20-4447629

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bonds in S_4N_4 ,^[13] and 286 pm in the S_8^{2+} ion.^[14] The latter is described as an S–S bond with π symmetry.

The radical nature of **1** follows from the EPR spectra^[15] of powder samples at $T=295$ and 115 K. Whereas at room temperature only one broad line at $g_0=2.031$ can be observed, an X-band EPR spectrum occurs at $T=115$ K (Figure 3),

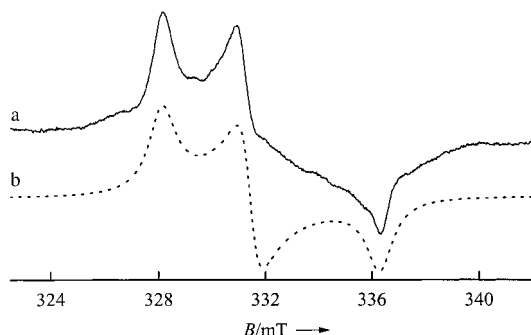


Figure 3. a) Experimental and b) simulated X-band EPR spectrum of a $(PPh_4)S_6$ powder sample at $T=115$ K.

which is characterized by a rhombic symmetric g tensor. The eigenvalues of the g tensor, $g_1=2.056$, $g_2=2.036$, and $g_3=2.003$ (g_i values ± 0.002), are similar to those found in numerous EPR spectra of ultramarine samples that are ascribed to S_2^- , S_4^- and, in particular, S_3^- radical anions.^[1, 2, 17] The g_1 value of **1** clearly deviates from the g_1 values of ≈ 2.049 determined for open-chain radicals. This is indicative for differences in s/p hybridization of the S atoms. The weak asymmetry observed for the signals of **1** in the g_1 and g_2 range has its origin in the crystallographic disorder of the S_6^- ions.

To better understand the nature of the S_6^- chair structure in Ph_4PS_6 , we have quantum chemically analyzed a series of cyclic S_6^- and S_6 systems using both density functional (BP86/TZ2P)^[18] and ab initio (MP2/6-31 + G^*)^[19] theory. All energies reported are corrected for zero-point vibrational effects ($\Delta E + ZPE$). For explorations of the potential energy surface (PES), see reference [20]. In agreement with the experimentally determined structure of S_6^- , we find a stationary point on the BP86/TZ2P PES that corresponds with the C_{2h} -symmetric chair conformation of **1a** (Figure 4). Species **1a** is composed of two loosely bound S_3 fragments ($d_{SS}=207.5$ pm) connected through somewhat longer sulfur–sulfur bonds of 246.0 pm. Interestingly, **1a** is not a stable minimum on the PES. It turns out, instead, to be a transition state for the automerization of a distorted C_2 -symmetric chair **1b**, which is slightly more stable, that is, by 8.4 kJ mol⁻¹. The S_3 fragments in **1b** are connected through a short (212.5 pm) and a long (294.3 pm) sulfur–sulfur bond (Figure 4). The relatively shallow PES explains the differences between the computed and the experimentally determined distances between the S_3 fragments (see below).

A detailed analysis of the electronic structure at the BP86/TZ2P level reveals two distinct bonding mechanisms that keep the S_3 units in the S_6^- six-membered ring systems **1a** and **1b** together (Figure 5): 1) an electron-pair bond between the $2b_1$ SOMOs of both fragments and 2) a three-electron bond between the $1a_2$ SOMO of the diradical $S_3^{2\cdot}$ and the $1a_2$

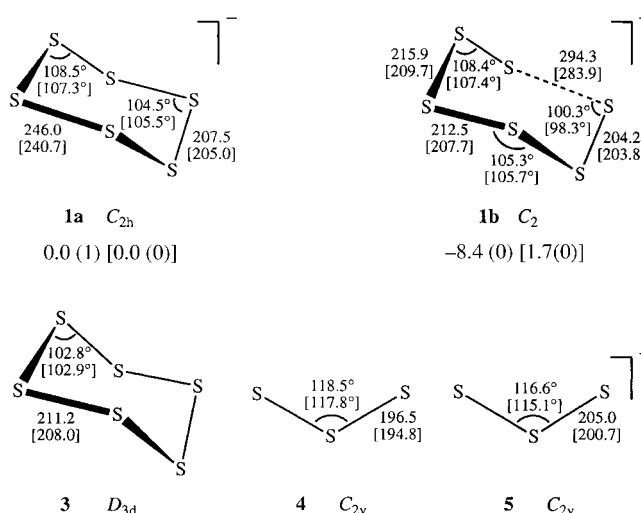


Figure 4. Structures [pm, °] optimized at BP86/TZ2P and MP2/6-31 + G^* (in square brackets). For **1a** and **1b**, relative energies [kJ mol⁻¹] and the number of normal modes with negative eigenvalues (in parentheses) are shown.

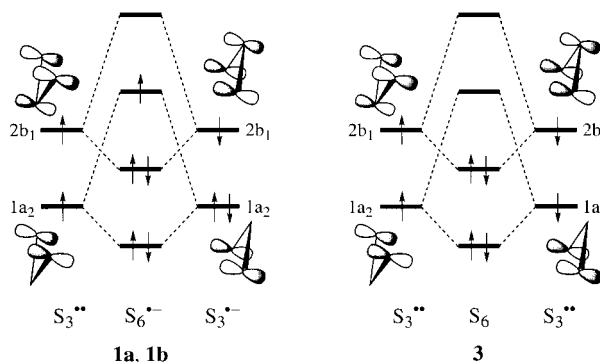


Figure 5. Schematic orbital interaction diagram for S_6^{2-} and S_6 based on the interactions of two S_3 fragments.

HOMO of $S_3^{2\cdot}$. Note that whereas neutral S_3 has a closed-shell ground state, it becomes a diradical after valence excitation of an electron from $1a_2$ to $2b_1$ (Figure 5). The overall bond energies relative to ground state S_3 (**4**) and $S_3^{2\cdot}$ (**5**) amount to -96.7 (**1a**) and -105.0 kJ mol⁻¹ (**1b**). An exhaustive discussion of sulfur–sulfur three-electron bonding is given in reference [21]. It is instructive to compare this with the interaction between two $S_3^{2\cdot}$ fragments in the neutral S_6 ring **3** (Figures 4 and 5). The loss of an antibonding electron turns the three-electron bond of S_6^{2-} into a more stabilizing electron-pair bond in S_6 (**3**). Consequently, the sulfur–sulfur bonds between the S_3 fragments in **3** are shorter (211.2 pm) and stronger (overall bond strength: -159.8 kJ mol⁻¹) than the ones in the ions **1a** and **1b**.

The chair conformations **1a** and **1b** are rather close in energy (BP86/TZ2P: 8.4 kJ mol⁻¹). This is confirmed by ab initio MP2/6-31 + G^* computations (Figure 4). Note, however, that at the MP2/6-31 + G^* level the chair conformation **1a** is a stable minimum which is 1.7 kJ mol⁻¹ lower in energy than **1b**. In view of these minimal energy differences, we expect that environment effects (e.g., packing, counterions) play a decisive role in determining which particular geometry

the S_6^{3-} ring eventually adopts in the crystal. Apparently, in Ph_4PS_6 , this is the chair conformation **1a**.

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Conical Intersections in Charge-Transfer Induced Quenching**

Adalgisa Sinicropi, Uwe Pischel, Riccardo Basosi, Werner M. Nau,* and Massimo Olivucci*

A detailed knowledge of the molecular mechanisms that govern the chemically unproductive and thus undesirable quenching of excited states by external additives is essential for the rational development of efficient photochemical reactions, artificial photosynthetic systems, and functional photonic devices. We are currently employing high-level quantum chemical methods to provide a comprehensive quenching path mapping for n,π^* -excited states of compounds such as ketones and azoalkanes. It was shown that quenching by hydrogen donors is induced by a conical intersection (CI) located halfway along the reaction coordinate for hydrogen abstraction (Figure 1a).^[1, 2] Herein, we introduce a new quenching mechanism by electron donors that operates in a similar way through a CI, which is located halfway along a

[*] Prof. Dr. W. M. Nau, Dipl.-Chem. U. Pischel
Departement Chemie der Universität
Klingelbergstrasse 80, 4056 Basel (Switzerland)
Fax: (+41) 61-267-3855
E-mail: Werner.Nau@unibas.ch

Prof. Dr. M. Olivucci, A. Sinicropi, Prof. Dr. R. Basosi
Dipartimento di Chimica, Università degli Studi di Siena
via Aldo Moro, 53100 Siena (Italy)
Fax: (+39) 0577-234278
E-mail: olivucci@unisi.it

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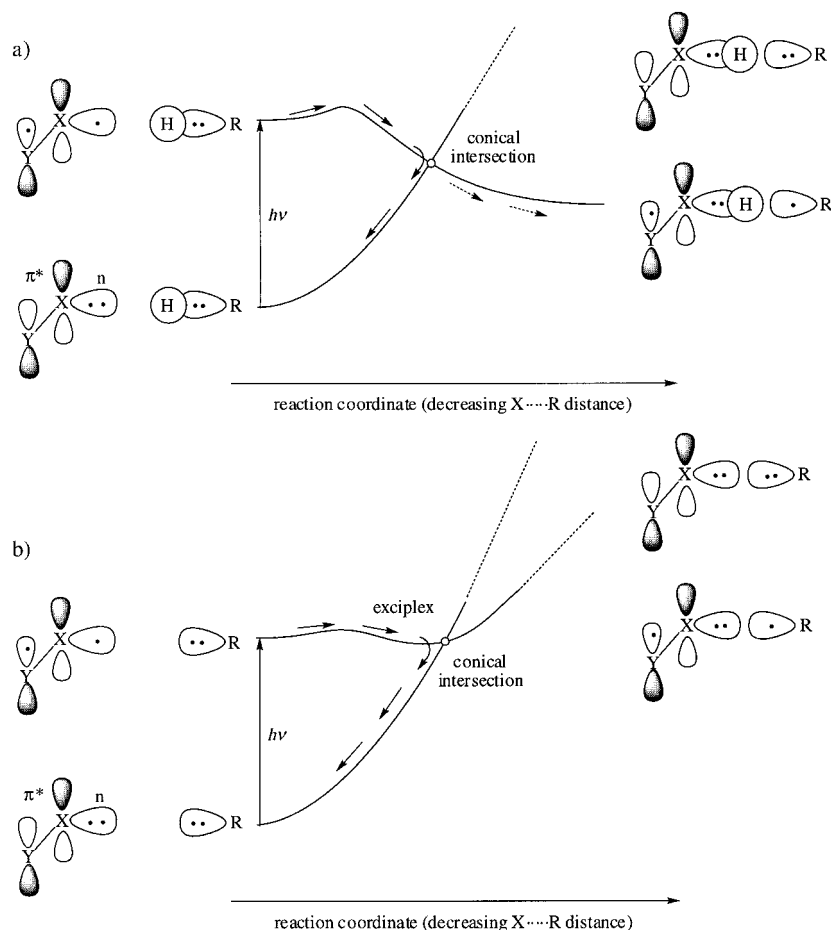
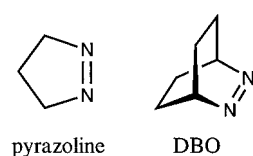


Figure 1. Modified correlation diagrams for the interaction of n, π^* -excited states ($X = O, N$; $Y = C, N$) with hydrogen donors H-R (a) and electron donors R (b). The diagrams reflect the occurrence of transition states, exciplexes, and conical intersections along the reaction pathway. Dashed lines lead to strongly repulsive states.

charge transfer (CT) reaction coordinate (Figure 1 b). While it is well known that quenchers can induce radiationless deactivation through reversible chemical reactions like hydrogen, proton, and electron transfer, the described quenching mechanisms are distinct since they require only a partial rather than a full hydrogen and electron transfer. This finding strongly supports the concept that common quenching mechanisms for excited states may be driven by photochemical reactions, including photoinduced electron transfer, which are interrupted (“aborted”) at conical intersections located along the excited-state reaction path.

Akin to our previous study on direct hydrogen abstraction,^[1] we have employed pyrazoline and 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO) as computational and experimental models of the CT-induced quenching of n, π^* -excited states. Most importantly, the azoalkanes allow a theoretical and experimental comparison of the singlet-state reaction, which is of primary interest in relation to the characterization of



conical intersections. For the calculations, trimethylamine and dimethyl ether were chosen as prototypical quenchers with powerful and poor electron-donor abilities to rationalize the observed switch-

over in quenching mechanism from CT (for amines and alkylbenzenes)^[3–12] to hydrogen transfer (for ethers and alcohols).

We have employed ab initio CASSCF^[13–16] and CASPT2^[17, 18] calculations to map the minimum energy path for the approach of NMe₃ and OMe₂ to n, π^* singlet-excited pyrazoline. The principal type of CT interaction (Figure 1 b) involves electron donation from the quencher lone pair to the excited state nonbonding orbital of one azo nitrogen atom, which is half-vacant and thus electron deficient.^[11, 12] Table 1 contains the calculated data for the two systems and Figure 2 shows the energy surfaces computed for the interaction of pyrazoline and NMe₃ or OMe₂.

The computed path starts at a long-distance point (LD) of negligible intermolecular interaction ($r \approx 3.7$ Å). Compression of the N...N bond length results in a slight (ca. 4 kJ mol^{−1}) increase in energy up to an energy maximum at $r \approx 3.3$ Å, which can be identified as a transition state (TS). Between $r \approx 3.3$ –2.1 Å the excited state complex gains about 17 kJ mol^{−1} stabilization (ca. 8 kJ mol^{−1} including zero-point energy (ZPE) correction). This is accompanied by a significant CT (ca. 0.2 electrons, Table 1), which characterizes the resulting minimum structure at $r = 2.1$ Å as an exciplex (EX) with partial CT character. The resulting N...N bond can be regarded as a weak, polarized two-center–three-electron bond and described as a mixture of a covalent (N=N...:NMe₃) and an ionic configuration (N=N⁺...[−]NMe₃).

Further progression along the reaction coordinate of Figure 2a leads to a conical intersection ($r = 2.0$ Å) and an additional increase of the CT character (Table 1). This S₁/S₀ potential energy surface crossing is located only about

Table 1. Calculated energies, dipole moments, and CT character of characteristic structures along the reaction coordinate for CT-induced quenching of n, π^* singlet-excited pyrazoline.

Quencher	Structure ^[a]	E [Hartree] ^[b]	ΔE	μ [kJ mol ^{−1}] ^[d]	Δq ^[e] [Debye] ^[f]
NMe ₃	LD (S ₀)	−400.48778 (0.71)	−265.14	2.985	0.007
	LD (S ₁)	−400.38670 (0.70)	≡ 0.00	2.684	0.006
	TS (S ₁)	−400.38481 (0.70)	4.97 [5.10]	2.534	0.001
	EX (S ₁)	−400.39113 (0.69)	−11.62 [−5.18]	1.840	0.226
	CI	−400.38997 (0.69)	−8.57	1.889	0.302
OMe ₂	LD (S ₀)	−381.16346 (0.74)	−268.11	2.573	0.001
	LD (S ₁)	−381.06125 (0.73)	≡ 0.00	2.240	0.001
	CI	−381.00545 (0.72)	146.34 ^[d]	2.216	0.192

[a] For the abbreviations LD, TS, EX, CI see Figure 2. [b] Calculated absolute energy at the CASPT2 level of theory. The weight of the CASSCF reference function in the first-order function is given in parentheses. [c] Calculated relative energies [LD(S₀) as reference]. ZPE-corrected values are given in square brackets. [d] Calculated (CASSCF level) dipole moments. [e] Amount of CT from the quencher to the azoalkane (CASSCF level). [f] The CASPT2 value of the lower root of the CASSCF optimized CI structure is given as an upper limit (circle at $r \approx 1.8$ Å in Figure 2b).

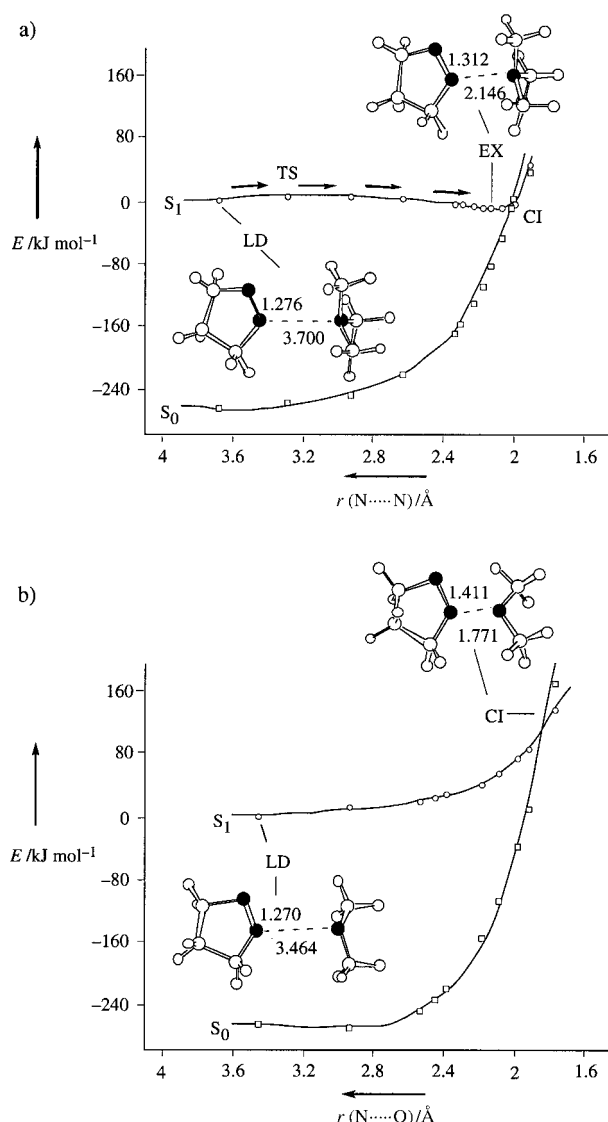


Figure 2. Energy profile (CASPT2 level) describing the quenching mechanism of pyrazoline by NMe₃ (a) and OMe₂ (b). The S₀ curve gives the ground state energy along the excited state reaction coordinate. The LD point represents a structure with a long bond length between the two involved nitrogen atoms. The reaction coordinate corresponds to a concurrent change of many geometrical parameters of the system (see ref.[13]). Only the values of the N...N bond lengths are reported for simplicity. LD = long-distance point; TS = transition state; EX = exciplex; CI = conical intersection.

3 kJ mol⁻¹ above the exciplex and lies below the initial LD structure. The steep rise of the ground state energy surface towards the CI is due to a destabilizing four-electron two-orbital interaction (N=N:···NMe₃). The strongly repulsive nature of both potential energy surfaces beyond the CI ($r < 1.9$ Å) rapidly leads to mixing with upper excited states, thus preventing a further analysis within the framework of the correlation diagram (dashed lines in Figure 1b). While the CI exists already at the CASSCF level of theory, the LD → TS → EX → CI energy profile along the excited-state reaction coordinate in Figure 2a emerges only after inclusion of dynamic electron correlation (CASPT2 level). Expectedly, the inclusion of dynamic electron correlation is particularly

important for structures with a mixed covalent CT character like exciplexes.^[19]

The reaction mechanism in Figure 2a is transferable to triplet n, π^* -excited states, where, however, the role of the CI is replaced by a singlet–triplet crossing.^[20] A calculation of the triplet reaction for pyrazoline/NMe₃ confirms the existence of a singlet–triplet crossing with a molecular structure featuring a N···N bond with $r = 2.5$ Å, which is located about 52 kJ mol⁻¹ above the corresponding triplet LD structure. Such a crossing provides a channel for intersystem crossing (ISC) to the S₀ surface and the rather pronounced radiationless deactivation in several triplet reactions of ketones and azoalkanes^[3–9] may proceed through this type of quenching mechanism.

CASPT2 computations on DBO/NEt₃, an experimentally accessible system, proved unpractical, but a CI structure has been located at the CASSCF level with geometrical parameters similar to the pyrazoline/NMe₃ system. Nevertheless, a comparison of the computed quenching mechanism for pyrazoline (Figure 2a) with the information from fluorescence quenching experiments of DBO was of great interest since pyrazoline has previously proven to be an excellent theoretical model for DBO.^[1] The experimental results for DBO are as follows: 1) Fluorescence quenching by amines leads to efficient radiationless deactivation (> 95 %).^[10, 11] 2) Steric effects suggest that exciplexes are involved.^[12] 3) The activation energies (this work, measured from 283–323 K) for NEt₃ are 5.4 ± 0.8 kJ mol⁻¹ in *n*-hexane and 7.1 ± 0.4 kJ mol⁻¹ in acetonitrile. 4) The quenching rate constants ($k_q = 10^7$ – 10^8 M⁻¹ s⁻¹) fall far below the diffusion-controlled limit^[11] and the pre-exponential factors are consequently low: $\lg[A/(M^{-1} s^{-1})] = 8.9 \pm 0.2$ for NEt₃ in both *n*-hexane and acetonitrile (this work). 5) The fluorescence quenching by amines and other electron donors exhibits an inverted solvent effect, since it is slowed down in polar solvents (e.g., $k_q = 1.4$ versus 0.44×10^8 M⁻¹ s⁻¹ for NEt₃ in cyclohexane and acetonitrile).^[11] 6) The quenching rate constants decrease for quenchers with weaker donor properties. For di-*n*-butyl ether, which has a much higher ionization potential than triethylamine (9.51 versus 7.50 eV)^[11, 21] the value of k_q drops to 6.4×10^5 M⁻¹ s⁻¹ in acetonitrile (this work).

In agreement with the experimental findings 1) and 2), the computed reaction mechanism predicts that the interaction of singlet-excited azoalkanes with amines leads to fast and efficient radiationless deactivation with the involvement of an intermediary exciplex. This exciplex should be very short-lived since it resides in the vicinity of the CI. The fact that most of the previously postulated^[3–12] exciplexes of n, π^* -excited states have remained spectroscopically elusive with very few exceptions^[4] may be due to their very short lifetime. Strikingly, with respect to the kinetic findings 3) and 4), the experimental activation energies and the computed TS for exciplex formation are in excellent agreement (ca. 4–8 kJ mol⁻¹). These values match those expected for solvent viscous flow,^[22] that is, the involved exciplexes are real “encounter complexes”.^[4–7] Accordingly, the quenching by amines can be classified as an entropically controlled reaction, which is experimentally manifested in the low absolute quenching rate constants and the low Arrhenius *A* factors.

Unfortunately, it cannot be decided whether this entropical control is related to stringent requirements to form the exciplex or undergo surface crossing at the CI.

With respect to the unusual observation of an inverted solvent effect (5), the dipole moments of the various structures have been suggested to play a key role.^[11] Regardless of the significant amount of CT along the reaction coordinate, the computed data (Table 1) reveal a rapid decrease in dipole moment upon progression from the initial LD state (2.684 D) to EX (1.840 D) and CI (1.889 D). Our calculations indicate that the dipole moment induced by CT (pointing from the amine to the azoalkane) is counterbalanced by the inherent molecular dipole moment (pointing from the azoalkane to the amine, that is, in the opposite direction). This computational result confirms the peculiarity of the recently observed solvent effect.^[11]

The viability of quenching by amines is made possible by a CT stabilization, which renders the CI and the exciplex energetically accessible. The degree of CT stabilization should decrease with decreasing donor ability of the quencher, which is reflected in the experimental finding 6) of a significantly slower quenching by ethers. Indeed, for OMe₂ as quencher of n,π* singlet-excited pyrazoline a CI along the reaction coordinate of decreasing N...O bond length is also computed (Figure 2b, Table 1). However, as expected from the poor donor properties of ethers, the amount of CT is lower than in the case of the amine (Table 1) and a reduced stabilization of the CI structure results upon inclusion of the dynamic electron correlation energy (CASPT2 level).^[19] This has the consequences that an LD → TS → EX → CI energy profile does not apply for the ether (Figure 2b) and that the computed crossing lies too high in energy (>100 kJ mol⁻¹) to be accessible within the short lifetime of an excited state. Hence, CT-induced quenching by ethers appears unlikely to compete with the hydrogen abstraction mechanism,^[1] which is expected to proceed with much lower activation energy. Indeed, our experiments (this work) yield an activation energy of 18.0 ± 0.4 kJ mol⁻¹ (for di-*n*-butyl ether in acetonitrile) and a large deuterium isotope effect (*k_q*(H)/*k_q*(D) = 4.2 in neat diethyl ether). These results support a switch-over in quenching mechanism from a hydrogen abstraction for ethers (Figure 1a) to a CT process for amines (Figure 1b).

In summary, the CT-induced quenching of singlet-excited azoalkanes by amines occurs according to the LD → TS → EX → CI mechanism in Figure 1b. After partial CT from the quencher to the azoalkane (ca. 20–30 %) the system enters an S₁/S₀ conical intersection which provides an efficient channel for radiationless deactivation to the ground state molecules. This CI occurs in the strict vicinity of the minimum energy point along the S₁ energy surface that corresponds to an exciplex with CT character. This novel quenching mechanism can be considered as an “aborted” charge transfer. Akin to the previously reported quenching through a partial hydrogen transfer^[1] the present mechanism involves a partial electron transfer.

There are two underlying general conclusions which can be drawn from the computed reaction coordinates in Figure 2. First, the principal occurrence of a conical intersection (or a singlet – triplet crossing) appears to be insensitive to the donor

and its electron-donating ability (cf. amine versus ether in Figure 2). This suggests that related quenching mechanisms of n,π*-excited states need also to be considered for other electron donors like alkylbenzenes^[3–8] and olefins,^[23–25] for which significant radiationless deactivation has also been observed. Second, one must consider the possibility of a full electron transfer as a follow-up reaction from the exciplex.^[26] Such a mechanism of electron transfer, which requires an intermediary state with close proximity and orbital overlap between the reactants, can be referred to as an “inner-sphere” electron transfer.^[5] This means that photoinduced electron transfer reactions, to the degree that they proceed by an inner-sphere mechanism, are likely to encounter competitive pathways of deactivation due to close-lying conical intersections and singlet – triplet crossings.

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- The geometry optimizations and reaction coordinate computations have been carried out at the CASSCF level of theory using a complete active space (CAS) including eight electrons in five orbitals [CAS(8,5)]. The orbitals comprise the π and π* N=N orbitals and the two N lone-pair orbitals of the pyrazoline fragment and the N lone-pair orbital of the amine NMe₃ or the p-type lone-pair orbital of the OMe₂ ether. The standard 6-31G* basis set (double-ζ + d-type polarization function on first- and second-row atoms) included in Gaussian 94^[14] has been used. In order to test the reliability of this basis set we have also carried out computations with the augmented 6-31 + G* basis set which includes sp-type diffuse functions on all the first-row atoms. The results showed that the change in the CASSCF energy difference between the CI and LD points (the initial and final point of the path) is less than 8 kJ mol⁻¹. The reaction coordinates have been computed according to the following procedure: 1) The CI between the excited state (S₁) and ground state (S₀) was optimized by using the methodology available in Gaussian 94. 2) An S₁ relaxation path was computed starting from the optimized CI point and using the IRD method described in refs. [15, 16]. In both cases the resulting relaxation path is barrierless and leads to the LD structure. In order to compute a more accurate energetics we reevaluated it along a selected series of reaction co-ordinate points at the multireference Möller–Plesset perturbation theory (CASPT2) level^[17] using MOLCAS-4.^[18]
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From a Racemate to a Pure Enantiomer by Laser Pulses: Quantum Model Simulations for H₂POSH**

Yuichi Fujimura, Leticia González, Kunihiro Hoki, Dominik Kröner, Jörn Manz,* and Yukiyo Ohtsuki

Laser pulse control of chemical reactions is a hot topic in femtosecond chemistry.^[1] After the theoretical design of various scenarios for laser pulse control,^[2–7] experimental verifications have been demonstrated, initially for small molecules^[8–10] but recently also for larger ones, for example selective laser pulse separation of different ligands from organometallic molecules.^[11] A particular challenge in this field is the design of laser pulses for the selective preparation

of enantiomers. The first suggestion was made by Shapiro and Brumer,^[12] in which a laser pulse was used to convert a prochiral initial state of a symmetric precursor molecule ABA' into a coherent superposition of two states with opposite symmetries, thus breaking the symmetry of the system. Subsequently, a second pulse should photodissociate the preexcited ABA' system into selective enantiomers, either A + BA' or AB + A'. A second scenario, presented for the model system H₂POSH assumed that the system is initially in its torsional ground state, which corresponds to a coherent 50%:50% superposition of left and right atropisomers.^[13] An optimal laser pulse then drives the system to a coherent near 50%:50% superposition of the two lowest torsional states which have opposite symmetries, thus preparing a pure enantiomer. An alternative approach, introduced by Quack, suggests that it should be possible to selectively excite enantiomers, because they have slightly different energies due to interactions.^[14, 15] Complementary to these proposals,^[12–15] various methods have been developed for the subsequent laser pulse control of pure enantiomers, for example their stabilization^[16] or the selective transformation of one enantiomer either into the opposite one^[17] or into a superposition of chiral wavefunctions.^[18]

Herein, we address the challenging problem of laser pulse preparation of a pure enantiomer from a racemate.^[19] The corresponding initial state is an incoherent superposition of states representing enantiomers with opposite chiralities, in contrast to the previous papers^[12, 13] which considered less demanding cases of pure initial states. Exemplarily, we shall simulate the laser pulse driven molecular dynamics from the racemate towards a pure enantiomer by using representative wavepackets for the torsional motion of the pre-oriented model system H₂POSH in the electronic ground state at low temperature.

H₂POSH forms a left and a right atropisomer depending on the negative or positive values of the P-O-S-H dihedral angle ϕ .^[13] The corresponding double-well potential V along the torsional angle ϕ is shown in Figure 1 (adapted from reference [13]) together with the levels $E_{v\pm}$ of the torsional

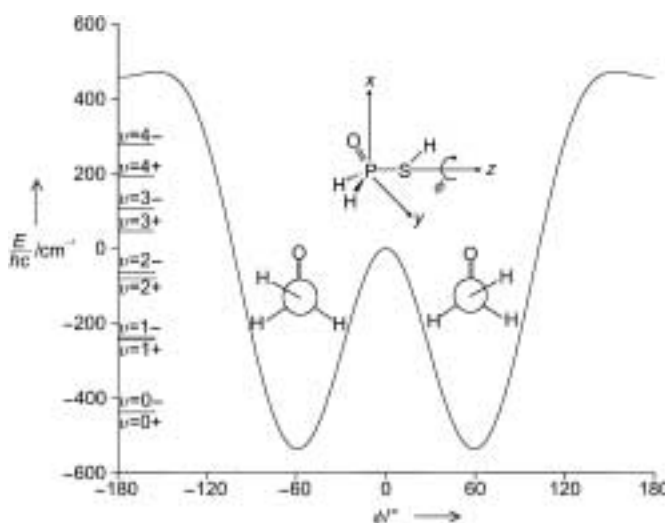


Figure 1. Double-well potential and torsional energy levels of the model H₂POSH (ab initio results adapted from ref. [13]).

[*] Prof. Dr. J. Manz, Dr. L. González, D. Kröner
Institut für Chemie, Physikalische und Theoretische Chemie
Freie Universität Berlin
Takustrasse 3, 14195 Berlin (Germany)
Fax: (+49)30-838-54792
E-mail: manz@chemie.fu-berlin.de
Prof. Y. Fujimura, K. Hoki, Dr. Y. Ohtsuki
Department of Chemistry, Graduate School of Science
Tohoku University, Sendai 980-8578 (Japan)

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eigenfunctions $\Phi_{v\pm}(\phi)$. All other degrees of freedom are neglected. For energies $E_{v\pm}$ below the potential barrier at $\phi = 0$, the $\Phi_{v\pm}(\phi)$ eigenfunctions occur as doublets of vibrational states labeled $v=0, 1, 2$ with + or – symmetries, with splittings corresponding to tunneling times τ_v of 630, 20.9, and 1.88 ps, respectively. The sums (+) or differences (–) of these eigenstates yield corresponding torsional states for the left and right enantiomers [Eq. (1a) and (1b), respectively],^[20] approximately embedded in the left and right potential wells, respectively. Torsional eigenstates $\Phi_{v\pm}(\phi)$ with higher energies, $v=3, 4$, etc., are delocalized.

$$\Psi_{vL} = \frac{1}{\sqrt{2}}(\Phi_{v+} + \Phi_{v-}) \quad (1a)$$

$$\Psi_{vR} = \frac{1}{\sqrt{2}}(\Phi_{v+} - \Phi_{v-}) \quad (1b)$$

For a given temperature T , the system is assumed to be in thermal equilibrium, described by the density ρ , where Q is the partition function [Eq. (2)].

$$\rho_{eq} = \sum_{v\pm} \Phi_{v\pm} \frac{e^{(-E_{v\pm}/k_B T)}}{Q} \Phi_{v\pm}^* \quad (2)$$

For simplicity, let us consider the particular case at low temperature ($T < 40$ K) where only the two torsional eigenstates of the lowest doublet are populated with nearly equal probabilities. Then, the initial thermal distribution [Eq. (2)] at $t=0$ can be rewritten as approximately the sum of equal densities for the left and right enantiomers [Eq. (3)].

$$\rho_{eq}(t=0) \approx \frac{1}{2}(\Phi_{0+} \Phi_{0+}^* + \Phi_{0-} \Phi_{0-}^*) = \frac{1}{2}(\Psi_{0L} \Psi_{0L}^* + \Psi_{0R} \Psi_{0R}^*) \quad (3)$$

Now the task is to design a laser pulse which drives the initial racemic state towards a final state which represents (nearly) a pure target enantiomer, for example the left enantiomer. The laser driven dynamics is simulated, in semi-classical dipole approximation, by solving the time-dependent Liouville–von Neumann equation [Eq. (4)].

$$i\hbar \frac{\partial}{\partial t} \rho = [\hat{H}, \rho] \quad (4)$$

In this equation $\hat{H}$ is the Hamiltonian operator with contributions for the kinetic torsional energy, for the torsional potential, and for the interaction of the molecular dipole $\vec{\mu}$ with the electric field $\vec{E}(t)$ of the laser pulse. We assume that $\vec{E}(t)$ propagates along the z axis, that is parallel to the P–S bond of the preoriented molecule. The laser field $\vec{E}(t)$ is then constructed by using the techniques given in reference [6] from five linearly x - or y -polarized subpulses which in accord with the symmetry selection rules induce nearly perfect state selective torsional transitions. Specifically, the dipole components μ_x and μ_y have + and – symmetry, respectively;^[13] thus, the interacting linearly x - and y -polarized subpulses induce transitions only between states with identical or opposite symmetries, respectively. Projection of the resulting density $\rho(t)$ on the states Ψ_{vL} and Ψ_{vR} , respectively, yields the populations P_{vL} and P_{vR} , respectively.

The laser pulse which drives the initial racemate towards a (nearly) pure enantiomer is shown in Figure 2b. The corresponding five subpulses have amplitudes 1.446, 0.611, 1.76,

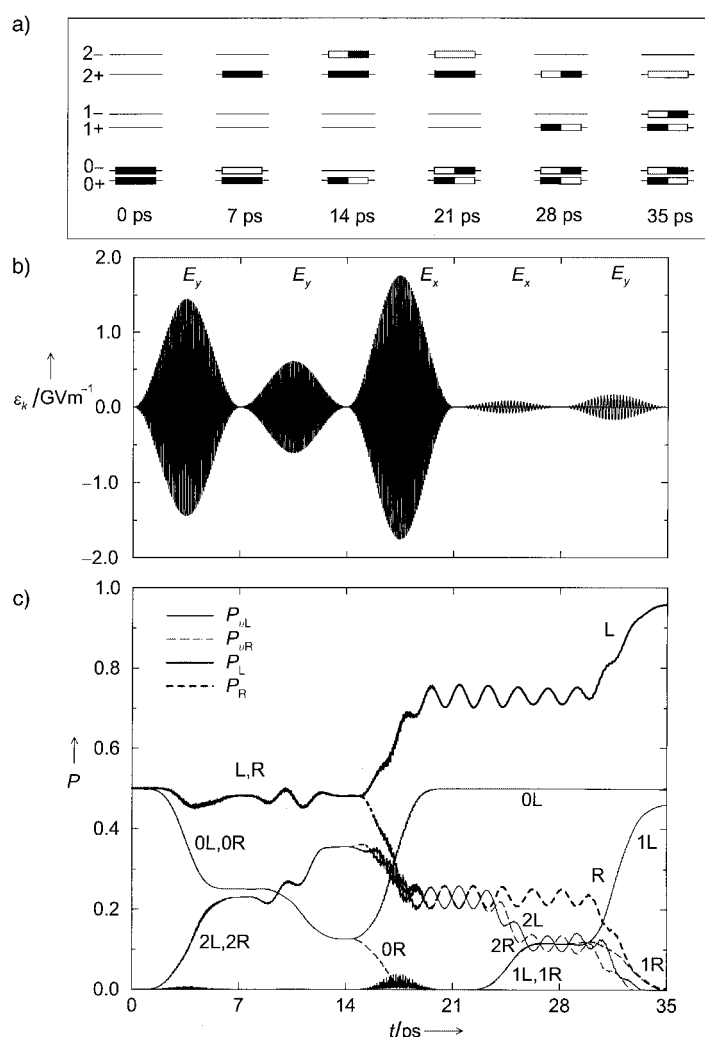


Figure 2. Selective preparation of the left enantiomer from a preoriented racemate of left and right atropisomers of the model H₂POSH, by means of a series of laser pulses. a) Selective transitions between the torsional states $\Phi_{v\pm}$, as induced by the subpulses (see b). b) Electrical fields of the linearly x - or y -polarized pulses. c) Resulting populations P_{vL} and P_{vR} of the torsional levels $v=0, 1, 2$, and the total populations P_L and P_R of the left (L) and right (R) enantiomers; marginal populations of more excited delocalized levels are not shown.

0.08, 0.17 GVm⁻¹, frequencies 352.75, 373.36, 372.7, 160.0, 161.0 cm⁻¹, phase shifts 0, 0, 25, 0, 180°, and durations of 7 ps. The resulting populations of the torsional states v of the left and right enantiomers are shown in Figure 2c. At the end of the laser pulse, the total population of the left enantiomer is close to 1, while the population of the right enantiomer is close to zero; the remaining small fraction is accounted for in the population of delocalized torsional states.

The underlying mechanism for the preparation of the left enantiomer is illustrated by the selective transitions induced by the five sequential subpulses, as indicated in Figure 2a. The first, y -polarized subpulse almost completely transfers the population of the initial state Φ_{0-} into the excited state Φ_{2+} , without influencing the population of the other initial state Φ_{0+} . Next, the second and third y - and x -polarized subpulses transfer the initial population of the other initial state Φ_{0+} into the ground state of the left enantiomer, Ψ_{0L} , while leaving the

“parking” population of state Φ_{2+} unaffected. This is achieved by first transferring half of the initial population of Φ_{0+} into the excited state Φ_{2-} and subsequently to Φ_{0-} , which then interferes coherently with the remaining fraction of Φ_{0+} leading to the formation of the target state of the left enantiomer Ψ_{0L} . Finally, the “parking” population of Φ_{2+} is dumped into the excited ($v=1$) state of the left enantiomer Ψ_{1L} , by means of the fourth and fifth subpulses which create the corresponding coherent superposition of states Φ_{1+} and Φ_{1-} , respectively, without influencing the population of Ψ_{0L} .

The net effect is conversion of the undesirable right portion of the racemate from its torsional ground state to the left enantiomer in its first torsional excited state, while keeping the initial fraction of the desired enantiomer in its torsional ground state. This is accomplished by transferring the incoherent population of two initial states (the racemate) into an incoherent superposition of two coherent states (the left enantiomer), where the coherences in each of the doublet states are introduced by the second to fifth subpulses. After the end of the laser pulses, the pure enantiomer tunnels back and forth towards the opposite enantiomer. Therefore, the resulting enantiomer selectivity remains, however, only for half of the tunneling time ($\tau_1/2$). This time is long enough, however, for subsequent laser control or reactions which may stabilize the target pure enantiomer.[16, 17]

Tunneling is also present during the laser pulse, but it does not impair the final localization, because it is exceedingly slow (630 ps) for Ψ_{0L} and, moreover, the more rapid tunneling of the intermediate excited states Ψ_{1L} and Ψ_{1R} or Ψ_{2L} and Ψ_{2R} compensate each other since their populations are designed to be nearly equal.

In conclusion, a preoriented racemate can be converted into a pure enantiomer by means of linearly polarized laser pulses. In our simulation the temperature is so low that only the lowest doublet of torsional states is populated. Extensions to higher temperatures are however straightforward, and involve transitions to other “parking” states followed by sequential transfers into appropriate excited states of the target enantiomer. Our approach can also be extended to rotating molecules, for example by means of alignment along the P–S axis but allowing free rotation around it.[21] The linear polarized laser pulses which define a left- or right-handed coordinate system in our model case, are then replaced by circular polarized laser pulses. Additional details of this extended general model will be published elsewhere. The present results should also serve as a reference for more complex situations such as the inclusion of the effects of intramolecular vibrational redistribution.

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Bis(pentafluorophenyl)xenon, $\text{Xe}(\text{C}_6\text{F}_5)_2$: A Homoleptic Diorganoxenon Derivative**

Nicola Maggiora, Dieter Naumann,* and
Wieland Tyrra

*Dedicated to Prof. Dr. Herbert Jacobs
on the occasion of his 65th birthday*

In 1989, pentafluorophenylxenon borates were synthesized as first examples of organoxenon derivatives.^[1, 2] In the following years, numerous new Xe–C compounds with the structural element $[\text{Xe}–\text{C}]^+$ were synthesized and characterized.^[3–8] The only $\text{C}_6\text{F}_5\text{Xe}^{\text{II}}$ compounds with two covalent bonds are $\text{C}_6\text{F}_5\text{XeCl}$, $[(\text{C}_6\text{F}_5\text{Xe})_2\text{Cl}]^+[\text{AsF}_6]^-$,^[9] and $\text{C}_6\text{F}_5\text{XeO-COC}_6\text{F}_5$.^[10] The existence of a xenon derivative with two xenon–carbon bonds has not yet been confirmed. Already in 1979, $\text{Xe}(\text{CF}_3)_2$ was described,^[11] however, these results could not be reproduced and are therefore the subject of controversy.

Investigations of the substitution reactions of XeF_2 suggested that the synthesis of a compound containing the fragment C–Xe–C and stable enough for spectroscopic detection should be possible by the reactions of XeF_2 and

[*] Prof. Dr. D. Naumann, Dr. N. Maggiora, Dr. W. Tyrra
Institut für Anorganische Chemie
Universität Köln
Greinstrasse 6, 50939 Cologne (Germany)
Fax: (+49) 221-470-5196
E-mail: d.naumann@uni-koeln.de

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arylsilanes, although in previous reports on similar reactions no concrete spectroscopic evidence for xenon–carbon compounds was mentioned.^[5, 12] The reaction of XeF_2 and $\text{Me}_3\text{-SiCF}_3$ yielded C_2F_6 as the major product, this result can be interpreted in terms of the formation of the intermediate $\text{Xe}(\text{CF}_3)_2$.^[13] Pentafluorophenyl derivatives should exhibit an increased thermal stability compared with non-aromatic perfluoroorganoxenon compounds. For this reason, we investigated the reaction of XeF_2 and $\text{Me}_3\text{SiC}_6\text{F}_5$.

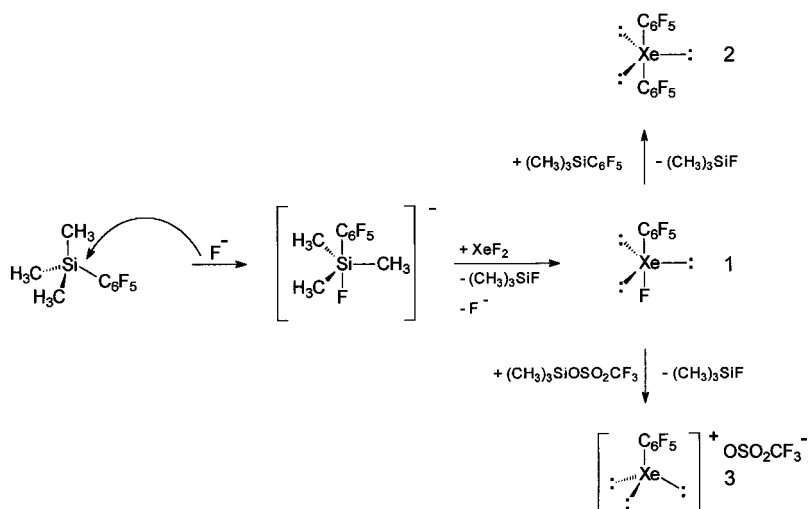
From the reactions of $\text{Me}_3\text{SiC}_6\text{F}_5/[\text{Me}_4\text{N}]\text{F}$ and XeF_2 in propionitrile, propionitrile/acetonitrile, acetonitrile, or CH_2Cl_2 , $\text{Xe}(\text{C}_6\text{F}_5)_2$ (**2**), the first [10-Xe-2] species with two xenon–carbon bonds is formed (Scheme 1) as a colorless solid that decomposes spontaneously at 20°C . The reactions proceed analogously to the fluoride-initiated trifluoromethylations^[14] via the reactive intermediates $[\text{Me}_3\text{Si}(\text{C}_6\text{F}_5)\text{F}]^-$ and $[\text{Me}_3\text{Si}(\text{C}_6\text{F}_5)_2]^-$, respectively. In the course of the reactions, $\text{C}_6\text{F}_5\text{XeF}$ (**1**) is formed as an intermediate which has been characterized by NMR spectroscopy. The by-products formed are $(\text{C}_6\text{F}_5)_2$ and $\text{C}_6\text{F}_5\text{H}$, the latter is formed by reactions of **2** and the solvent.

Solutions of **2** in the basic solvent MeCN are rather unstable at -40°C and decompose quantitatively within 24 h to give $(\text{C}_6\text{F}_5)_2$ and small amounts of $\text{C}_6\text{F}_5\text{H}$. Above -20°C an uncontrollable, spontaneous reaction occurs. In contrast, solutions of **2** either in a mixture of propionitrile/MeCN or in propionitrile, dichloromethane, or $[\text{D}_6]\text{acetone}$ are stable at -65°C for several days. Above -45°C a continuous increase in the formation of $(\text{C}_6\text{F}_5)_2$, $\text{C}_6\text{F}_5\text{H}$, and $\text{C}_6\text{F}_5\text{D}$, respectively, was observed. The solubility of **2** is significantly better in MeCN and CD_3CN than in EtCN, CH_2Cl_2 , or acetone.

Attempts to prepare **1** as a pure substance have so far been unsuccessful. Compound **1** always contained small quantities of **2** (<5%). The reaction of **1** and $\text{Me}_3\text{SiOSO}_2\text{CF}_3$ yields **3**. This reaction suggests that **3** is an excellent tool for the synthesis of further covalent $\text{C}_6\text{F}_5\text{Xe-R}$ compounds.

Compound **2** reacts with elemental mercury in MeCN between -40°C and room temperature to give $\text{Hg}(\text{C}_6\text{F}_5)_2$ as well as $(\text{C}_6\text{F}_5)_2$ and traces of $\text{C}_6\text{F}_5\text{H}$. All the products are unambiguously identified by their NMR spectra.

The NMR spectra of **2** shows an $[[\text{AC}]_2\text{B}]_2\text{X}$ spin system. The splitting of the $[[\text{AC}]_2\text{B}]_2$ part is comparable to that of an



$[[\text{AZ}]_2\text{M}]_2$ system. To aid analysis of the ^{129}Xe NMR spectrum, the $[[\text{AZ}]_2\text{M}]_2$ part of the model substance, $(\text{C}_6\text{F}_5)_2\text{SiBr}_2$,^[15] is used, the effects of higher order on the resonance signals of the ^{129}Xe spectrum are small and can be neglected. The absolute values determined from the analysis of the ^{129}Xe NMR spectrum of **2** ($[\text{D}_6]\text{acetone}$, -58°C ; Figure 1 A) are $^3J(\text{Xe},\text{F}) = 45.1\text{ Hz}$, $^4J(\text{Xe},\text{F}) = 34.7\text{ Hz}$, and $^5J(\text{Xe},\text{F}) = 9.5\text{ Hz}$. The calculated ^{129}Xe NMR spectrum (C) matches well with the measured ones (A and B).

In the ^{19}F NMR spectrum of **1**, the resonance signal of the fluorine atom directly bound to xenon is detected in propionitrile/MeCN at $\delta = 2.3$ (Figure 2) and in CH_2Cl_2 at $\delta = -1.6$ as a ψ -septet with splittings of 9.8 Hz. In MeCN solution, this signal is detected as a broad resonance at

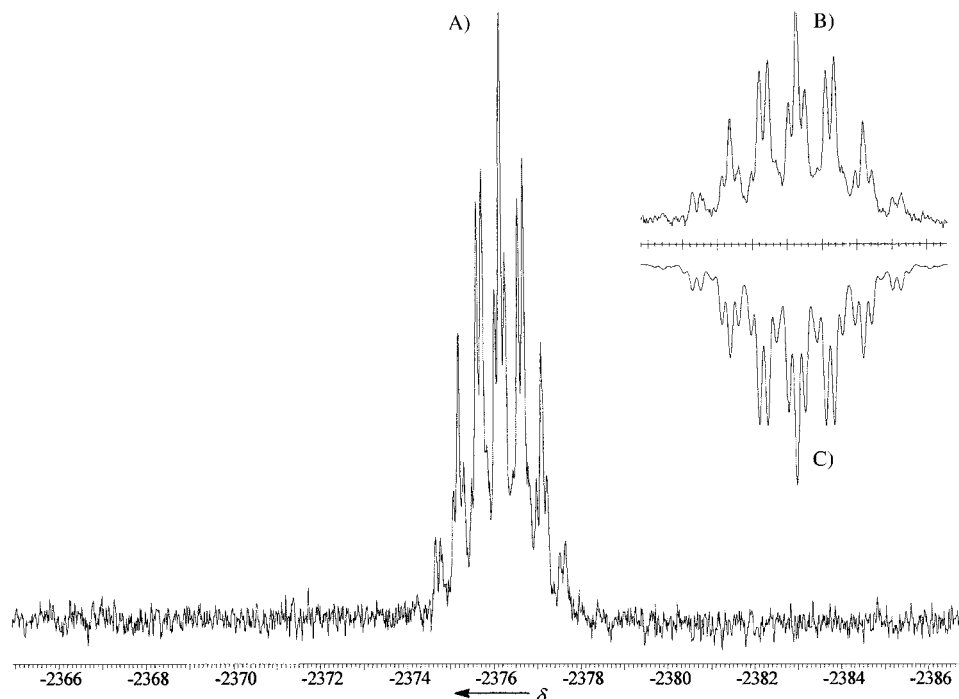


Figure 1. A) ^{129}Xe NMR spectrum of **2** measured in $[\text{D}_6]\text{acetone}$ at -58°C ; B) section of the spectrum determined by experiment; C) corresponding section of the simulated experiment.

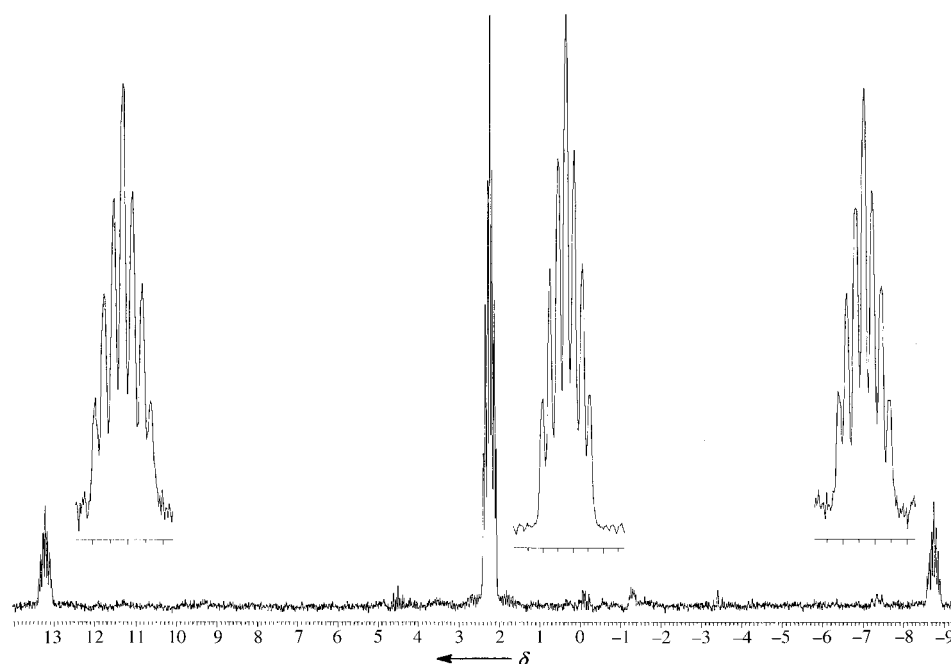


Figure 2. ^{19}F NMR spectrum of **1** (FXe part) in EtCN/MeCN at -58°C .

$\delta = 4.5$ which is converted, after addition of $[\text{Me}_4\text{N}]\text{F}$, into a poorly resolved multiplet with a line width of $\tau_{1/2} = 10$ Hz. This suggests by analogy to the pentafluoroxenate(iv) anion^[16] a rapid exchange of the terminal fluorine atom in the basic good-coordinating solvent MeCN. The ^{129}Xe NMR signal of **1** in acetonitrile is detected at $\delta = -2004.3$ and as expected is split into a doublet $^1J(\text{Xe},\text{F}) = 4099.6$ Hz of triplets $^3J(\text{Xe},\text{F}) = 82$ Hz of multiplets.

The comparison of the ^{19}F and ^{129}Xe NMR spectroscopic data of the ion $[\text{C}_6\text{F}_5\text{Xe}]^+$ (**3**)^[1,2] with that of $\text{C}_6\text{F}_5\text{XeO-COC}_6\text{F}_5$,^[10] $\text{C}_6\text{F}_5\text{XeCl}$, $[(\text{C}_6\text{F}_5\text{Xe})_2\text{Cl}][\text{AsF}_6]$,^[9] **1**, and **2** (Table 1) is noteworthy. With increasing covalency, the resonance signals of the *p*-fluorine are significantly shifted to higher field. This effect is strongly marked by $\Delta\delta \approx 11$ of **2** compared with the cation and $\Delta\delta \approx 5$ and 6, respectively, compared with the unsymmetrically substituted derivatives.

This high-field shift implies a decrease of mesomeric interactions between the C_6F_5 group and the Xe^{II} center.^[10] The high-field shift of the ^{129}Xe resonance signal of **2** and the simultaneous low-field shift of the C-1 resonance compared

with data of other pentafluorophenylxenon derivatives (Table 1) verify that the effective charge at the xenon atom is significantly lower and at the C-1 atoms is significantly higher than in all the compounds known so far.

Experimental Section

2: XeF_2 (1.10 g, 6.50 mmol) was suspended in a Schlenk tube at -78°C in the appropriate solvent (40 mL, EtCN; EtCN/MeCN (2:1); MeCN (-40°C); CH_2Cl_2). $\text{Me}_3\text{SiC}_6\text{F}_5$ (3.43 g, 2.72 mL, 14.29 mmol) was added. The stirred mixture was warmed to -60°C ; with MeCN all manipulations were made at -40°C . To this suspension precooled $[\text{Me}_4\text{N}]\text{F}$ (0.01 g, 0.11 mmol) was added.^[14] The mixture was warmed to -40°C for a short period and then cooled to -60°C . A transparent, colorless solution was formed. Additional stirring for 4 h at -60°C led to the formation of **2** as a white precipitate,

whereas the educts XeF_2 and $\text{Me}_3\text{SiC}_6\text{F}_5$ vanished completely (^{19}F NMR). To this suspension, with the exception of the solvent MeCN, pentane precooled to -78°C was added to complete the precipitation of **2**. The solution was decanted and the remaining solid was washed several times with pentane. In contrast to **2**, the by-products $(\text{C}_6\text{F}_5)_2$, Me_3SiF , and $\text{C}_6\text{F}_5\text{H}$ are readily soluble in pentane and are therefore easily removed. The product **2** was dried at 10^{-3} mbar and -50 to -35°C and obtained as a colorless solid. Compound **2** was dissolved in $[\text{D}_6]\text{acetone}$ and investigated by NMR spectroscopy (^{129}Xe , ^{19}F , ^{13}C). **Caution!** Even very small amounts of impurities lower the decomposition temperature of the isolated solid **2**. Crystallization was unsuccessful.

Compound **1** was obtained as the major product under analogous conditions with a stoichiometry $\text{XeF}_2:\text{Me}_3\text{SiC}_6\text{F}_5 = 1:1$.

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Table 1. NMR spectroscopic data of selected pentafluorophenylxenon compounds.^[a]

Compound	$\delta(\text{F-2,6})$	$\delta(\text{F-3,5})$	$\delta(\text{F-4})$	$\delta(\text{Xe})$	$^3J(\text{Xe},\text{F})$	$\delta(\text{C-1})$	$^1J(\text{Xe},\text{C})$	Solvent (T [$^\circ\text{C}$])
$[\text{C}_6\text{F}_5\text{Xe}][\text{B}(\text{C}_6\text{F}_5)_2\text{F}_2]^{[2b]}$	–125.2	–154.7	–142.0	–1980	69	82.8	119	MeCN/(–30)
$\text{C}_6\text{F}_5\text{XeOCOC}_6\text{F}_5^{[b],[10]}$	–128.3	–154.8	–144.7	–2030	86	91.3	n.b.	CD_2Cl_2 /(–10)
$[(\text{C}_6\text{F}_5\text{Xe})_2\text{Cl}][\text{AsF}_6]^{[9]}$	–128.6	–155.5	–144.3	–2116	81	92.9	195	CD_3CN /(–40)
$\text{C}_6\text{F}_5\text{XeF}$ 1	–129.3	–157.8	–148.4 ^[c]	–2004	82			MeCN/(–30)
	–128.8	–156.1	–146.5 ^[d]	–1994	80			CH_2Cl_2 /(–30)
	–129.9	–158.1	–148.6 ^[e]		81			EtCN/MeCN/(–58)
	–130.8	–155.5	–146.2	–2295	94	101.6	208	CD_2Cl_2 /(–60)
$\text{Xe}(\text{C}_6\text{F}_5)_2$ 2	–131.4	–158.4	–153.4	–2376	45 ^[f]	123.2 ^[g]	320	$(\text{CD}_3)_2\text{CO}$ /(–58)

[a] NMR spectra were recorded by using Bruker AMX 300 and AC 200 spectrometers. Chemical shifts were determined with respect to external XeF_2 (^{129}Xe), CCl_3F (^{19}F), and TMS (^{13}C). [b] $\delta(^{129}\text{Xe})$ of $\text{C}_6\text{F}_5\text{XeOCOC}_6\text{F}_5$ calculated according to ref. [17]: $\delta(\text{XeF}_2) = \delta(\text{XeF}_2\text{O}) + 1822$. [c] $\delta = 4.5$ (tm, broad, $^1J(\text{Xe},\text{F}) = 4099.6$ Hz; FXe). [d] $\delta = -1.6$ (tm, $^1J(\text{Xe},\text{F}) = 4035.9$ Hz; FXe). [e] $\delta = 2.3$ (*ψ*-sept, $^1J(\text{Xe},\text{F}) = 4125.0$ Hz; FXe). [f] $^4J(\text{Xe},\text{F}) = 34.7$ Hz; $^5J(\text{Xe},\text{F}) = 9.5$ Hz. [g] $^{13}\text{C}\{^{19}\text{F}\}$ NMR: $\delta = 143.4$ (s, $^2J(\text{Xe},\text{C}) = 148$ Hz; C-2,6), 136.7 (s; C-3,5), 141.1 (s; C-4).

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C₆F₅XeF, A Key Substrate in Xenon–Carbon Chemistry: Synthesis of Symmetric and Asymmetric Pentafluorophenylxenon(II) Derivatives**

Hermann-Josef Frohn* and Michael Theißen

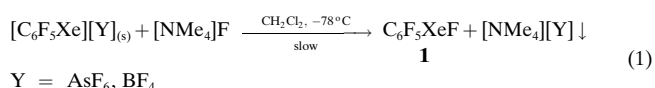
Salt-like compounds with a C–Xe bond in the cationic part [RXe]⁺ have been known since 1989, where R represents an aryl,^[1] alkenyl,^[2] or alkynyl group^[3]. In contrast C₆F₅XeO₂CC₆F₅,^[4] C₆F₅XeCl and [(C₆F₅Xe)₂Cl]⁺^[5] contain weaker covalent C–Xe bonds (3c–4e bonds^[6], asymmetric, hypervalent bonds with different distinct heteropolar components). The existence of the symmetric, hypervalent, molecular compound Xe(CF₃)₂^[7] is extremely doubtful, and unambiguous proof of its constitution has not yet been provided.

[*] Prof. Dr. H.-J. Frohn, Dipl.-Chem. M. Theißen
Fachgebiet Anorganische Chemie
Gerhard-Mercator-Universität Duisburg
Lotharstrasse 1, 47048 Duisburg (Germany)
Fax: (+49)203-379-2231
E-mail: frohn@uni-duisburg.de

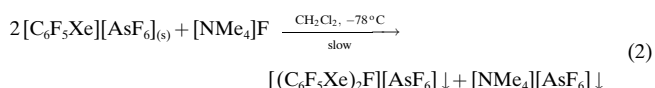
[**] This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemische Industrie.

On the basis of high values of group electronegativity, for example, Xe(C₆F₅)₂ and Xe(CN)₂ should be favored for a new class of symmetric C–Xe–C molecules, since their C(1) atom is part of a polarizable π system and the strongly electron-withdrawing components make the C–ligand electron-poor.

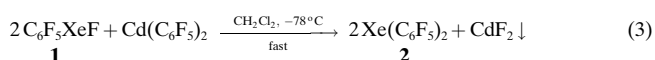
Herein we present a new concept for the synthesis of covalent C₆F₅Xe–C and C₆F₅Xe–Y compounds, specific examples being Xe(C₆F₅)₂ and C₆F₅XeCN^[8]. This concept is based on the new molecule C₆F₅XeF (**1**)^[9] which is the decisive key substrate. The asymmetric hypervalent molecule **1** is formed in 70 % yield as the soluble product of the heterogeneous low-temperature reaction of [C₆F₅Xe]⁺ salts with “naked” fluoride [NMe₄]F in CH₂Cl₂ [Eq. (1)]. The alternative approach, the electrophilic substitution of E in C₆F₅–E with [FXe]⁺ does not lead to **1**, because the oxidation potential of [FXe]⁺ is too high even for fluoroaromatics.



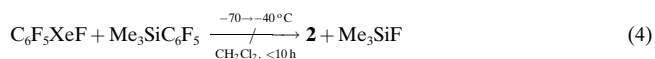
If the soluble source of fluoride in Equation (1) is used in a smaller than stoichiometric amount, multinuclear fluoro-bridged xenonium species result [Eq. (2)]:



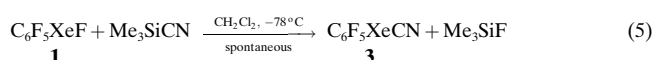
Ab initio calculations (comparison in the gas phase) show that the C–Xe distance in **1** is longer than in the [C₆F₅Xe]⁺ ion and the Xe–F distance is greater than in XeF₂. The latter feature makes the F[–] ion a good leaving group. The permanent dipole moment in **1** makes the successful attack of nucleophiles on the electrophilic Xe center easier. With Cd(C₆F₅)₂ as an aryl transfer reagent it is possible to introduce a second aryl group into **1** [Eq. (3)]:



The direct introduction of the C₆F₅ group into XeF₂ within the thermal-existence range of **2** is not successful with Cd(C₆F₅)₂, as the nucleophilicity of the aryl group (no permanent dipole moment) does not suffice for the substitution on XeF₂. The nucleophilicity of the aryl group in Me₃SiC₆F₅ in CH₂Cl₂ is not high enough for the successful F–C₆F₅ substitution in **1** [Eq. (4)] (see also ref. [10]):



However, with Me₃SiCN the CN group can be introduced successfully into **1** [Eq. (5)]:



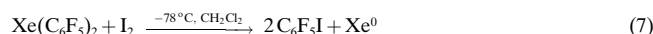
We attribute the different reactivities of Me₃SiC₆F₅ and Me₃SiCN more to differences in Lewis acidity than to steric effects.

The symmetric C-Xe-C molecule **2** as well as the asymmetric molecules **1** and **3** are soluble in polar, weak-coordinating solvents, such as CH₂Cl₂. When dissolved in basic, strongly coordinating MeCN compound **1** shows no heterolysis to the [C₆F₅Xe]⁺ and F[−] ions. Compounds **1–3** are unstable at room temperature and even at −78 °C their CH₂Cl₂ solutions decompose within a few weeks. The products of decomposition point to homolytic breakage of bonds followed by radical recombinations and radical attacks on the solvent.

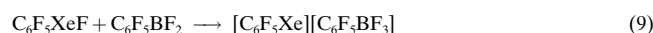
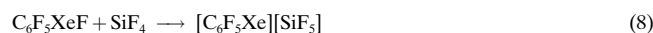
Both electronegative aryl groups in **2** show a relatively high anionic character. In the superacidic solvent aHF (anhydrous HF) one of the aryl groups can be quantitatively split off through electrophilic attack forming equimolar amounts of [C₆F₅Xe]⁺ and C₆F₅H [Eq. (6)]:



In the presence of I₂ compound **2** behaves as an arylating agent [Eq. (7)]:



The reactions of **3** with I₂ or HF proceed in a more complex manner because of the inequivalent C–Xe bonds. The reaction of the C₆F₅ group in **3** was monitored by ¹⁹F NMR spectroscopy and the products C₆F₅I, C₆F₅H, and C₆F₅CN (5:3:1) or [C₆F₅Xe]⁺, C₆F₅H, and C₆F₅CN (4:1:3) were detected. As a result of its good fluoride donor ability **1** reacts even with weak Lewis acids such as SiF₄ or C₆F₅BF₂ [Eqs. (8), (9)]:



The constitution of **1–3** was confirmed by heteronuclear NMR spectroscopy (¹⁹F, ¹²⁹Xe, ¹³C, ¹⁵N; Table 1) in CH₂Cl₂ solutions. The coupling constants were determined in some cases by ¹⁹F- or ¹²⁹Xe-decoupling experiments. The ¹²⁹Xe NMR spectrum of **1** shows the large ¹J_{Xe,F} (doublet, 4014 Hz) and the smaller ³J_{Xe,o-F} coupling (triplet, 82 Hz) that confirm unambiguously the constitution of **1**. In the ¹²⁹Xe NMR spectrum of **2** the resonance signal at δ = −4152 (the lowest frequency of a Xe^{II} species!) appears as a not fully resolved multiplet. The accompanying o-F signal at δ = −133.05 shows ¹²⁹Xe satellites of appropriate intensity. The ³J_{F,Xe} coupling is determined (by using selective m-F decoupling) to be 43 Hz. The ¹²⁹Xe NMR spectrum of C₆F₅XeCN shows a triplet (³J_{Xe,F} = 86 Hz) at δ = −3883.2. In the labeled compound C₆F₅Xe¹³CN (Figure 1) an additional doublet (¹J_{Xe,C} = 1060 Hz) is observed. In the labeled compound C₆F₅XeC¹⁵N the ¹²⁹Xe NMR resonance signal (¹⁹F decoupled) appears as a doublet (²J_{Xe,N} = 21 Hz).

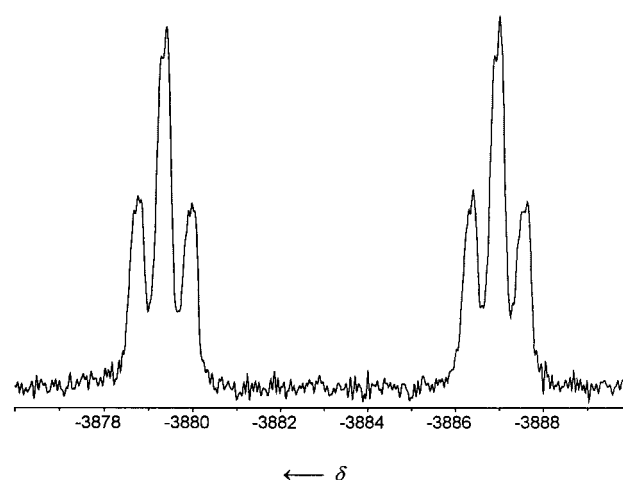


Figure 1. ¹²⁹Xe NMR spectrum of C₆F₅Xe¹³CN in CD₂Cl₂ at −78 °C.

Table 1. NMR spectroscopic characterization of compounds **1–3**[a].

¹⁹ F NMR		δ(o-F)	δ(p-F)	δ(m-F)	δ(Xe-F)	³ J _{p-F}	³ J _{F,Xe}	⁴ J _{F,Xe-F}	¹ J _{F,Xe}
RXeF	1	−129.35	−146.87	−156.49	−3.50 ^[b]	20	81 ^[c]	19 ^[c]	4010
RXeR	2	−133.05	−154.14	−159.04		21	43 ^[c]		
RXeCN	3	−131.54	−147.50	−156.39		21	87		
¹³ C[¹⁹ F] NMR		δC(1)	δC(2,6)	δC(3,5)	δC(4)	¹ J _{C,Xe}	² J _{C(1),o-F}	² J _{C,Xe-F}	³ J _{C,Xe-F}
RXeF	1	86.35	143.90	137.14	143.09	111 ^[d]	28	115 ^[d]	ca. 6 ^[c]
RXeR	2	122.59	143.01	136.31	140.64				
RXeCN	3 ^[e]	103.07	143.11	137.10	142.82	66			
¹²⁹ Xe		Xe				³ J _{Xe,F}	¹ J _{Xe,F}		
RXeF	1	−3789.2				82	4014		
RXeR	2	−4152 ^[f]							
RXeCN	3	−3883.2				86			
selected δ and J-values of the labeled compound 3 :									
¹³ C		δCN				¹ J _{CN,Xe}	² J _{CN,C(1)}		
RXe ¹³ CN		125.68				1060	142		
¹⁵ N		δCN				² J _{N,Xe}			
RXeC ¹⁵ N		123.7				22			

[a] R = C₆F₅. The NMR measurements proceeded in FEP sample tube liners (FEP = tetrafluoromethylene hexafluoropropylene copolymer) in CD₂Cl₂ at −78 °C with a Bruker AVANCE-DRX-500 spectrometer. The absolute values of the coupling constants J in Hz. The ¹⁹F, ¹²⁹Xe, ¹³C, and ¹⁵N chemical shift values are relative to the standards C₆F₆ (δ(CCl₃F) = −162.9 ppm), XeOF₄ (24 °C), TMS, and CD₃NO₂ (24 °C), respectively, at the corresponding measurement temperature. [b] s, br, τ_{1/2} becomes smaller after addition of F[−] ions.^[11] [c] From ¹⁹F-decoupling experiments. [d] From insensitive nuclei enhancement by polarization transfer (INEPT) experiments. [e] δ(CN) = 125.22. [f] m, τ_{1/2} ≈ 150 Hz.

Table 2. Calculated (Gaussian 94, RHF, LANL2DZ) geometric parameters and charges (Mulliken) of C_6F_5Xe-Z molecules^[a].

C_6F_5Xe-Z	Molecule	Sym.	Selected geometric parameters ^[b]			Selected Mulliken charges			
			C(1)-Xe	Xe-Z	C(2)-C(1)-C(6)	Xe	C_6F_5	C(1)	Z
C_6F_5Xe-F	1	C_s	2.20	2.13	117.7	1.148	-0.415	-1.001	-0.733
$C_6F_5Xe-C_6F_5$	2	C_1	2.34	2.34	117.4	0.980	-0.490	-0.687	-0.490
C_6F_5Xe-CN	3	C_s	2.24	2.38	118.1	0.967	-0.403	-0.853	-0.564 ^[c]
for comparison see ref [12]									
$C_6F_5Xe \cdots F-AsF_5$		$\approx C_s$	2.12	2.56	121.3	1.083	-0.134	-1.032	-0.948
$[C_6F_5Xe]^+$		C_s	2.16	—	122.7	0.886	0.114	-0.870	—
$FXeF$		$D_{\infty h}$	—	2.03	—	1.306	—	—	-0.653

[a] Z = second ligand bound to Xe^{II} ; [b] in [Å] or [°], respectively; [c] Mulliken charges of C in the CN ligand: -0.466.

The results of ab initio calculations for **1–3** show the following sequence of C-Xe distances: **2** > **3** > **1**, thus opposite to the sequence of Mulliken charges of the ligand Z in C_6F_5XeZ (Table 2). The comparison of data for **1** and $C_6F_5Xe \cdots FAsF_5$ ^[12] elucidates clearly the change when going from the asymmetric hypervalent C-Xe-F bond to a significant C-Xe $\cdots$ F contact: the negative charge on Z gets closer to -1 whereas the negative charge of the C_6F_5 group decreases significantly. The high anionic character of the C_6F_5 group in **1–3** agrees with the observed reactivities towards electrophiles and explains the lower-frequency chemical shifts of the p-F atom compared to $[C_6F_5Xe]^+$.

Experimental Section

1: A cold solution of $[NMe_4]F$ (25 mg, 0.27 mmol) in CH_2Cl_2 (1 mL) was added to a suspension of $[C_6F_5Xe][AsF_6]$ (131 mg, 0.27 mmol) in CH_2Cl_2 (1.5 mL) at $-78^\circ C$ in an 8 mm FEP trap. The suspension was stirred over 2 days at $-78^\circ C$ until all the fluoride was consumed. The mother liquor was separated from solid $[NMe_4][AsF_6]$ and the quantity of **1** was determined (^{19}F NMR): 0.19 mmol, 70%. The other reactions were usually performed directly with the cold solutions of **1**. By evaporating CH_2Cl_2 at 10^{-2} hPa/ $\leq -55^\circ C$ and later drying at $\leq -40^\circ C$ **1** was obtained as a colorless solid, which after warming to $20^\circ C$ decomposed totally within 4 h. In CH_2Cl_2 solution noticeable decomposition proceeded above $-30^\circ C$ with the formation of C_6F_5H and traces of C_6F_5Cl .

2: A cold solution of $Cd(C_6F_5)_2$ (17 mg, 0.04 mmol) in CH_2Cl_2 (0.5 mL) was added to a solution of **1** (0.08 mmol) in CH_2Cl_2 (1.5 mL) at $-78^\circ C$. After 5 min of stirring CdF_2 precipitated. The reaction was complete (^{19}F NMR) after further 10 min. The mother liquor was collected. In addition to **2** (0.06 mmol, 75%) the solution contained C_6F_5H (4 μ mol) and $(C_6F_5)_2$ (2 μ mol). The isolation of solid **2** was achieved as described for **1**. Solid **2** decomposes completely at room temperature within 1 h and in CH_2Cl_2 solution at $-40^\circ C$ within 9 h [$C_6F_5H:(C_6F_5)_2 = 1:0.1$].

3: A cold solution of Me_3SiCN (14 μ L, 0.10 mmol; or the labeled ^{13}CN and $C^{15}N$ derivatives) in CH_2Cl_2 (0.5 mL) was added to a solution of **1** (0.10 mmol) and CH_2Cl_2 (1.5 mL) at $-78^\circ C$ and stirred. After 5 min the reaction was complete (^{19}F NMR: quantitative reaction) giving a 1:1 mixture of **3** and Me_3SiF . CH_2Cl_2 and Me_3SiF were distilled at $\leq -55^\circ C/10^{-2}$ hPa and the white solid product was dried at $\leq -40^\circ C/10^{-2}$ hPa. The solid spontaneously decomposed during the rapid warming to room temperature. CH_2Cl_2 solutions of **3** decomposed completely at $-40^\circ C$ within 2 h with the formation of C_6F_5CN and C_6F_5H (4:1).

Checking the purity of the cold solid products after dissolution in CH_2Cl_2 at $-78^\circ C$ showed in the case of **1** and **2** degrees of decomposition of up to 10% and for the thermally more sensitive product **3** up to 30%.

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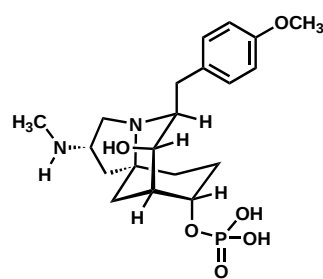
An Enantiospecific Synthesis of the Potent Immunosuppressant FR901483**

Goetz Scheffler, Hirofumi Seike, and Erik J. Sorensen*

Bond formations induced by phenol oxidations have a rich history in organic chemistry. The influential two-step synthesis of usnic acid by Sir Derek Barton and co-workers^[1] followed a set of simple rules that provided guidelines for rationalizing the course of oxidative phenolic radical couplings occurring in the biogeneses of a number of natural products.^[2] We were

[*] Prof. Dr. E. J. Sorensen, Dr. G. Scheffler, H. Seike
Department of Chemistry and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-2798
E-mail: sorensen@scripps.edu

[**] Dr. G. Scheffler and H. Seike contributed equally to this work. We thank Dr. L. B. Pasternack and Dr. D. H. Huang for NMR spectroscopic assistance, and Dr. G. Siuzdak for mass spectrometric assistance. We also thank Dr. S. Takase and Dr. H. Setoi from the Fujisawa Pharmaceutical Co. for physical data for FR901483. This work was generously supported by The Skaggs Institute for Chemical Biology, a grant from the Merck Research Laboratories, a Beckman Young Investigator Award to E.J.S., and a predoctoral fellowship from the Heiwa Nakajima Foundation to H.S.



1: FR901483

drawn to the possibility that the azaspiro[4.5]decane nucleus of the powerful immunosuppressant FR901483 (**1**)^[3] may form during the course of an oxidation of a tyrosine phenol moiety. This fungal-derived natural product comprises a novel tricyclic core framework and a salient monophosphate ester. At the outset, we noted that the intriguing structure of **1** seemed to conceal most of the atoms of two molecules of tyrosine and surmised that a laboratory synthesis of this substance could be facilitated by oxidative azaspiroannulation and intramolecular aldol addition reactions.^[4] This general analysis was guided by the supposition that such reaction processes may have counterparts in the biosynthesis of this natural product. Our goal was to evaluate the feasibility of these reactions in the course of a synthesis of **1**. In this communication, we describe our convergent, enantiospecific synthesis of this promising immunosuppressant from *L*-tyrosine, which was executed along these lines.^[5]

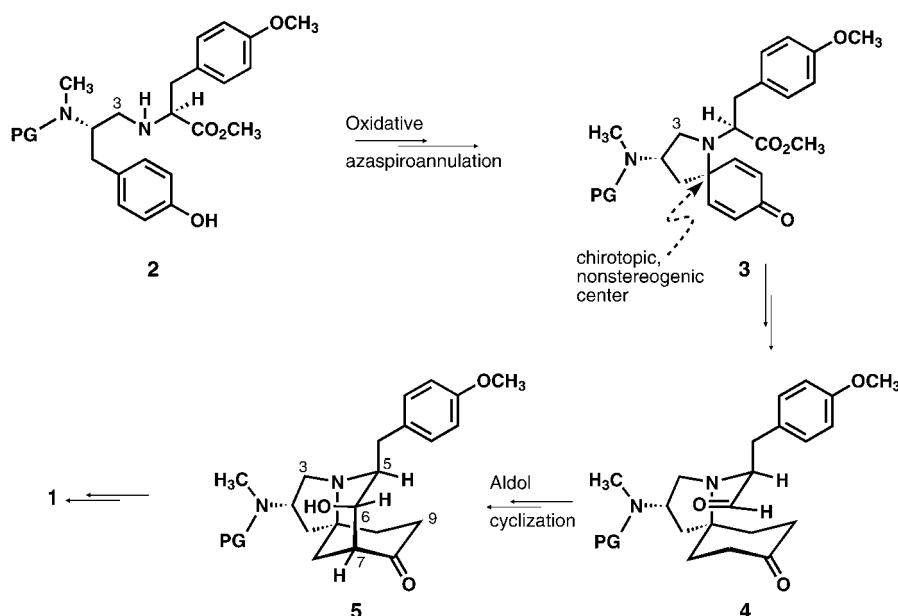
We anticipated that the N–C3 bond of an amino phenol **2** (Scheme 1) could be established in the course of a reductive coupling of two derivatives of *L*-tyrosine. The cornerstone of our strategy is the idea that exposure of **2** to an appropriate oxidant, such as iodobenzene diacetate,^[6] could give rise to **3**. This appealing ring-closure would create the azaspiro[4.5]decane substructure of **1** and would not be complicated by the formation of diastereoisomers.^[7–9] Our goal was to effect intramolecular carbon–nitrogen bond formations with substrates possessing the natural oxidation state at C-3, and we maintained our commitment to this plan even in the face of a potentially problematic oxidation of the secondary amine of **2**

by hypervalent iodine-based oxidants. To construct the remaining ring and C-6 stereocenter of **1**, we hoped to utilize an intramolecular aldol reaction (see **4**→**5**).^[10] Despite concerns about diastereoselectivity issues and potential epimerization problems, this process was deemed well-suited for the task of completing the architecture of **1**.^[4] A synthesis of **5** would then allow us to explore an uncommon strategy for introducing the conspicuous phosphate ester of **1** (see below). A laboratory synthesis of **1** based on these concepts was achieved as described below.

By straightforward reaction sequences, aldehyde **6** and amine **7** (Scheme 2) were created from commercially available *N*-Boc-*L*-tyrosine^[11] and subsequently joined under reducing conditions to give **2** (PG = *p*NO₂C₆H₄SO₂).^[12] After much experimentation, we found that exposure of a solution of **2** in 1,1,1,3,3,3-hexafluoro-2-propanol to iodobenzene diacetate at room temperature resulted in the formation of azaspiro[4.5]decadienone **3** in 51 % yield (based on 70 % consumed **2**). The outcome of this ring closure, which established a significant portion of the structure of **1**, is noteworthy because analogous amino phenols are converted into fused ring systems under similar reaction conditions.^[9c] At this stage, it was necessary to replace the 4-nitrobenzenesulfonamide protecting group^[13] in **3** with a more compatible *tert*-butoxycarbonyl group (**3**→**8**, Scheme 2). Treatment of a solution of **8** in EtOAc/EtOH with Raney nickel under an atmosphere of hydrogen caused a complete reduction of the dienone moiety and afforded a mixture of epimeric alcohols shown as **9**. A reduction of the methyl ester with lithium aluminum hydride then produced a mixture of diols that could subsequently be converted by Swern oxidation into a single ketoaldehyde **4** in good overall yield.^[14, 15]

Our intention was to create the C6–C7 bond and the remaining ring of **1** in the course of an aldol cyclization, although we were aware that this objective could be difficult to achieve owing to the various sites at which compound **4** could undergo deprotonation in the presence of bases. Nevertheless, we were mindful of the work of Snider and co-workers^[4] and could convert **4** into the desired compound **5** in 34 % yield under the conditions shown.^[16] When **4** was dissolved in THF and treated with pyrrolidine (0.3 equiv) and AcOH (1.2 equiv) at 0 °C→RT, the equatorial C-6 epimer of **5** was formed in 51 % yield.^[17] Despite the modest yield for the methoxide-induced aldol cyclization of **4**, we could procure significant quantities of hydroxyketone **5**.

Owing to its rigid and concave architecture, hydroxyketone **5** was converted into diol **10** in a completely diastereoselective fashion by hydrogenation. To achieve a synthesis of **1** from **10**, inversion of stereochemistry at C-8 and production of a C-8 monophosphate ester are required. Gratify-



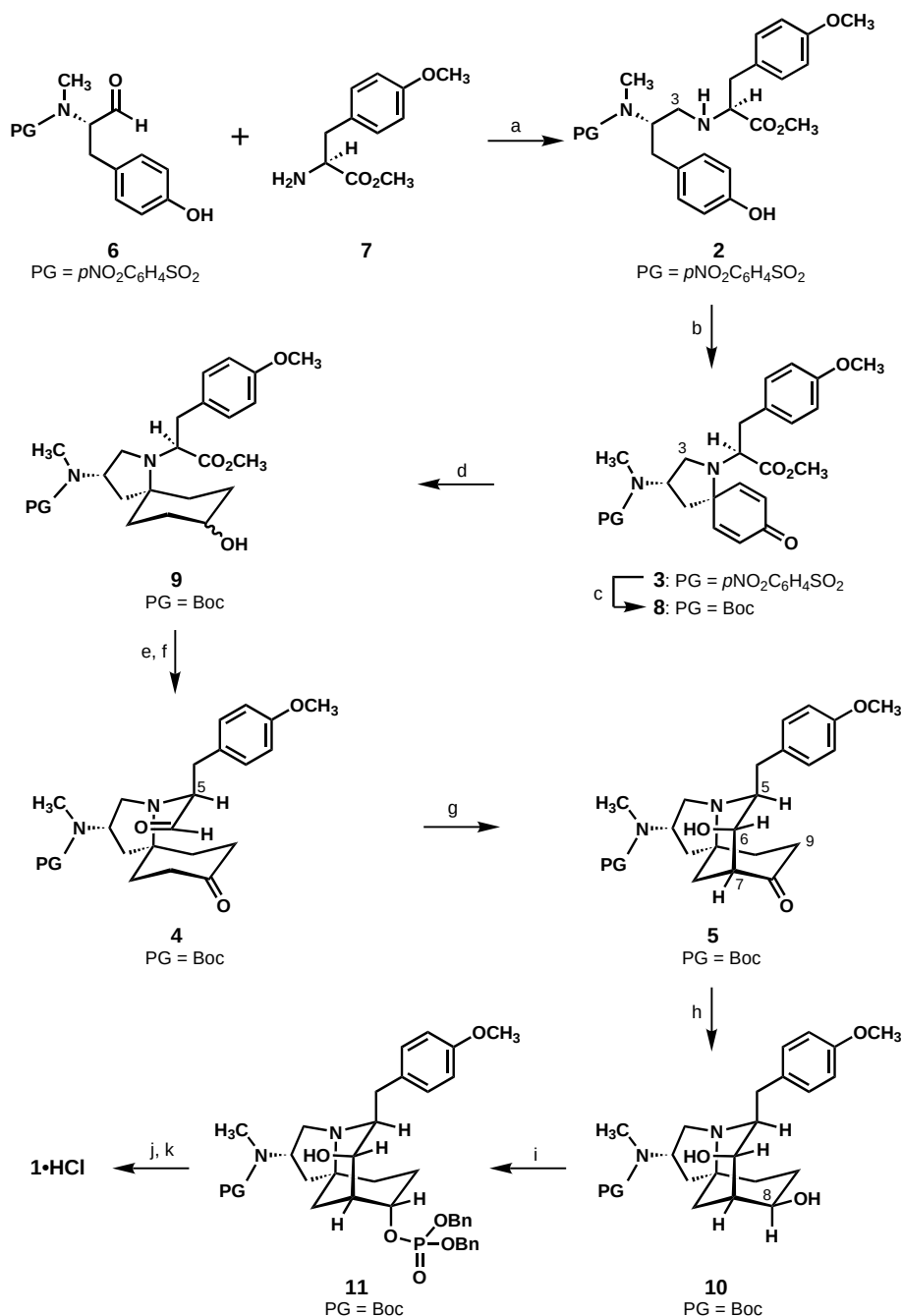
Scheme 1. A strategy for the synthesis of FR901483 (**1**) featuring oxidative azaspiroannulation and aldol cyclization reactions. PG = protecting group.

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- [2] For selected reviews, see: a) D. H. R. Barton, T. Cohen, *Festschr. Arthur Stoll* **1957**, 117–143; b) D. H. R. Barton, *Chem. Br.* **1967**, 330–337; c) A. I. Scott, *Q. Rev. Chem. Soc.* **1965**, 19, 1–35; c) T. Kametani, K. Fukumoto, *Synthesis* **1972**, 657–674; d) D. H. R. Barton, *Half A Century of Free Radical Chemistry*, Cambridge University Press, New York, **1993**, pp. 7–20.
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- [4] After we initiated our studies, Snider and co-workers demonstrated the feasibility of an intramolecular aldol addition in the course of a synthesis of demethylamino-FR901483 (see B. B. Snider, H. Lin, B. M. Foxman, *J. Org. Chem.* **1998**, 63, 6442–6443). In the following year, Snider and Lin reported the first synthesis and absolute stereochemistry of FR901483 (**1**) (see B. B. Snider, H. Lin, *J. Am. Chem. Soc.* **1999**, 121, 7778–7786). The authors of this pioneering report also postulated that an oxidative cyclization of a methylated tyrosine–tyrosine dipeptide may be a key step in the biosynthesis of **1**.
- [5] For an informative review of enantio-specific syntheses of heterocycles from α -amino acids, see: F. J. Sardina, H. Rapoport, *Chem. Rev.* **1996**, 96, 1825–1872.

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[8] Kita and co-workers pioneered oxidations of phenolic amides with hypervalent iodine reagents. See: a) Y. Tamura, T. Yakura, J.-i. Haruta, Y. Kita, *J. Org. Chem.* **1987**, 52, 3927–3930; b) Y. Kita, T. Yakura, H. Tohma, K. Kikuchi, Y. Tamura, *Tetrahedron Lett.* **1989**, 30, 1119–1120; c) Y. Kita, H. Tohma, K. Kikuchi, M. Inagaki, T. Yakura, *J. Org. Chem.* **1991**, 56, 435–438.



Scheme 2. Synthesis of FR901483 (**1**): HCl: a) **6** (1.0 equiv), **7** (1.5 equiv), NaBH(OAc)₃ (1.5 equiv), 4 Å MS, 0 °C, 1 h, then RT, 24 h, 80%; b) PhI(OAc)₂, (1.1 equiv), (CF₃)₂CHOH, RT, 10 min, 51% (based on 70% consumed **2**); c) NaSPh (1.5 equiv), DMSO, RT, 3 h; then (Boc)₂O (2 equiv), pyr (2 equiv), RT, 12 h, 71% from **3**; d) H₂, Raney Ni, EtOAc/EtOH (2:1), RT, 15 h, 93%; e) LiAlH₄ (3 equiv), THF, -78 °C, 1 h then 0 °C, 2 h, 90%; f) (COCl)₂ (8 equiv), DMSO (16 equiv), CH₂Cl₂, -78 °C, 30 min; then *i*Pr₂NH (20 equiv), -78 °C, 30 min; then 0 °C, 1 h, ≈100%; g) NaOMe (1.1 equiv), MeOH, 0 °C, 2 h, 34% of **5** (74% total yield of aldol adducts); h) H₂, Raney Ni, EtOAc/EtOH (1:10), RT, 4 h, 92%; i) dibenzyl hydrogen phosphate (6 equiv), tris(4-chlorophenyl)phosphine (3 equiv), DIAD (3 equiv), Et₃N (10 equiv), toluene, RT, 26 h, 37% of **11**+17% of alkene; j) H₂, Pd/C, MeOH, RT, 5 h, 95%; k) 4N HCl, dioxane, 0 °C, 30 min; then RT, 2 h, 84% of **1**: HCl (precipitated from MeOH with Et₂O). MS = molecular sieves, RT = room temperature, Boc = *tert*-butoxycarbonyl, pyr = pyridine, DIAD = diisopropyl azodicarboxylate, Bn = benzyl.

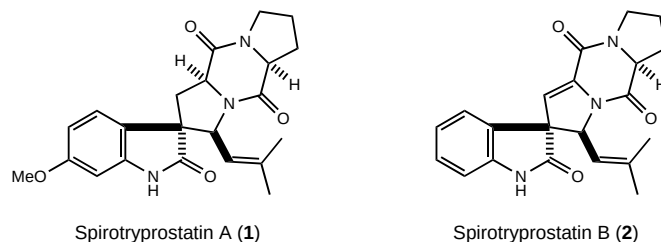
ingly, it was possible to accomplish both objectives in a single step by a Mitsunobu reaction between **10** and dibenzyl hydrogen phosphate (**10**→**11**).^[18, 19] Despite its modest yield, this type of reaction is, to our knowledge, unprecedented in complex natural-product total synthesis, can be achieved in

- [9] As we were attempting to achieve oxidative azaspiroannulations, Ciufolini and co-workers described novel oxidative cyclizations of oxazolines produced from tyrosine–tyrosine dipeptides, which thus demonstrated for the first time that oxidative azaspiroannulations are viable reaction processes. See: a) N. A. Braun, M. A. Ciufolini, K. Peters, E.-M. Peters, *Tetrahedron Lett.* **1998**, 39, 4667–4670; b) N. A. Braun, J. D. Bray, M. A. Ciufolini, *Tetrahedron Lett.* **1999**, 40, 4985–4988; c) N. A. Braun, M. Ousmer, J. D. Bray, D. Bouchu, K. Peters, E.-M. Peters, M. A. Ciufolini, *J. Org. Chem.* **2000**, 65, 4397–4408. This provocative reaction type is related to several interesting oxidative spiroannulations. For examples, see: d) A. V. R. Rao, M. K. Gurjar, P. A. Sharma, *Tetrahedron Lett.* **1991**, 32, 6613–6616; e) P. Wipf, Y. Kim, *Tetrahedron Lett.* **1992**, 33, 5477–5480; f) P. Wipf, Y. Kim, P. C. Fritch, *J. Org. Chem.* **1993**, 58, 7195–7203; g) P. Wipf, Y. Kim, D. M. Goldstein, *J. Am. Chem. Soc.* **1995**, 117, 11106–11112; h) A. McKillop, L. McLaren, R. J. K. Taylor, R. J. Watson, N. Lewis, *Synlett* **1992**, 201–203; i) A. McKillop, L. McLaren, R. J. K. Taylor, R. J. Watson, N. J. Lewis, *J. Chem. Soc. Perkin Trans. 1* **1996**, 1385–1393; j) K. Marshall Aubart, C. H. Heathcock, *J. Org. Chem.* **1999**, 64, 16–22; k) D. Yang, M.-K. Wong, Z. Yan, *J. Org. Chem.* **2000**, 65, 4179–4184.
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- [13] T. Fukuyama, C.-K. Jow, M. Cheung, *Tetrahedron Lett.* **1995**, 36, 6373–6374.
- [14] A. J. Mancuso, D. Swern, *Synthesis* **1981**, 165–185.
- [15] Aldehyde **4** was used in crude form because partial epimerization at C-5 occurred during chromatography on silica. For stimulating discussions of the stability and chemistry of optically active α -amino aldehydes, see: A. G. Myers, D. W. Kung, B. Zhong, *J. Am. Chem. Soc.* **2000**, 122, 3236–3237.
- [16] Compound **5** was the major component of a mixture of three aldol cyclization products. The equatorial C-6 epimer of **5** was formed in approximately 19% yield and a compound tentatively assigned as a C6–C9 aldol adduct was obtained in approximately 21% yield.
- [17] This aldol procedure affords **5** in 17% yield and a substance that we tentatively assigned as the C6–C9 aldol adduct in 12% yield (see ref. [16]). We have not yet been able to utilize the C-6 epimer of **5** in our synthesis.
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- [19] The results of COSY, HMQC, and ROESY NMR spectroscopy experiments were fully consistent with the structure of compound **11**. In addition to **11**, a C8–C9 alkene was formed in 17% yield due to elimination.
- [20] Mitsunobu reactions are sensitive to steric environments; therefore, site-selective modification of polyols is feasible. For examples, see: a) D. A. Evans, J. R. Gage, J. L. Leighton, *J. Am. Chem. Soc.* **1992**, 114, 9434–9453; b) R. S. Coleman, J. R. Fraser, *J. Org. Chem.* **1993**, 58, 385–392.

Total Synthesis of (–)-Spirotryprostatin B and Three Stereoisomers**

Larry E. Overman* and Mark D. Rosen

Small-molecule natural products play an important role in contemporary studies to understand and control cellular proliferation.^[1] From the fermentation broth of the fungus *Aspergillus fumigatus*, Osada and co-workers recently identified a group of novel diketopiperazine alkaloids that inhibit G2/M phase progression of the mammalian cell cycle at micromolar concentrations.^[2, 3] Spirotryprostatins A (**1**) and B (**2**) are the most complex of these alkaloids,^[2] all of which appear to arise biosynthetically by prenylation of a diketopiperazine derived from tryptophan and proline.



The novelty of their structures and the potential utility of cell-cycle inhibitors make the spirotryprostatins attractive targets for total synthesis. If such an undertaking were to be stereocontrolled, a central challenge would be to relate the stereochemistry of the quaternary spiro carbon to the adjacent stereocenter bearing the 2-methylpropenyl side chain. In 1998, Danishefsky and co-workers reported the total synthesis of spirotryprostatin A (**1**), which constituted the first total synthesis in this area.^[4] Earlier this year, the groups of Williams,^[5] Danishefsky,^[6] and Ganesan^[7] disclosed inaugural total syntheses of (–)-spirotryprostatin B (**2**).

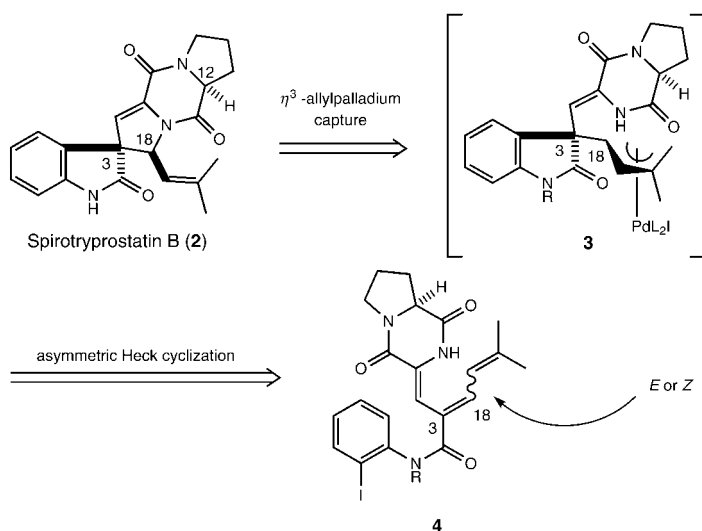
Our approach to spirotryprostatin B (**2**) and congeners is distinctly different from the previous syntheses (Scheme 1). The logic of our strategy is to correlate the relative configurations of C3 and C18 in **2**^[8] to the geometry of the internal double bond of a triene cyclization substrate **4** by capitalizing on the stereochemical selectivity of two palladium-catalyzed

[*] Prof. L. E. Overman, M. D. Rosen
Department of Chemistry
University of California, Irvine
516 Rowland Hall, Irvine, CA 92697-2025 (USA)
Fax: (+1) 949-824-3866
E-mail: leoverma@uci.edu

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



Scheme 1. Retrosynthesis of spirotryprostatin B (**2**). L = ligand.

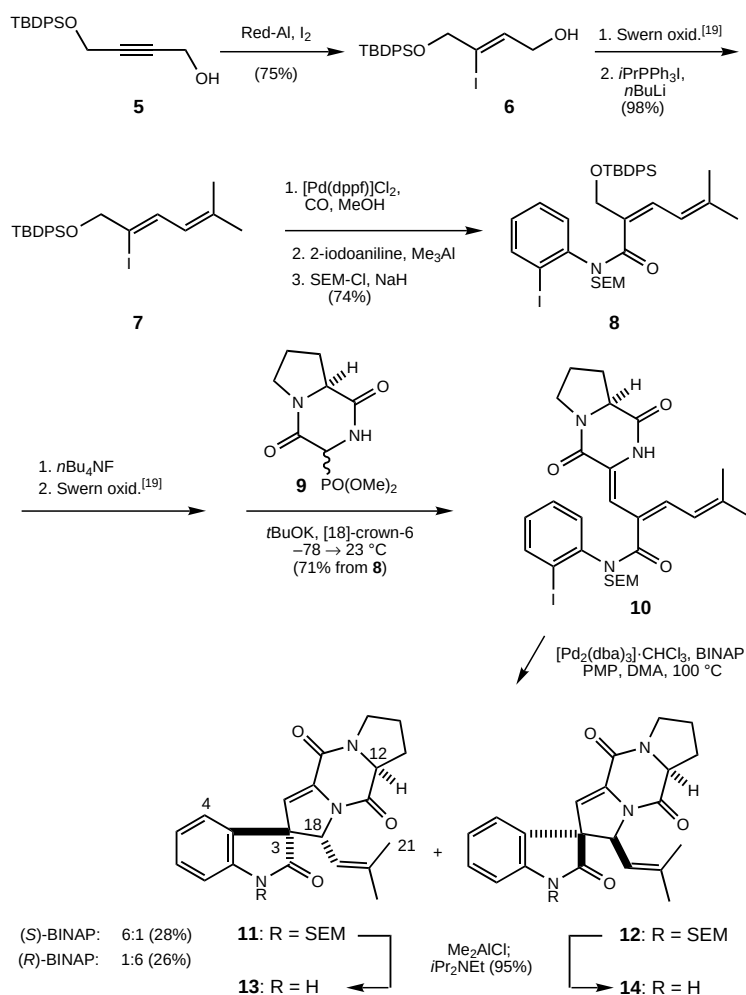
reactions: Heck insertions^[9] and trapping of η^3 -allylpalladium intermediates by nitrogen nucleophiles.^[10] Under control of an appropriate chiral ligand,^[11] a suprafacial intramolecular Heck reaction of (*E*)-**4** in a favored 5-exo sense could generate η^3 -allylpalladium intermediate **3**, which would not be expected to undergo facile stereomutation.^[12] If reaction of intermediate **3** with the proximal nitrogen of the tethered diketopiperazine occurred *anti* to the metal, spirotryprostatin B (**2**) would be produced; trapping *syn* to the metal would lead to 18-*epi*-spirotryprostatin B.^[13] Our strategy would have the flexibility to deal with either stereochemical outcome of the second step by modulation of the stereochemistry of the internal double bond of triene **4**.^[14–16] Herein we disclose an investigation of the strategy adumbrated in Scheme 1, which culminated in enantioselective total syntheses of (–)-spirotryprostatin B (**2**) and three stereoisomers.

The preparation of triene cyclization substrate **10** began with alcohol **5**,^[17] which was converted in high yield into dienyl iodide **7** as shown in Scheme 2.^[18, 19] Palladium-catalyzed carbonylation of **7** in the presence of methanol,^[20] aminolysis of the resulting ester with 2-iodoaniline,^[21] and protection of the amide nitrogen with (2-trimethylsilyl)ethoxymethyl chloride (SEM-Cl) gave anilide **8** in 74% yield. Removal of the *tert*-butyldiphenylsilyl (TBDPS) group of **8**, followed by Swern oxidation^[19] and reaction of the resulting aldehyde with the potassium salt of diketopiperazine phosphonate **9**^[22] delivered isomerically pure **10** in 71% yield from **8**.

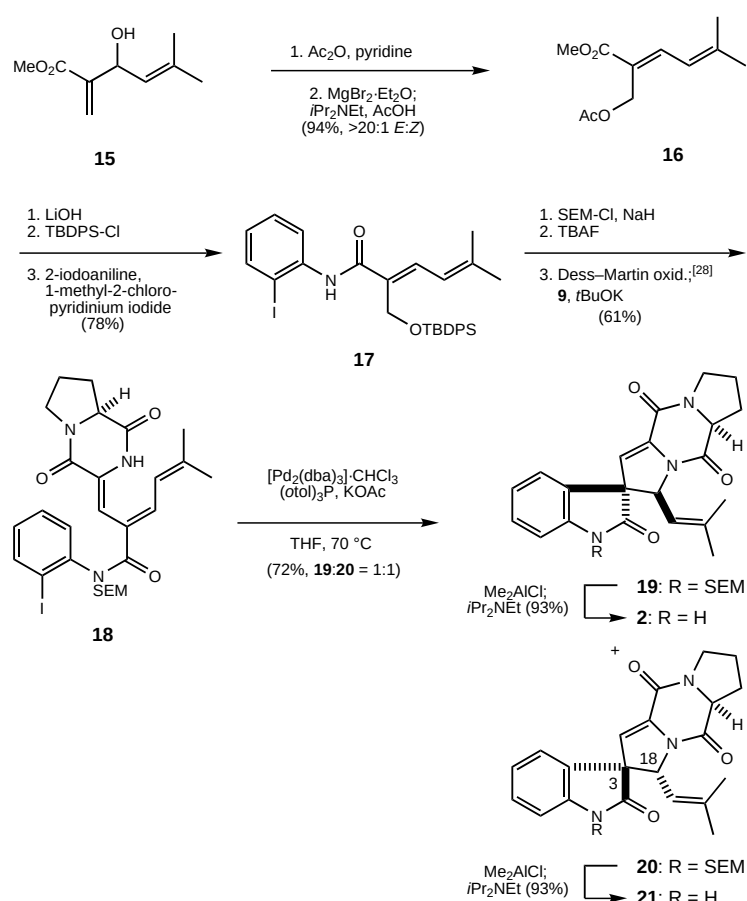
Cyclization of **10** with 20% Pd/(*S*)-BINAP and excess 1,2,2,6,6-pentamethylpiperidine (PMP) in *N,N*-dimethylacetamide (DMA) at 100°C^[11] produced a 6:1 mixture of pentacycles **11** and **12** in 28% yield, while identical cyclization of **10** using Pd/(*R*)-BINAP proceeded with similar efficiency and selectivity to deliver **12** as

the major pentacyclic product (**12**:**11** = 6:1). After some experimentation, we found that the SEM protecting group could be discharged in high yield from these delicate products by initial exposure to six equivalents of Me₂AlCl, followed by heating *N*-hydroxymethyl derivatives with diisopropylethylamine in methanol to remove the unit of formaldehyde. Analytically pure samples of 18-*epi*-spirotryprostatin B (**13**) and 3-*epi*-spirotryprostatin B (**14**) could be obtained from these mixtures by preparative HPLC. The *trans* relationship of the phenyl and 2-methylpropenyl groups of **13** and **14** was apparent from the large NOE enhancements observed in each case between H4 and H18, while the stereorelationship of the pyrrolidine and dihydropyrrole rings was established from long-range NOE enhancements observed between H12 and H21 of **13** by DPGFSE NOE experiments.^[23, 24]

These Pd/BINAP-catalyzed double cyclizations of **10** demonstrate that the η^3 -allylpalladium intermediate is generated and captured with high stereochemical fidelity, and that the nitrogen of the tethered diketopiperazine attacks the allylpalladium complex *anti* to the metal center. Consequently, we turned to the synthesis of the stereoisomeric cyclization



Scheme 2. Synthesis of 18-*epi*-spirotryprostatin B (**13**) and 3-*epi*-spirotryprostatin B (**14**). TBDPS = *t*BuPh₂Si, Red-Al = [(MeOCH₂CH₂O)₂AlH₂]Na, dppf = 1,1'-bis(diphenylphosphanyl)ferrocene, SEM = CH₂OCH₂CH₂SiMe₃, BINAP = 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl, dba = (*E,E*)-dibenzylideneacetone, PMP = 1,2,2,6,6-tetramethylpiperidine, DMA = *N,N*-dimethylacetamide.



Scheme 3. Synthesis of spirotryprostatin B (**2**) and 3,18-bis-*epi*-spirotryprostatin B (**21**). TBAF = tetrabutylammonium fluoride, *otol* = *ortho*-tolyl, see Scheme 2 for other abbreviations.

substrate **18** that should lead to spirotryprostatin B (**2**; Scheme 3).

The preparation of **18** began with allylic alcohol **15**.^[25] The acetate derivative of **15** reacted cleanly with $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$ to give the primary allylic bromide,^[26] which was displaced with acetate to provide (*E*)-dienoate **16**. Conversion of **16** into the siloxycarboxylic acid and coupling^[27] of this intermediate with 2-iodoaniline provided **17** in excellent overall yield. Following a sequence similar to that employed in the stereoisomeric series (**8** → **10**), **17** was elaborated to form isomerically pure **18** in 61% overall yield.^[28, 29]

Cyclization of **18** with $\text{Pd}(\text{S})$ -BINAP under conditions identical to those employed with stereoisomer **10** unexpectedly led to the formation of pentacycle **11**. Control experiments conducted in the absence of $\text{Pd}(\text{S})$ -BINAP indicated that **18** underwent rapid isomerization of the internal double bond of the triene to give **10** when heated above 80°C in DMA with excess PMP. As a result, a wide variety of reaction conditions, including many with scavengers of HI less basic than PMP, were surveyed in an attempt to accomplish the asymmetric double cyclization under conditions that did not isomerize the triene unit of **18**. While these attempts met with failure, we did discover that cyclization of **18** with 10% $[\text{Pd}_2(\text{dba})_3] \cdot \text{CHCl}_3$, 40 mol% tri-*o*-tolylphosphane, and excess KOAc in THF at 70°C cleanly led to the formation of a 1:1 mixture of pentacycles **19** and **20**. Removal of the SEM

group from these products and chromatographic purification provided pure (–)-spirotryprostatin B (**2**; $[\alpha]_{\text{D}}^{23} = -159$ ($c = 0.40$, CHCl_3)) in 21% overall yield from **18**, as well as 3,18-bis-*epi*-spirotryprostatin B (**21**).^[30]

In summary, (–)-spirotryprostatin B (**2**) was synthesized in 9% yield from methyl acrylate and 3-methyl-2-butenal (the commercially available precursors of **15**) by way of ten isolated intermediates. This synthesis and the related syntheses of stereoisomers **13**, **14**, and **21**, introduce a new strategy for stereocontrolled construction of quaternary spiro and adjacent stereocenters. Moreover, this investigation shows for the first time that a) intramolecular Heck insertions of conjugated trienes can proceed with high regioselectivity, b) η^3 -allylpalladium intermediates can be trapped by the nitrogen of a tethered diketopiperazines, and c) the latter reactions proceed with *anti* stereochemistry. These methodological advances further expand the potential applications of organopalladium chemistry in the construction of complex heterocyclic ring systems.

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- [7] H. Wang, A. Ganesan, *J. Org. Chem.* **2000**, 65, 4685–4693.
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Reconstitution of Channel Proteins in (Polymerized) ABA Triblock Copolymer Membranes**

Wolfgang Meier,* Corinne Nardin, and Mathias Winterhalter

Lipid bilayers are the basic constituent of biological membranes. The lipids serve as a fluid matrix for membrane or membrane-associated proteins, which are responsible for various key functions such as signaling or transport. Many of these membrane proteins are pharmacologically important or have biotechnological potential. For such applications one has to immobilize them in an artificial membrane system. This creates a biosensor which can be used, for example, for rapid drug screening. The great advantage of planar freestanding films is the direct access to both sides of the membrane. Thus, these can be applied to carry out, for example, conductance measurements to monitor transport processes across membranes or to detect minor changes on reconstituted channel-forming membrane proteins. An early model system for freestanding films was the so-called “black lipid membranes”, named as such because during the thinning out of the membrane the intensity of the reflected light vanishes and the membrane appears black in the reflected light. In addition to their biological functions, lipid membranes have unique material properties; for instance, they are extremely flexible but at the same time mechanically very stable. In contrast, artificial, freestanding membranes were always fragile and thus of little technological interest. Above a certain size, supported lipid membranes contain defects, which rules out conductance measurements as a recording technique. Herein we present a new type of matrix for membrane proteins. This material is stable and defect-free and allows the formation of giant dense planar membranes.

Similar to conventional lipids, amphiphilic block copolymers may also form membrane-like superstructures in aqueous solution.^[1–6] Hence they can be regarded as higher molecular weight analogues of lipids. Moreover, the high diversity of block copolymer chemistry may lead to a plethora of new artificial membrane structures inaccessible with conventional lipids. For example, it is possible to vary the molecular weight, the block–length ratio, the chemical constitution, or even the molecular architecture of these molecules. Owing to their larger size and their slower dynamics, amphiphilic block copolymers may lead to signifi-

[*] Priv.-Doz. Dr. W. Meier, Dipl.-Phys. C. Nardin
Institut für Physikalische Chemie
Universität Basel
Klingelbergstrasse 80, 4056 Basel (Switzerland)
Fax: (+41)61-2673855
E-mail: wolfgang.meier@unibas.ch

Prof. Dr. M. Winterhalter
IPBS-CNRS UPR 9062, Université Paul Sabatier
31077 Toulouse (France)

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cantly more stable aggregates than those formed from conventional lipids.^[1–3, 5–8] Additionally, the structure of such aggregates is considerably strengthened if the individual block copolymers are interconnected through covalent bonds to form giant supermacromolecules.^[3, 4, 6] Recently we reported the synthesis of a new type of amphiphilic ABA triblock copolymer.^[3] The polymer consisted of a flexible, hydrophobic poly(dimethylsiloxane) (PDMS) middle block and two water-soluble poly(2-methyloxazoline) (PMOXA) side blocks. Additionally, the ends of this PMOXA-PDMS-PMOXA triblock copolymer carried methacrylate groups which allow a crosslinking polymerization. The phase behavior of aqueous solutions of the PMOXA-PDMS-PMOXA triblock copolymer ($M_{n,PMOXA} = 1800$ Da, $M_{n,PDMS} = 5400$ Da; $M_w/M_n = 1.7$) between 0°C and 90°C is shown in Figure 1. At

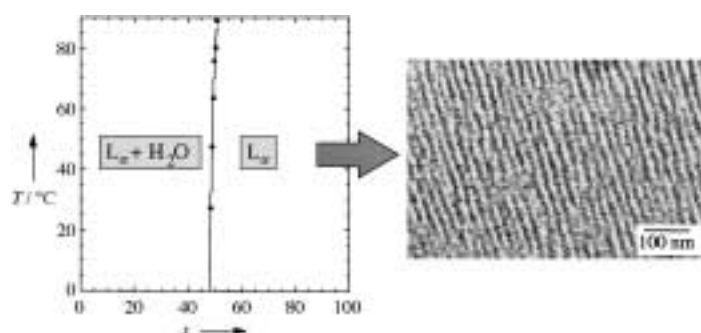


Figure 1. Phase diagram of the PMOXA-PDMS-PMOXA triblock copolymer in water, and Cryo-TEM image of the lamellar phase formed at $x = 50$; x = polymer fraction in water in wt%; L_α = lamellar liquid crystalline phase.

block copolymer concentrations above 48 wt % the system forms a homogeneous lamellar liquid crystalline phase. The lamellar phase was directly imaged by cryo-transmission electron microscopy (cryo-TEM). The samples were prepared as thin films (thickness below 500 nm) by depositing a drop of the solution on a polymer-carbon-coated TEM grid and blotting it with filter paper. The samples were then vitrified by plunging them into liquid ethane. Imaging was performed at temperatures below -160°C with a few micrometers under-focus to increase phase contrast. A representative cryo-TEM image of the lamellar phase formed at 50 wt % triblock copolymer is also shown in Figure 1. The dark ordered lines are interpreted as the triblock copolymer membranes observed from the side. The lamellar spacing can be estimated to be about 20 nm at this composition and the hydrophobic thickness of the lamellae formed by the PDMS blocks can be estimated to be approximately 10 nm. This is in good agreement with capacitance measurements on freestanding triblock copolymer membranes which yielded a hydrophobic thickness of 10 nm.^[6] Below 48 wt % polymer, the system shows a broad miscibility gap where the lamellar phase coexists with excess water. That means that over the whole composition range the basic morphological unit are lamellae with a hydrophobic PDMS core and a hydrated PMOXA corona. It has to be emphasized that the phase behavior of the triblock copolymer is similar to that of typical bilayer-forming lipids like lecithin.^[9]

Recently we showed that in dilute aqueous solution this PMOXA-PDMS-PMOXA triblock copolymer forms vesicular structures that consist of spherically closed triblock copolymer membranes.^[3] Furthermore, freestanding films with an area of up to 1 mm^2 and a thickness of 10 nm could be formed.^[6] Interestingly, the methacrylate end-groups could be polymerized within these self-assembled structures under preservation of the characteristic membrane structure. As a result of the crosslinking polymerization, the individual triblock copolymer molecules are covalently linked together, which leads to a considerable mechanical stabilization of the membranes. Since the polymerizable groups of the macromonomers are attached to the very ends of the hydrophilic blocks, the hydrophobic PDMS middle block preserves a certain mobility within the membrane despite the crosslinking reaction.^[3, 6, 10, 11]

Conventional black lipid membranes are frequently employed as model membranes to reconstitute transmembrane proteins (Figure 2).^[12, 15] Here planar freestanding PMOXA-PDMS-PMOXA triblock copolymer membranes with an area

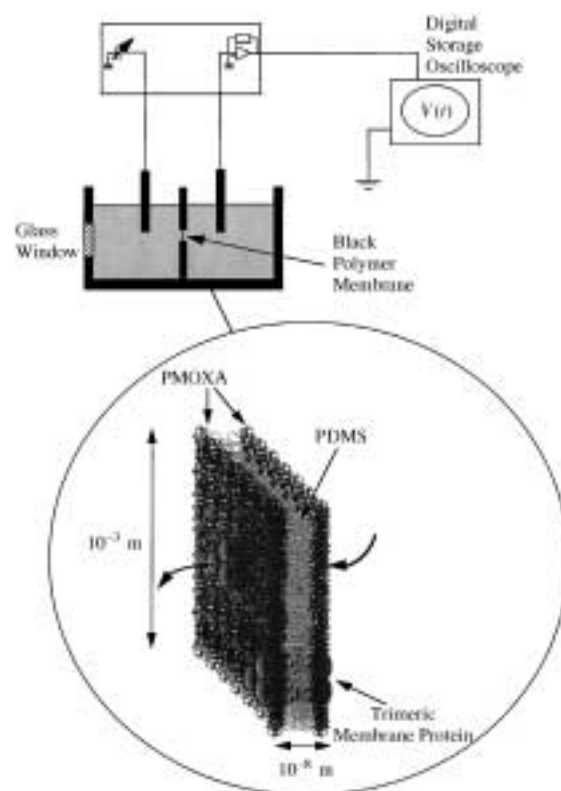


Figure 2. Schematic representation of a protein-containing (polymerized) PMOXA-PDMS-PMOXA triblock copolymer membrane (bottom) and of the experimental setup used to record the conductance across such membranes (top).

of 0.02 mm^2 were prepared by using an adapted standard procedure known from conventional low molecular weight lipids.^[6, 13, 15] The block copolymer membranes are usually thicker than conventional lipid bilayers due to the larger size of the underlying block copolymer molecules.^[3, 5, 6] The hydrophobic–hydrophilic pattern of membrane proteins is naturally optimized with respect to the thinner biological

membranes and the hydrophobic part of, for example, channel-forming proteins may therefore be too small to fit through the polymer membranes. This raises the question as to whether and when to what extent the proteins preserve their activity within a block copolymer membrane. On the other hand, the high flexibility and the conformational freedom of the polymer molecules may allow a block copolymer membrane to adapt to the specific geometric and dynamic requirements of membrane proteins without considerable loss of free energy. In such a case it is expected that the protein will remain functional.

For the reconstitution experiments we used the well-characterized bacterial porins OmpF and maltoporin as model systems.^[14–17] Both porins are transmembrane proteins that form trimeric channels in the outer membrane of Gram-negative bacteria. These water-filled channels allow passive diffusion of small solutes like ions, nutrients, or antibiotics across the membrane. The incorporation of such channels into planar freestanding films can be monitored directly by conductivity measurements.^[12, 15]

A first set of experiments was devoted to whether such transmembrane proteins can indeed be reconstituted in a preformed PMOXA-PDMS-PMOXA triblock copolymer membrane. For this purpose about 1–5 μL of a porin stock solution (about 0.2–1.5 mg mL^{-1} in 1% octyl-polyoxyethylene, 1 mM NaN_3 , 100 mM NaCl, and 2.5 mM Hepes, pH 7.6) was added on both sides of the chamber (each having a volume of 5 mL). The incorporation of porin into the triblock copolymer membranes was favored by applying a potential of 20 mV across the membrane. The conductance across the freestanding film was measured by using Ag/AgCl electrodes and a current amplifier (BLM120, Biologic, Claix, France; see Figure 2).

Figure 3 shows the characteristic time course of the conductance across the triblock copolymer membrane in the presence of OmpF. Surprisingly despite the extreme thickness of this polymer membrane a few minutes after the addition of the porin from the stock solution the conductance increases in a stepwise manner due to multiple OmpF trimer insertions in agreement with previous measurements.^[18] In natural lipid membranes each OmpF trimer contributes a conductance of

2 nS under the given experimental conditions (buffer, temperature).^[18] Under the assumption that the conductance of a porin channel in the block copolymer membrane is the same as in a natural lipid membrane, an insertion of three OmpF trimers is deduced from the conductance increase of 6 nS in Figure 3.

We also tested the voltage-dependent closure of reconstituted OmpF;^[12, 19, 20] no closure was observed up to 200 mV. Similar results were reported for OmpF in conventional solvent-containing black lipid films.^[20] The occurrence of the voltage gating seems to depend significantly on the preparation procedure of the membrane. For soft membranes the OmpF porin will close only at very high voltages. In contrast solvent-free membranes which are stiffer, close at about 100 mV. However, the solvent-free preparation method failed with the PMOXA-PDMS-PMOXA triblock copolymer.

PMOXA-PDMS-PMOXA triblock copolymers carry methacrylate groups at both ends which can be polymerized within the membranes by a UV-induced free radical polymerization. Then the individual triblock copolymers are all interconnected through covalent bonds, which freezes in the whole superstructure. The formation of a covalently cross-linked network structure can be expected to change both the lateral mobility and the conformation of the triblock copolymer molecules within the membrane. Therefore, after polymerization protein insertion is inhibited.

In a second series of experiments we used a second bacterial porin, LamB protein or maltoporin. Maltoporin forms very narrow channels of about 150 pS at 1 M KCl^[15, 18] and possesses stereospecific binding sites for maltooligosaccharides inside the aqueous channels which enhances passive diffusion of the sugars.^[14–16, 21, 22] Using the above-mentioned set-up (Figure 2), we monitored directly the incorporation of maltoporin into the polymer membranes. Titration of sugar drives the sugar into the channels in a concentration-dependent manner and causes the channels to close. The maltooligosaccharide affinity can be obtained from the decrease in conductance as a function of sugar concentration.^[15]

Figure 4 shows the time course of the conductance of a maltoporin-containing triblock copolymer membrane. For comparison the conductance of a protein-free membrane is also included. Initially the conductance of the maltoporin-

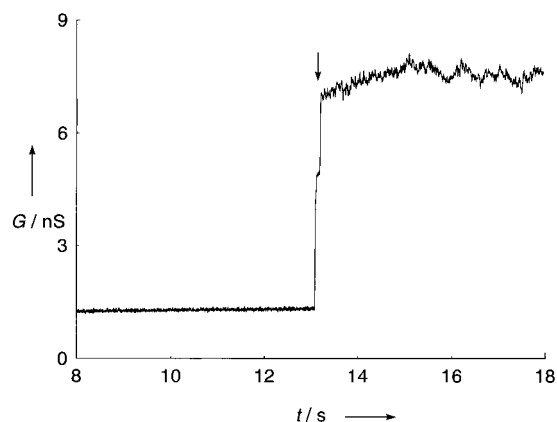


Figure 3. Characteristic time course of the conductance across a PMOXA-PDMS-PMOXA triblock copolymer membrane in the presence of the bacterial porin OmpF. The arrow marks the OmpF insertion.

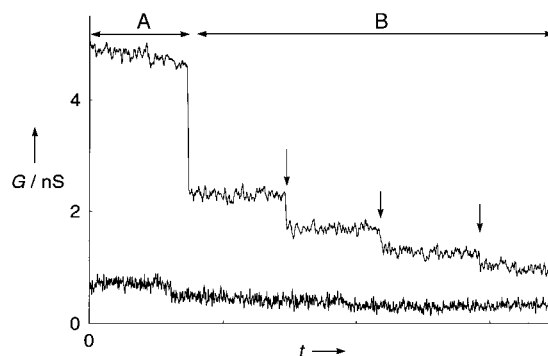


Figure 4. Conductance of a maltoporin-containing PMOXA-PDMS-PMOXA triblock copolymer membrane (upper curve) and a protein-free membrane (lower curve) A) before and B) after polymerization as well as on the stepwise addition of 6 μL of a 10^{-1}M maltohexaose solution (vertical arrows).

containing triblock copolymer membrane is about 4 nS higher than that of the protein-free membrane. This suggests that 27 maltoporin trimers are inserted. The same preparation was subsequently polymerized with UV light. Interestingly the conductance decreases considerably. This could reflect a closure or an expulsion of some of the channels during the crosslinking reaction, probably due to internal stress occurring in the membrane during the polymer chain reaction which may lead to a steric contraction of the hydrophilic blocks of the polymers.^[3, 6] Such a steric contraction should depend sensitively on the length of the hydrophilic blocks. This remains to be clarified. This argumentation is also supported by the slight decrease in conductance observed in the protein-free membrane during the crosslinking reaction (see Figure 4). Presumably, the polymerization induces a reorganization within the films that allows small membrane defects to be healed.

The remaining maltoporin-trimers in the polymerized PMOXA-PDMS-PMOXA triblock copolymer membrane were subsequently titrated with maltooligosaccharides (malto-dextrin). Figure 4 shows the conductance of the polymerized membrane during stepwise addition of 6 μL of a 10^{-1}M maltohexaose solution. Analysis of the conductance data in the usual way^[15, 18] gave a binding constant of $K = 7100\text{M}^{-1}$ between the proteins and the sugar. The sugar affinity constants for maltoporin within the polymerized triblock copolymer membrane were the same and in good agreement with previous investigations on maltoporin in conventional lipid membranes.^[12, 15] Evidently, the conformation of the protein is not influenced by the surrounding membrane and its functionality is fully preserved.

The present investigations show clearly that a functional reconstitution of membrane proteins can be achieved in completely artificial ultra-thin films. This opens the possibility to benefit from the enhanced stability and diversity of such block copolymer aggregates and to incorporate membrane proteins into such a complete artificial polymer membrane. The resulting protein-polymer hybrid materials can be expected to possess great potential for applications in the area of diagnostics, sensor technology, protein crystallization, and even drug delivery.

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Direct Observation of the Lower Critical Solution Temperature of Surface-Attached Thermo-Responsive Hydrogels by Surface Plasmon Resonance**

Erik Wischerhoff,* Thomas Zacher,
André Laschewsky,* and El Djouhar Rekaï

Affinity biosensing is an important method for the investigation of biochemical binding processes: it allows the monitoring of biomolecular interaction in real time and therefore enables the user to deduce kinetic constants from experimental data.^[1] One of the most popular transduction principles in affinity biosensing is surface plasmon resonance (SPR).^[2] The excitation of surface plasmons in thin metal films—usually gold—by totally internal reflected light can be observed as a minimum in the intensity of the reflected light at a certain angle of incidence θ_{SPR} , the resonance angle. Changes of the refractive index in close proximity to the gold surface lead to a shift in the resonance angle. SPR offers the advantage of interaction measurements in real time and does not require labeled analytes.^[3]

An important prerequisite for the success of affinity biosensing is a chemically tailored sensor interface. Typically, hydrogels are employed as a biocompatible matrix to prohibit the denaturation and to maintain the unique functions of biomolecules which are covalently immobilized at sensor

[*] Dr. E. Wischerhoff,^[+] Dipl.-Phys. T. Zacher^[+]
BioTuL AG
Gollierstrasse 70, 80339 München (Germany)
E-mail: e.wischerhoff@pharm.uu.nl
Prof. Dr. A. Laschewsky, Dr. El D. Rekaï
Department of Chemistry
Université catholique de Louvain
Place L. Pasteur 1, 1348 Louvain-la-Neuve (Belgium)
Fax: (+32)10-472836
E-mail: laschewsky@cico.ucl.ac.be

[+] New address:
Department of Pharmaceutical Proteomics
Universiteit Utrecht
Sorbonnelaan 16, 3508 TB Utrecht (The Netherlands)
Fax: (+31)30-251-8219

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interfaces.^[4] Frequently, polysaccharides have been chosen for such hydrogels.^[5] Although they fulfil this task in many respects satisfactorily, synthetic hydrophilic polymers are attractive alternatives as they offer some advantages. First, they may be more resistant to harsh conditions (strong acids, fouling etc.). Second, they provide the opportunity to create polymers with new physical properties by tailoring their structure. An impressive example for the potential provided by tailored hydrogels are so-called thermo-responsive or thermo-sensitive hydrogels.^[6]

The behavior of thermo-responsive polymers in aqueous media often contradicts intuition because they generally exhibit a lower critical solution temperature (LCST). Upon heating, when the temperature increases beyond a certain value commonly referred to as the cloud point, they precipitate from solution. Thermo-responsive hydrogels should undergo an analogous transition even under the particular conditions existing in confined space at a surface.^[7] Such a behavior is of special interest for applications at sensor surfaces because it allows one to switch their properties, or to introduce new functionalities. Some applications already utilize the phase transition on a solid surface.^[8] However, such a transition has not yet been directly observed at an interface up to now. Herein, we report the direct observation of the LCST of a new thermo-responsive hydrogel linked to a gold surface by using surface plasmon resonance.

The specific balance of hydrophilic and hydrophobic groups is a characteristic feature for many thermo-responsive polymers exhibiting a LCST in aqueous solution.^[9] To control the thermal transition temperature, we have modified water-soluble precursor polymers by partial acylation.^[10] Compared to the widely applied approach of employing copolymerization, this strategy facilitates the making of chemically homogeneous, random copolymers, thus enabling a sharp transition in principle. The precursor polymer chosen for this work was poly-*N*-[tris(hydroxymethyl)methyl]acrylamide (P-THMA; see inset in Figure 1) that is known for biomedical

applications.^[11] Partially acetylated P-THMA derivatives exhibit a LCST in aqueous solution,^[10] the observed cloud points depending on the concentration. P-THMA bearing 40 mol % of acetoxy groups covers the physiologically interesting temperature range of 20 to 45 °C (Figure 1).

However, it is not evident how polymers that are thermo-responsive in solution will behave when grafted to a surface. For measuring the temperature-dependent conformational state of the grafted polymers, we have used a SPR-prototype setup (BioTul AG, Munich, Germany). Because the evanescent field of the surface plasmons decays exponentially from the surface, the collapse of the hydrogel when passing the cloud point will change the local average refractive index (Figure 2). Consequently, it should be possible to track the collapse by monitoring the angle shift in the intensity minimum.

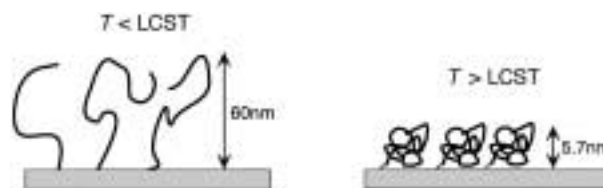


Figure 2. Schematic representation of the thermo-responsive effect of acetylated P-THMA attached to a gold surface as determined and quantified with SPR. The phase transition occurs at $LCST \approx 26^\circ C$.

The SPR setup used employs a diode laser ($\lambda = 784 nm$) as light source and an angular resolution of 0.005° is available. The monochromatic light is directed through a cylindrical prism to a gold-coated glass slide (BK7 glass, $n = 1.511$) coupled through immersion oil to the glass prism. The intensity of the reflected light was measured by a photodiode as a function of the angle of incidence.

In the experiments, pure P-THMA and partially acetylated P-THMA were chemisorbed on gold through their terminal disulfide groups and then the coated substrates were immersed in water which resided in a flow chamber. Water heated stepwise to certain temperatures was injected into the flow chamber and SPR measurements were carried out simultaneously (Figure 3). Several single measurements were averaged to filter the noise.

A polynomial fit to the data points of simple P-THMA (Figure 3, open circles) serves as a reference curve and reflects the temperature dependence of the refractive index of water in accordance with the data listed by Schiebener et al.^[12] The average slope of the curve amounts to $-0.011 \text{ deg K}^{-1}$ which equals a temperature-dependent change of the refractive index of water of $-1.0 \times 10^{-4} \text{ K}^{-1}$.

The analogous measurements carried out on modified P-THMA bearing 40% of acetylated hydroxy groups are shown in Figure 3 (filled circles). Apart from the

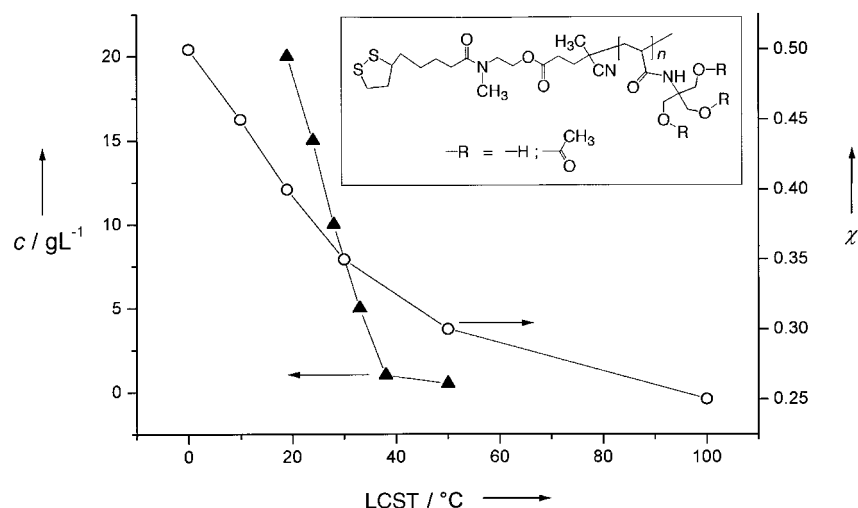


Figure 1. Dependence of the LCST of aqueous solutions of acetylated P-THMA on the molar fraction of acetylation χ (concentration 20 gL^{-1} ; open circles) as well as on the polymer concentration c (degree of acetylation 40%; filled triangles). The lines only serve as a guide to the eye and have no physical meaning. The inset shows the structure of P-THMA with the azo initiator fragment which bears disulfide moieties.

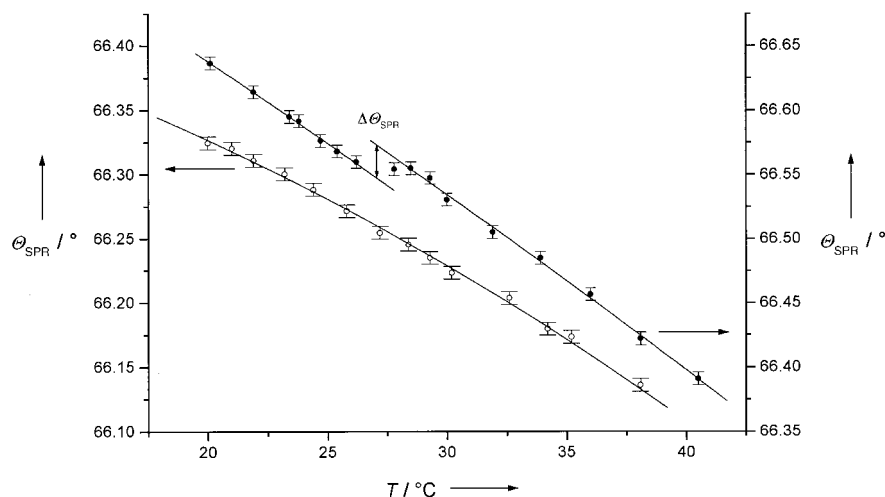


Figure 3. Open circles: $\Theta_{\text{SPR}}(T)$ of non-stimuli-responsive P-THMA. The curve reflects the evolution of the refractive index of water with temperature and serves as a reference. Filled circles: $\Theta_{\text{SPR}}(T)$ of acetylated P-THMA (degree of acetylation 40 %). The collapse of the hydrogel at $T = \text{LCST}$ leads to a significant shift $\Delta\Theta_{\text{SPR}}$, indicating the phase transition as visualized in Figure 2.

overall decrease of the resonance angle with temperature, there is a distinct change in the slope between about 26 °C and 28.5 °C, leading to an offset of $\Delta\Theta_{\text{SPR}} = 0.026^\circ$. This indicates the phase transition of the acetylated P-THMA triggered by the increasing temperature. Calculations based on applying Fresnel equations^[13] to a model layer system assuming homogeneous layers reveal that the measured values of $\Theta_{\text{SPR}}(T)$ and the observed shift of $\Delta\Theta_{\text{SPR}} = 0.026^\circ$ correspond to a swollen hydrogel film which is approximately 60 nm thick at $T < \text{LCST}$, and which collapses to a 5.7 nm thick film when the temperature exceeds the LCST. For the collapsed hydrogel a refractive index of 1.478 was assumed, for the swollen hydrogel a mixed refractive index of 1.344 calculated according to Garnet.^[14] The width ΔT of the phase transition can be estimated to amount to $\Delta T \approx 2.5^\circ\text{C}$ and is believed to be caused by the polydispersity of the compound. (A fully acetylated sample from the same batch was analyzed by gel-permeation chromatography. The resulting number for the apparent polydispersity was 8.)

The thermal transition on the gold surface occurs at $T \approx 26^\circ\text{C}$ which would correspond to a concentration of about 15 g L^{-1} in aqueous solutions. However, the dimensions of the collapsed and swollen hydrogel film as determined by the SPR measurements imply a local concentration of the polymer in the chemisorbed swollen hydrogel film of about 120 g L^{-1} , assuming a density of 1.2 g cm^{-3} for the polymer. Two factors may explain this difference: first, the temperatures measured may be systematically slightly too high due to heat flow effects, but, more important, it is very likely the steric restrictions for the polymer chains at the interface have a significant influence on the phase transition,^[7] shifting the transition temperature to higher values for polymer chains in confined space. An analogous increase of transition temperatures was reported, for example, for surface-attached brushes of liquid crystalline polymers.^[15]

In conclusion, controlled chemical modification allows one to induce and to tune the LCST of well-known water-soluble polymers, as in the case of acetylated P-THMA. The

chemisorption of such polymers leads to thermo-responsive hydrogels on gold surfaces. We have directly evidenced the thermal transition by SPR and quantified the thermo-responsive effect by comparison of the measurements with calculated values. Owing to their straightforward structure together with a marked thermo-responsiveness, the presented hydrogels have the potential for interesting biochemical applications, for example control of binding processes at sensor surfaces by variation of the temperature.

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Asymmetric Synthesis of α -Substituted β -Nitrophosphonic Acids by Phospho-Analogous Michael Addition to Aromatic Nitroalkenes**

Dieter Enders,* Livio Tedeschi, and Jan Willem Bats

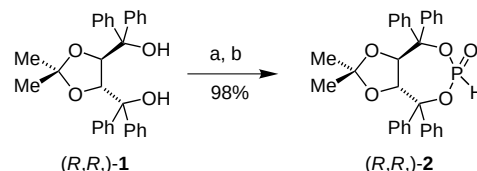
Dedicated to Professor Hans Jürgen Bestmann on the occasion his 75th birthday

Considering the enormous significance of organophosphorus compounds in nature, it is surprising that naturally occurring phosphonates^[1] that contain a C–P bond, have only been known since 1959^[2] when (aminoethyl)phosphonic acid was isolated from sheep rumen. Since then numerous compounds of this class have been isolated, synthesized, and tested for their biological activity. In particular, phosphonates^[3] bearing heteroatomic substituents in the α - or β -position to the phosphorus atom have shown strong activities as antibiotics, anticancer drugs, herbicides, and enzyme inhibitors.^[4] Their activity may be attributed to the similarity to the natural α - and β -amino acids and to the relative hydrolytic stability of the C–P bond compared to that of the P–O bond. Optically active α -hydroxy and α -aminophosphonic acids can nowadays be prepared by a variety of methods.^[5]

In contrast, only a few methods have been developed for the asymmetric synthesis of β -aminophosphonic acids^[6] and of their precursors. A convenient route to β -aminophosphonic acids is provided by the addition of phosphorus compounds bearing a labile phosphorus–hydrogen bond to nitroalkenes, first developed by Pudovik et al.,^[7] and the subsequent reduction of the nitro group.^[8] However, the addition of dialkylphosphites to aromatic nitroalkenes leads to extensive polymerization of the nitro compounds. Examples of addition of di- and trialkylphosphites to unsaturated nitro compounds were also described by Yoshimura et al.,^[9] who were able to add diethylphosphite to aliphatic nitroalkenes at 100 °C in moderate yields. Yamamoto et al.^[10] developed the first substrate-controlled diastereoselective addition of dialkylphosphites to unsaturated nitrosaccharides, albeit only with moderate stereoselectivities. An efficient general method for the asymmetric Michael addition of phosphorus nucleophiles to unsaturated nitro compounds has not yet been described. Chiral catalysts^[11] and chiral phosphorus nucleophiles^[12, 13] have already been employed in the asymmetric hydrophosphonylation of aldehydes and imines by 1,2-addition.

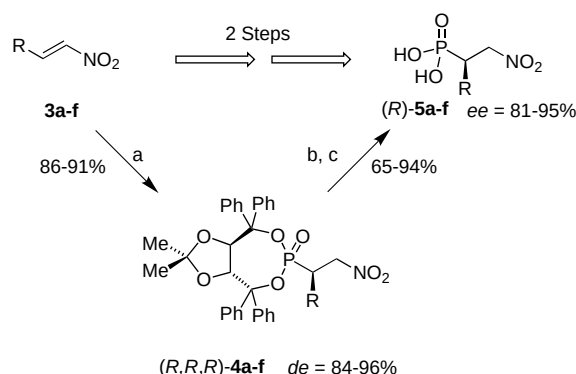
We report here on the synthesis of an enantiomerically pure phosphite and its application as a chiral phosphorus nucleophile in the asymmetric Michael addition to aromatic nitroalkenes.

We obtained phosphite (*R,R*)-**2** in a two-step synthesis starting from TADDOL^[14] ((*R,R*)-4,5-bis(diphenylhydroxymethyl)-2,2-dimethyl-1,3-dioxolane) (*R,R*)-**1**, which was converted into the corresponding chloride with PCl_3 and finally hydrolyzed in almost quantitative yield (Scheme 1).^[15] The C_2 -symmetry of the ligand avoided the formation of a new stereogenic center at phosphorus and the otherwise necessary separation of diastereomers.



Scheme 1. Synthesis of phosphite (*R,R*)-**2**. a) 1.3 equiv $\text{PCl}_3/\text{Et}_3\text{N}$, THF, 0 °C; b) $\text{H}_2\text{O}/\text{Et}_3\text{N}$, THF, 0 °C.

Phosphite (*R,R*)-**2** was allowed to react with nitroalkenes **3a–f** in the presence of Et_2Zn and *N,N,N',N'*-tetramethylethylenediamine (TMEDA) and the resulting β -nitrophosphonates **4a–f** were obtained in very good yields and very high diastereomeric excesses ($de = 84–96\%$) (Scheme 2, Table 1).



Scheme 2. Synthesis of β -nitrophosphonic acids (*R*)-**5**. a) (*R,R*)-**2**, TMEDA, Et_2Zn , THF, –78 °C; b) TMSCl , NaI, CH_3CN , reflux; c) $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, room temperature.

The reaction of diethylzinc with diphenylphosphane leads to the formation of reactive organozinc–phosphorus adducts, which were already described by Noltes^[16] as very reactive but very insoluble. Also in our case we observed, after the addition of Et_2Zn to (*R,R*)-**2**, the precipitation of a solid substance which proved to be unreactive at –78 °C but easily underwent addition to the nitroalkenes at 0 °C. The solubility of the adduct was greatly improved by addition of TMEDA, so that it was possible to perform the reaction at low temperature and thus to obtain the addition products with higher de values (Table 1).

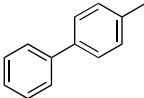
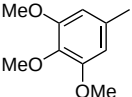
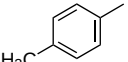
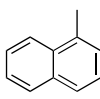
The configuration of the newly formed stereogenic center was determined to be *R* by single-crystal X-ray analysis for the

[*] Prof. Dr. D. Enders, Dipl.-Chem. L. Tedeschi
Institut für Organische Chemie der RWTH Aachen
Professor-Pirlet-Strasse 1, 52074 Aachen (Germany)
Fax: (+49) 241-8888-127
E-mail: Enders@RWTH-aachen.de

Dr. J. W. Bats
Institut für Organische Chemie der Universität Frankfurt
Marie-Curie-Strasse 11, 60439 Frankfurt am Main (Germany)

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Table 1. Michael addition of phosphite **2** to nitroalkenes **3** to give β -nitrophosphonates **4**.

4	R	Yield [%]	<i>de</i> [%] ^[a]	$\delta^{31}\text{P}$	$[\alpha]_{\text{D}}^{20}$
a		89	89	17.0/16.8	−195.5
b		88	92(>96) ^[b]	15.9/15.5	−158.4
c		91	88(>96) ^[b]	17.2/16.8	−203.3
d		86	96	17.2/16.9	−187.3
e		87	93(>96) ^[b]	15.2/15.9	−219.3
f		86	84	17.7/17.5	−84.4

[a] Determined by ^{31}P NMR spectroscopy and by HPLC (Whelk 01, cyclohexane/2-propanol 9/1). [b] *de* values after chromatographic epimer separation (prep. HPLC: Merck Lichrosorb Si60, pentane/diethyl ether 1/1).

case of the 1,4-addition product **4b** (Figure 1).^[17] Since we can postulate a common reaction mechanism all the described examples **4a–f** should possess this configuration.

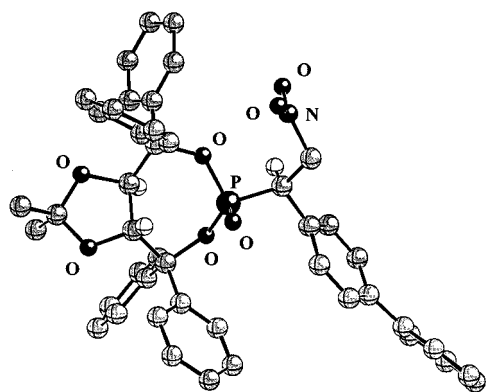


Figure 1. X-ray crystal structure of **4b**.^[17]

The chiral auxiliary was cleaved under mild conditions by a modification of a procedure by Morita et al.^[18] Reaction of nitrophosphonates **4a–f** with trimethylsilyl chloride (TMSCl) and NaI in acetonitrile led to bis(trimethylsilyl)phosphonates, which were finally hydrolyzed in a mixture of water/dichloromethane (Scheme 2, Table 2).

Dimethyl- or diethylphosphonates can be converted to the corresponding silyl esters with NaI/TMSCl at room temperature or 40 °C, respectively, and then hydrolyzed with water. Owing to the steric hindrance of the esters **4a–f** it was necessary in our case to use longer reaction times and a higher temperature. The free nitrophosphonic acids **5a–f** were

Table 2. Cleavage of TADDOL auxiliary to yield β -nitrophosphonic acids **5**.

(<i>R,R</i>)- 5	Yield [%]	<i>ee</i> [%] ^[a]	$\delta^{31}\text{P}$	$[\alpha]_{\text{D}}^{20}$
a	87	92	19.7	+9.2
b	88	88	20.1	+11.1
c	92	86	20.5	+9.8
d	94	95	20.4	+15.3
e	65	91	20.2	+8.7
f	85	81	19.9	+4.7

[a] Determined by HPLC over a chiral stationary phase of the methyl ester derivatives (Whelk 01, *n*-heptane/2-propanol 9/1).

isolated from the aqueous phase after separation of the organic phase. We could show by HPLC over a chiral stationary phase that the cleavage of the auxiliary occurred practically without racemization (*ee* = 81–95 %). The β -nitrophosphonic acids were esterified with trimethylsilyl diazomethane and the enantiomeric excesses determined by HPLC (Table 2).

Aliphatic nitroalkenes (R = Me, Et) can also be used as Michael acceptors (yields: 85–95 %), but the asymmetric inductions are currently still unsatisfactory.

The described asymmetric phospho-analogous Michael addition to aromatic nitroalkenes provides an efficient route to optically active β -nitrophosphonic acids in good yields and enantiomeric excesses by a C–P bond-forming reaction. The products are valuable synthetic bifunctional building blocks and constitute potential precursors of β -aminophosphonic acids.

Experimental Section

(*R,R*)-**2**: In an oven-dried Schlenk flask triethylamine (1.3 equiv) was added under argon at 0 °C to a solution of phosphorus trichloride (1.3 equiv) in dry degassed THF (3 mL mmol^{−1}). A solution of TADDOL **1** (1 equiv) in THF (7 mL mmol^{−1}) was then added dropwise over 30 min. After one hour the solid triethylammonium chloride was filtered off under argon, and a mixture of water (1 equiv) and triethylamine (1 equiv) was added to the solution. The resulting mixture was stirred for 1 h, filtered, and the product was purified by chromatography (SiO₂, pentane/diethyl ether 1/1). (*R,R*)-**2** was obtained as a colorless solid in 98 % yield.

(*R,R,R*)-**4a–f**: In an oven-dried Schlenk flask phosphite (*R,R*)-**2** was added under argon at 0 °C to a solution of TMEDA (1 equiv) in THF (2 mL mmol^{−1}), and then Et₂Zn (1 equiv) was added dropwise. The nitroalkenes **3a–f** dissolved in THF (2 mL mmol^{−1}) were added directly and the resulting solution was stirred for 12 h at −78 °C. The reaction was quenched at 0 °C with a saturated aqueous solution of ammonium chloride, extracted three times with diethyl ether, and the organic phase was washed with brine. After removal of the solvent under reduced pressure the β -nitrophosphonates (*R,R,R*)-**4a–f** were obtained as colorless solids which were purified by chromatography (SiO₂, pentane/diethyl ether).

(*R*)-**5a–f**: In a round-bottomed flask NaI (1 equiv) and TMSCl (1 equiv) were added to a solution of nitrophosphonates (*R,R,R*)-**4a–f** (1 equiv) in CH₃CN (10 mL mmol^{−1}) and the resulting mixture was refluxed for 12 h. The precipitated NaCl was filtered off. After removal of the solvent under reduced pressure, a mixture of CH₂Cl₂ (3 mL mmol^{−1}) and H₂O (2.5 mL mmol^{−1}) was added. After the mixture had been stirred at room temperature for 2 h and the organic phase had been removed, the water was removed under high vacuum and the β -nitrophosphonic acids (*R,R,R*)-**5a–f** were obtained as colorless solids, which were purified by recrystallization.

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- [17] Crystal data for **4b**: single crystals were obtained by recrystallization from diethyl ether. The substance ($C_{45}H_{40}NO_7P$ $M_r = 737.75$) crystallized in the monoclinic space group $P2_1$, $a = 15.052(2)$, $b = 10.1503(14)$, $c = 27.203(4)$ Å, $\beta = 95.738^\circ$, $V = 4135.2(10)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.185$ g cm⁻³, $F(000) = 1552$, $T = 147(2)$ K. Data collection: Siemens-Smart diffractometer with graphite-monochromated MoK α radiation ($\lambda = 0.71073$ Å). Crystal dimensions: $0.03 \times 0.08 \times 1.55$ mm $3.0^\circ \leq 2\theta \leq 54.4^\circ$; 41782 measured reflections, of which 16113 were independent and were used for the structure refinement of 994 parameters. Empirical absorption correction (SADABS). The structure was solved by direct methods (SHELXS), the hydrogen atoms were placed at idealized positions and refined according to the riding model. Disordered solvent was described with five isotropic C and O atoms. The structure was refined according to the full-matrix least-squares method on F^2 (SHELX-96). At convergence $R1 = 0.0618$ ($I > 2\sigma(I)$) and $wR2 = 0.1036$; min./max. residual electron density: $-0.26/+0.41$ e Å⁻³, Flack parameter 0.07(9). Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-147316. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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Breakdown of Bond Length–Bond Strength Correlation: A Case Study**

Martin Kaupp,* Bernhard Metz, and Hermann Stoll

“Shorter bonds are stronger bonds”. This is still the tenet of essentially all interpretations of molecular structure, for example in the analysis of crystallographic data,^[1] formalized by a plethora of empirical bond length–bond strength correlations.^[2] However, the number of cases where shorter bonds have been found to exhibit smaller bond energies or force constants has increased slowly but steadily during the past decade. Most examples (see, however, refs. [3, 4]) pertain to the effects of substitution by very electronegative groups, such as fluorine. Thus, for example, increased fluorine substitution in the $NH_{3-n}F_n$ and $OH_{2-n}F_n$ series shortens the N–F or O–F bonds but apparently reduces the force constants and dissociation energies.^[5] Fluorine substitution in lead(IV) compounds $PbR_{4-n}F_n$ ($R = H, CH_3$) was shown computationally to contract bonds but to facilitate reductive elimination.^[6] Similarly, in a number of titanium–phosphane complexes $[Ti(2,4-C_7H_{11})_2PX_3]$, Ti–P bond lengths and dissociation energies were found experimentally to decrease along the series $X = CH_3, OC_2H_5, F$.^[7] In contrast, in some polyfluorinated hydrocarbons, C–C bond lengths and dissociation energies apparently increase with increasing fluorine substitution.^[8]

There is clearly a need for systematic studies aimed at elucidating under which circumstances and in which direction deviations from the usual correlations may be expected. Here we report a high-level ab initio case study on the Sn–Sn bond lengths, homolytic dissociation energies, and force constants in distannane, Sn_2H_6 , and several of its fluorine-substituted derivatives. This particular choice of model systems has several advantages: 1) a homonuclear bond is studied, and thus polar bonding contributions may be minimized or, for symmetrical substitution, eliminated; 2) in contrast to first-row compounds,^[8] complicating effects of negative hyperconjugation are expected to be minor for the properties discussed; 3) the systems are still sufficiently small to obtain definite results from highly accurate coupled-cluster calculations with extended basis sets.^[9]

Table 1 provides computed Sn–Sn bond lengths, dissociation energies, and force constants.^[9] We will concentrate on the CCSD data, as the available computational resources did not allow CCSD(T) calculations at this basis-set level for all systems (the available data indicate that contributions from triple excitations will not alter the conclusions). Figure 1 shows clearly that bond lengths and dissociation energies do

[*] Prof. Dr. M. Kaupp
Institut für Anorganische Chemie, Universität Würzburg
Am Hubland, 97074 Würzburg (Germany)
Fax: (+49) 0931-888-7135
E-mail: kaupp@mail.uni-wuerzburg.de
Dipl.-Math. B. Metz, Prof. Dr. H. Stoll
Institut für Theoretische Chemie, Universität Stuttgart
Pfaffenwaldring 55, 70569 Stuttgart (Germany)

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Table 1. Sn–Sn bond lengths d , homolytic dissociation energies D_e , and force constants k of $\text{Sn}_2\text{H}_{6-n}\text{F}_n$.^[a]

Compound	d [Å]		D_e [kJ mol ⁻¹] ^[b]		k [10 ⁻² au] ^[c]	
	CCSD	CCSD(T)	CCSD	CCSD(T)	CCSD	CCSD(T)
$\text{H}_3\text{Sn-SnH}_3$	2.759	2.758	248.7 (250.9)	256.4 (258.5)	8.26–8.28	8.13–8.15
$\text{H}_2\text{FSn-SnFH}_2$	2.765	2.767	242.5 (245.4)	249.1 (251.9)	8.34–8.35	8.13–8.14
$\text{HF}_2\text{Sn-SnF}_2\text{H}$	2.764	2.767	226.0 (230.0)	231.5 (236.3)	8.47–8.49	8.17–8.18
$\text{F}_3\text{Sn-SnF}_3$	2.758	–	206.8 (208.6)	–	8.59–8.61	–
$\text{F}_3\text{Sn-SnH}_3$	2.767	–	230.1 (235.1)	–	7.91–7.93	–
$\text{H}_3\text{Sn-SnFH}_2$	2.761	2.761	246.9 (249.1)	253.9 (256.0)	8.23–8.24	8.08–8.09

[a] Bond angles, Sn–H, and Sn–F distances, as well as monomer structures, optimized at the MP2 level. [b] Values in parentheses were computed with monomers frozen at the optimized dimer structural parameters. [c] A range of values is given, due to the inherent uncertainties of the fitting procedure.

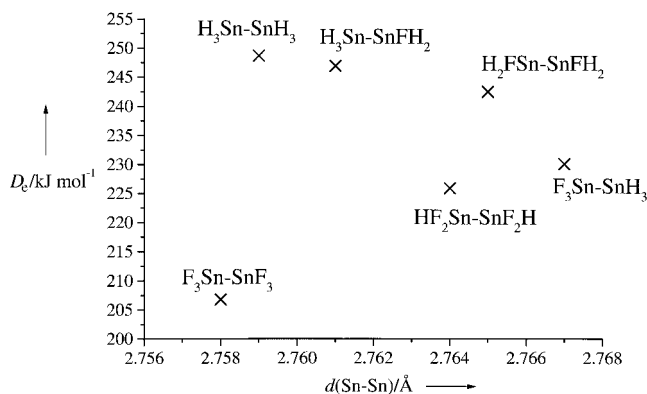


Figure 1. Plot of CCSD dissociation energy D_e versus CCSD bond length d .

not correlate. While the dissociation energies decrease significantly with increasing fluorine substitution, the bond lengths change very little, in a nonmonotonous fashion. Nuclear reorganization energies of the fragments amount to only a few kJ mol⁻¹ (cf. Table 1). Electronic reorganization of the monomers is of course substantial. However, it appears that the breakdown of bond length–bond strength correlation in this series of systems is related predominantly to intrinsic properties of the Sn–Sn bond in the dimers (see below).

The range of force constants is significantly smaller (ca. 8%) than that of the dissociation energies (ca. 20%) and becomes even more attenuated at the CCSD(T) level. Figure 2 indicates that, for the symmetrical systems, larger dissociation energies are accompanied by *smaller* force constants. Interestingly, the unsymmetrically substituted

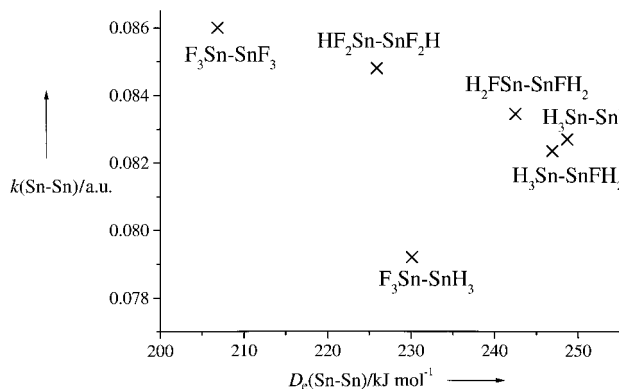


Figure 2. Plot of CCSD force constant k versus CCSD dissociation energy D_e .

$\text{F}_3\text{Sn-SnH}_3$ has by far the lowest force constant, in spite of exhibiting an intermediate dissociation energy. As a consequence of this nonintuitive behavior, no correlation between force constants and bond lengths may be discerned either (Table 1).

What is the reason for the weaker but partly even shorter bonds in the fluorine-substituted species? It has already been shown that fluorine substitution removes electronic charge from the central atom (in this case Sn) and thereby reduces its effective size. At the same time, the relative size of the valence s and p orbitals on Sn is rendered even more disparate than before.^[6] As a consequence, “hybridization defects”^[10] become more dramatic: the p orbitals are too large to effectively hybridize with the compact s orbitals. Thus, they cannot contribute efficiently to covalent bonding. This weakens the Sn–Sn bonds, but also the Sn–H and Sn–F bonds.^[6] Figure 3 shows that, indeed, the relative p character of the Sn hybrids

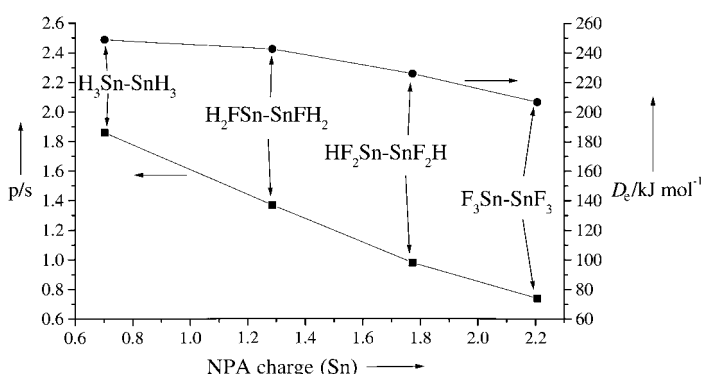


Figure 3. Dependence of p/s hybridization ratios (■) of the Sn–Sn bonding natural localized molecular orbitals (NLMO), and of the dissociation energies (●) on the natural population analysis (NPA) charges for the symmetrical substituted distannanes. Bonding analyses for HF wavefunctions at the MP2-optimized structures; CCSD dissociation energies taken from Table 1.

used for the homonuclear bond (and also for the other bonds to Sn; cf. Figure 4) decreases dramatically with increasing charge. “Hybridization-deficient” bonds are weaker, and thus the dissociation energies decrease (Figure 3). However, the weaker bonds do not become longer, as the effect is compensated (or partly even overcompensated) by the reduced size, and the related larger net s character, of the Sn atom. Weakening of the bonds would also be expected to reduce the force constants. However, the reduced atom size may again contribute in the opposite direction.

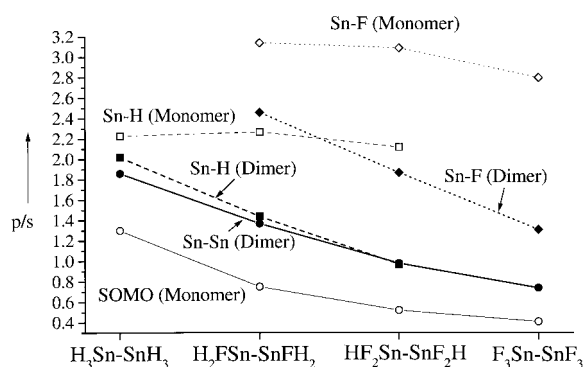


Figure 4. Changes in p/s hybridization ratios of various NLMO upon Sn-Sn bond dissociation. Bonding analyses for HF wavefunctions at the MP2-optimized structures. Average of α and β NLMO hybridization for the monomers.

Figure 4 shows the p/s hybridization ratio of the relevant localized MOs. In the dimers, hybridization defects increase dramatically with increasing fluorine substitution, not only for the Sn-Sn bonds (cf. also Figure 3), but also for the Sn-H and Sn-F bonds. In the monomers, the s character may concentrate more in the singly occupied molecular orbital (SOMO). The effect of fluorine substitution on Sn-H and Sn-F bonding (cf. corresponding NLMO hybridization) is therefore much less pronounced than in the dimers. As the hybridization defects are directly related to the bond strength, it is clear that changes in the bonding of the dimers are largely responsible for the observed trend in the dissociation energies. Our bonding analyses confirm furthermore, that delocalization effects are of only minor importance. The distannane series provides thus a particularly clearcut case in which hybridization defects are directly responsible for the observed lack of correlation between bond lengths, force constants, and dissociation energies.

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- [3] It has long been known that monoionization of the alkali-dimers increases the bond lengths and binding energies; see, for example: W. Müller, W. Meyer, *J. Chem. Phys.* **1984**, 80, 3311, and references therein.
- [4] The (formal) double bonds in disilenes are shorter but weaker than the single bonds in the corresponding disilanes. The same holds for the heavier homologues of silicon; see, for example: H. Jacobsen, T. Ziegler, *J. Am. Chem. Soc.* **1994**, 116, 3667, and references therein.
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- [8] See, for example: J. M. Martell, R. J. Boyd, Z. Shi, *J. Phys. Chem.* **1993**, 97, 7208; B. E. Smart in *Molecular Structures and Energetics*, Vol. 3 (Eds.: J. F. Liebmann, A. Greenberg), Verlag Chemie, Weinheim, **1986**, pp. 141–189, and references therein.
- [9] a) We used a modern quasirelativistic small-core (22-valence-electron) pseudopotential and extended (12s12p9d3f)/[6s6p4d3f] valence basis for Sn^[9b] together with extended (11s6p2d)/[5s4p2d] and (6s3p1d)/[4s3p1d] all-electron basis sets for F and H, respectively.^[9c] The structures of monomers and dimers were first fully optimized at the MP2 level of theory with the Gaussian98 program.^[9d] Subsequently, the Sn-Sn distances were optimized numerically at the coupled-cluster singles–doubles (CCSD) and partly at the CCSD(T) levels (i.e. with a perturbational treatment of triple excitations), keeping the other degrees of freedom (and the monomer structures) at their MP2 values (five equidistant points in intervals of ca. 0.02–0.04 Å around the minimum distance were fitted to a polynomial, to determine the minimum and the force constant). The MOLPRO program^[9e] was employed. Open-shell fragments were calculated at RCCSD, RCCSD(T), and UMP2 levels. Natural population and hybridization analyses (A. E. Reed, L. A. Curtiss, F. Weinhold, *Chem. Rev.* **1988**, 88, 899) used the MP2-optimized structures, the Hartree–Fock (HF) densities, and the built-in subroutines of the Gaussian98 program. We have restricted our study to ethane-like staggered structures. A detailed exploration of the potential energy surface in these systems is beyond the scope of the present work. b) B. Metz, H. Stoll, M. Dolg, *J. Chem. Phys.* **2000**, 113, 2563; c) These basis sets were derived from the aug-cc-pVTZ basis (T. H. Dunning, Jr., *J. Chem. Phys.* **1989**, 90, 1007; R. A. Kendall, T. H. Dunning, Jr., R. J. Harrison, *J. Chem. Phys.* **1992**, 96, 6796), but truncated in the polarization space. d) Gaussian98, Revision A.7, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Lui, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, J. L. Andres, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh, PA, **1998**; e) MOLPRO is a package of ab initio programs written by H.-J. Werner and P. J. Knowles, with contributions from R. D. Amos, A. Berning, D. L. Cooper, M. J. O. Deegan, A. J. Dobbyn, F. Eckert, C. Hampel, G. Hetzer, T. Leininger, R. Lindh, A. W. Lloyd, W. Meyer, M. E. Mura, A. Nicklass, P. Palmieri, K. Peterson, R. Pitzer, P. Pulay, G. Rauhut, M. Schuetz, H. Stoll, A. J. Stone, and T. Thorsteinsson.
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Transition Metal Catalyzed Preparation of Grignard Compounds**

Borislav Bogdanović* and Manfred Schwickardi

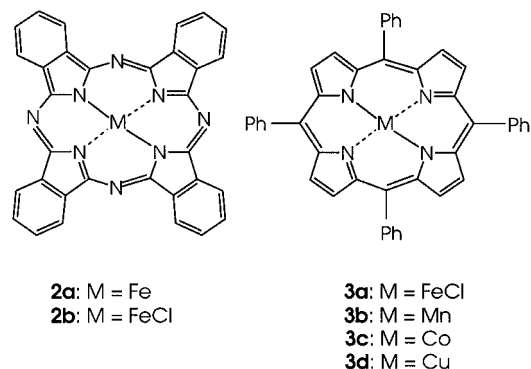
A multitude of organo halide compounds, in particular aromatic, heteroaromatic, and vinylic chloro compounds react slowly in low yield, if at all, with magnesium powder or turnings. In the course of our work on the so-called inorganic Grignard reagents [IGR, see Eq. (1)]^[1] we have developed efficient transition metal catalysts for the transformation of inactive organo chlorides into the corresponding Grignard compounds, commercial magnesium powder is used in this procedure.^[2] Herein this method is introduced for several Grignard compounds that are considered to be difficult to prepare and the possible catalytic role of the IGR is discussed.

The catalysts for the preparation of Grignard compounds are produced through the reaction of transition metal halides, alcohols, or amides (precatalysts) in the presence of an excess of magnesium, and a Grignard or other organo magnesium compounds, usually in THF. The Grignard compound required for the preparation of the catalyst is preferably prepared in situ from an organo halide, such as ethyl bromide, and the excess magnesium present. To date Fe catalysts have been found to be the most active and the best cocatalysts are MgCl_2 and 9,10-diphenylanthracene (DPA).^[2]

The influence that varying the precatalyst, the cocatalyst, and the reaction conditions has on the catalysis of the Grignard formation were investigated for, among others, 1-chloronaphthalene as the inactive organo chloride (Table 1). With the catalyst generated from Mg, EtBr, FeCl_2 , and MgCl_2

the compound 1-naphthylmagnesium chloride (**1**) is formed in 76.7% yield in under 1 h (entry 1).^[3] The best results are obtained when the drop-wise addition of the organo halide is rapid and is started within a few minutes of the addition of the transition metal component, this is because the catalytic activity decreases with time (entry 2). THF is the preferred solvent, but mono- (entry 4) and diglyme can also be used. As well as the iron compounds other compounds, in particular the corresponding manganese precatalysts, such as MnCl_2 , can be considered (entry 5).

As precatalysts iron phthalocyanin (**2a, b**) or the porphine complexes of iron, manganese, cobalt, or copper (**3a–d**) can be used. With these compounds, and particularly in the case of



the iron complexes (entries 6–8) highly active catalysts for Grignard formation are generated even without a cocatalyst (MgCl_2). The catalysts formed from these amides seem to be more stable in THF than the catalysts generated from transition metal halides such as FeCl_2 . The aging of the catalysts before the start of the Grignard reaction (40–100 min) has in these cases no negative effect on the catalytic activity (entry 6–8). In general however, for every single organohalide substrate the optimal catalysts has to be identified. In most cases we use as first choice the catalytic system derived from Mg, EtBr, FeCl_2 , and MgCl_2 .^[6] This system allows the conversion of the aminal-protected chlorobenzaldehyde **4a**,^[7] the oxygen substituted chloroarene 5-chloro-1,3-benzodioxol **5a**, and the oxygen substituted chloroheteroarene 2-chloro-6-methoxypyridine **6a** into the corre-

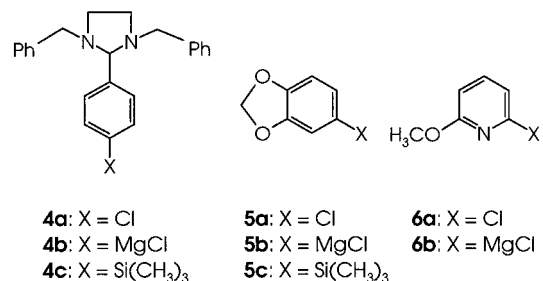


Table 1. Catalytic preparation of 1-naphthylmagnesium chloride **1** from 1-chloronaphthalene and Mg powder in THF.

Entry	EtBr ^[a] [mol %]	Precatalyst ^[a] (2.0 mol %)	MgCl_2 ^[a] [mol %]	Aging ^[b] [min]	T [°C]	Yield [%] ^[c] (t [h]) ^[d]
1	1.1	FeCl_2	5.0	2	22–b.p.	76.7 (1), 82.3 (18)
2	1.1	FeCl_2	5.0	70	22–26	16.7 (3), 35.2 (18)
3	1.1	FeCl_2	–	3	22–b.p.	68.5 (1), 73.7 (20)
4 ^[e]	0.5	FeCl_2	5.0	10	21–b.p.	80.6 (1), 82.7 (4)
5	0.5	MnCl_2	5.0	30	23–62	62.2 (2), 66.5 (19)
6	1.1	2a	–	100	22–b.p.	77.0 (1), 84.0 (6)
7	0.5	2b	–	40	22–b.p.	87 (2)
8	0.5	3a	–	60	22–b.p.	95 (2)
9	0.9	3c	–	45	22–b.p.	69 (0.5)
10	0.9	3d	–	45	22–b.p.	78 (4)

[a] Calculated from 1-chloronaphthalene. [b] Interval between the addition of the precatalyst and commencement of the 1-chloronaphthalene addition. [c] Determined by acidimetric titration. [d] After the end of the 1-chloronaphthalene addition. [e] Reaction in monoglyme; in addition to MgCl_2 in this experiment DPA^[a] (2 mol %) was also used as cocatalyst.

[*] Prof. Dr. B. Bogdanović, M. Schwickardi
Max-Planck-Institut für Kohlenforschung
Kaiser-Wilhelm-Platz 1
45470 Mülheim an der Ruhr (Germany)
Fax: (+49)208-306-2980
E-mail: bogdanovic@mpi-muelheim.mpg.de

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sponding Grignard compounds in high yield (Table 2). In the preparation of the Grignard compound **5b** the yield could be increased from 40 to 76% (after 2.5 h) by increasing the $\text{EtMgBr}/\text{FeCl}_2$ ratio from 0.2 to 2.0 (entries 2 and 3).^[8] After hydrolysis and silation the corresponding benzodioxol and the

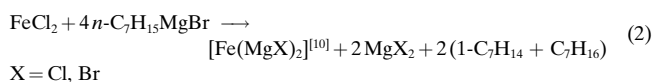
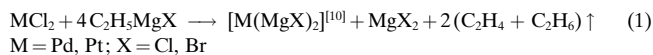
Table 2. Catalytic preparation of the Grignard compounds **4b–6b** from **4a–6a** and Mg powder.

Entry	EtBr ^[a] [mol %]	FeCl ₂ ^[a] [mol %]	MgCl ₂ ^[a] [mol %]	Aging ^[b] [min]	Substrate	T [°C]	Yield [%] ^[c] (t [h]) ^[d]
1	1.0	(2.1)	5.0	10–15	4a ^[7]	20–45	88 (10) ^[e,f]
2 ^[g]	1.0	(5.1)	5.0	3	5a	21–36	40 (2.5)
3	10.9	(5.1)	5.0	3	5a	22–52	76 (2.5), 80.7 (8.5), 82.9 (23) ^[h,i]
4 ^[j]	1.0	(5.1)	5.0	3	6a	22–33	37 (2.5), 52 (9)
5	10.9	(5.1)	5.0	3	6a	22–45	62 (2.5), 83 (9), 96 (23.5) ^[k]

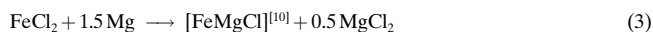
[a] Calculated from the substrate. [b] Interval between the addition of FeCl₂ and commencement of the substrate addition. [c] Determined through acidimetric titration, unless otherwise stated. [d] After the end of the substrate addition. [e] Yield calculated from the amount of the trimethylsilyl derivative **4c**. [f] During the reaction the suspension was stirred with a paddle stirrer in the presence of glass sphere (i.d. = 5 mm; 5 mL) grinding elements. [g] Comparative experiment to entry 3. [h] Yield derived from the GC analysis of the benzodioxol formed by hydrolysis. [i] Comparative experiment without FeCl₂ and MgCl₂: 1.3 % yield after 38.5 h at 22 °C. [j] Comparative experiment to entry 5. [k] Comparative experiment without FeCl₂ and MgCl₂: 12.8 % yield after 30 h at 22 °C.

5-trimethylsilyl-1,3-benzodioxol **5c** were isolated in yields of 83 and 70 %, respectively. In the same manner the yield of the Grignard compound **6b** (entries 4 and 5) could be increased from 52 to 83 % (after 9 h). Without the Fe catalyst the Grignard compounds **4b–6b** could be prepared only in low yield, if at all.

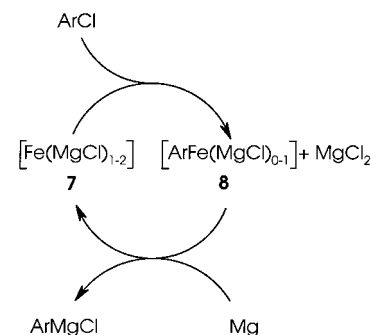
To investigate the catalyst generation a catalyst solution, prepared with a Mg/*n*-C₇H₁₅Br/FeCl₂ ratio of 25/4/1, was analyzed. After a reaction time of 8.5 h the centrifuged catalyst solution contained an amount of magnesium and of bromine equivalent to the amount of *n*-heptyl bromide initially present and 95 and 89 % of the initial FeCl₂ as iron and chlorine, respectively. A GC analysis of the vacuum distillate of the catalyst solution showed 58.6 % of a 1-heptene/heptane mixture (calculated from heptyl bromide) was found by GC analysis.^[9] From these results and based on the formation of Pd- and Pt-IGR from PdCl₂ and PtCl₂, respectively, and ethylmagnesium halides [Eq. (1)] or diethylmagnesium^[11a,c] allows the formation of the catalyst to be described according to Eq. (2).



If in the generation of the catalyst the Grignard compound is not available in sufficient quantities for the reaction to proceed according to Eq. (2) then the known Fe-IGR [FeMgCl]^[11a] [Eq. (3)] can be formed from the FeCl₂ and the excess Mg powder. In the preparation of the Fe catalysts for the Grignard reaction Fe-IGR [Fe(MgCl)₂] [Eq. (1)] and [FeMgCl] [Eq. (3)] can be formed together.



For the Fe-catalyzed Grignard formation (Scheme 1) we assume that the aryl iron intermediate **8** is formed from the mixture **7** and the chloroarene. This step is plausible bearing in mind the known high reactivity of IGR compared to perchlorovinyl and aryl compounds.^[11c] In the next step of the catalysis the Grignard can be released from **8** by a Fe/Mg transmetalation^[11, 12] with the excess Mg, this regenerates the catalytic species **7**. The cocatalytic function of MgCl₂ is



Scheme 1. Proposed mechanism for the iron-catalyzed preparation of Grignard compounds; Ar = aryl, heteroaryl.

probably to stabilize the catalytically active species, in particular **8**, by preventing the precipitation of Fe⁰, the MgCl₂ is also necessary for the transmetalation step.

With the transition metal catalyzed preparation of Grignard compounds the IGR species have demonstrated themselves to be useful homogenous catalysts, which extends the applications of the Grignard reaction to include inactive chloroarene compounds.^[13]

Experimental Section

Typical procedure: The reactions were carried out under an argon atmosphere. THF was distilled over magnesium anthracene·3 THF. Magnesium was used in the form of a commercially available (Eckart Werke, Fürth, Germany) powder (270 mesh). Anhydrous MgCl₂ was prepared from 1,2-dichloroethane and Mg powder in THF. The anhydrous FeCl₂ was obtained from Alfa. The organo chloride was dried over molecular sieves.

Mg powder (2.43 g; 100 mmol) in THF (30 mL) was treated with ethyl bromide (0.22 mL; 3 mmol to 0.56 mL 7.5 mmol depending upon the reactivity of the organo chloride to be used) and the mixture stirred for 1 h by using a magnetic stirrer bar. To mechanically activate the Mg surface, grinding elements (ca. 5 vol %), for example, glass spheres with a diameter of 5 mm can be added (Table 2, entry 1).^[8] After the addition of FeCl₂ (0.19 g, 1.5 mmol to 0.48 g, 3.8 mmol) and a solution of MgCl₂ (7.7 mL, 0.485 molar in THF, 3.7 mmol) the reaction mixture was then stirred for a total of 3 min during which time the solution turned dark brown. Immediately afterwards the organo chloride (75 mmol) was added drop wise over 30 min. In the course of the reaction the solution heated up, sometimes even reaching the boiling point of the solvent used. During the reaction samples were removed, and, after removal of the excess magnesium and hydrolysis, acidimetric titrated (Tables 1 and 2). To identify and quantify the reaction product an aliquot of the reaction solution was hydrolyzed and the organic phase examined by gas chromatography (Table 2, entry 3). When a sample of the Grignard reaction was treated with

chlorotrimethylsilane, the corresponding organotrimethylsilane compound of the Grignard compound was obtained, which could be identified and quantified by GC/MS coupling (Table 2, entry 1).

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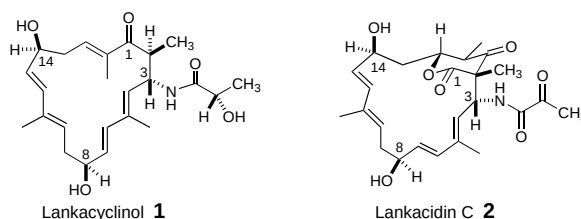
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- [7] We thank the firm Bayer AG, Leverkusen, for providing a sample of **4a**.
- [8] Patent registered.
- [9] The rest is most likely to be the dimerization product C₁₄H₃₀.
- [10] In light of EXAFS investigations (EXAFS = extended x-ray absorption fine structure), particularly of “[Pt(MgCl)₂]_n”,^[16] we assume that the empirical formula [M(MgCl)_m]_p(thf), (*m* = 1, 2, 3; *p* ≥ 2) refers to the metal clusters M_p that are bound directly to the ligands MgCl(thf). The simplified form, such as “[Fe(MgCl)₂]_n” etc. denotes a fragment 1/*p* of this sort of cluster.
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Total Synthesis of Lankacyclinol**

David R. Williams,* Guillermo S. Cortez, Stéphane L. Bogen, and Christian M. Rojas

The lankacidins are a family of structurally unique antibiotics isolated from the fermentation broths of *Streptomyces griseofuscus*, *S. violaceoniger*, and *S. rochei* var.^[1] These me-

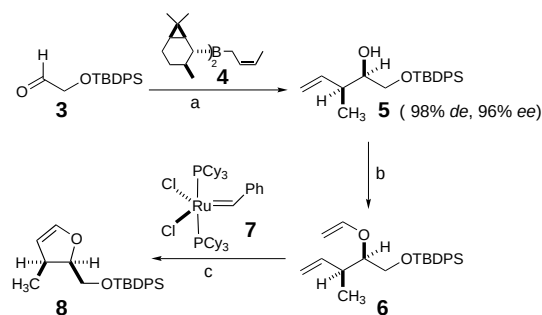
tabolites show strong antitumor activity against L1210 leukemia, B16 melanoma, and solid lymphosarcoma cells.^[2] Lankacyclinol (**1**), which is also identified as T-2636 G,^[3] is a rare example of a seventeen-membered carbocycle, and incorporates two independent pentadienyl alcohol systems as well as a novel β-amido ketone moiety. The assignments of relative



stereochemistry at C3, C8, and C14, and the absolute configuration of **1** have been considered by comparison with lankacidin C (**2**) which was unambiguously characterized by X-ray crystallography.^[4] Indeed, biosynthesis studies have suggested that enzymatic reduction of the 2'-oxopropionamide of **2** followed by base-induced decarboxylation provides lankacyclinol.^[5]

However, the asymmetry at C2 of **1** has remained undefined in spite of substantial advancements leading to the synthesis of **2** by Kende and co-workers.^[6] Our recent studies, stemming from 4,5-dihydrofurans, of tandem acyl nitrene insertions and Wittig reactions have demonstrated a stereocontrolled route to unique β-amido esters.^[7] These results have provided the opportunity to address the challenges inherent in a proposed synthesis of **1**, particularly with regard to serious issues of acid and base instability and stereochemical concerns. In this communication, we describe the first enantioselective synthesis of (–)-lankacyclinol by a convergent pathway which establishes the relative and absolute configuration as illustrated in **1**.

An enantiocontrolled preparation of the C1–C6 fragment was developed, which utilized *cis*-disubstituted dihydrofuran **8** and incorporated a ring-closing metathesis (RCM) strategy (Scheme 1). Addition of the (*Z*)-crotyl-di-(2-*isocaranyl*)borane **4**, as described by Brown and co-workers,^[8] to aldehyde **3** gave the *syn*-homoallylic alcohol **5** as a single diastereomer.^[9] Transesterification to afford the vinyl ether **6** was induced by treatment with ethyl vinyl ether in the presence of small amounts of mercuric trifluoroacetate.^[10] Cyclization with the RCM protocol using the ruthenium Grubbs' catalyst **7**^[11]



Scheme 1. a) **4**, BF₃·Et₂O, THF, –78 °C, 62%; b) Hg(O₂CCF₃)₂, ethyl vinyl ether, Et₃N, reflux, 60%; c) **7**, CH₂Cl₂, reflux, 48%. TBDPS = *tert*-butyldiphenylsilyl, Cy = cyclohexyl.

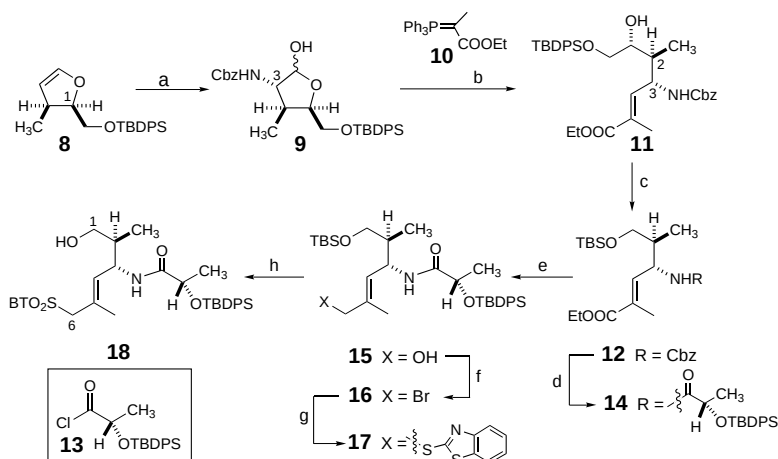
[*] Prof. D. R. Williams, G. S. Cortez, Dr. S. L. Bogen, C. M. Rojas
Department of Chemistry
Indiana University
800 East Kirkwood Avenue, Bloomington, IN 47405 (USA)
Fax: (+1) 812-855-8300
E-mail: williamd@indiana.edu

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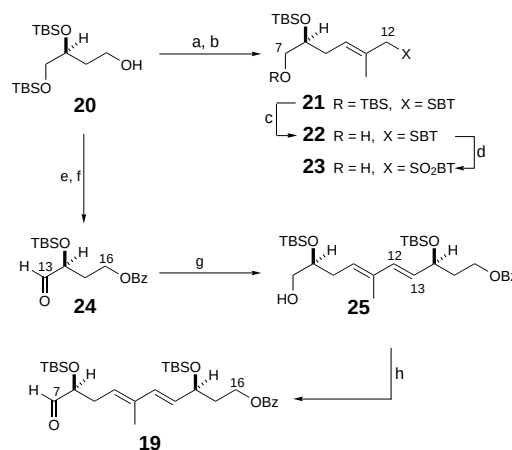
cleanly gave the 4,5-dihydrofuran **8** as a single product, which proved to be identical to material prepared from D-glutamic acid by an alternative route.^[12, 13]

Introduction of the C3-amido appendage was undertaken by the C=C insertion of a reactive acyl nitrene in situ reorganization to a *cis*-fused bicyclic oxazoline intermediate at room temperature.^[7] As illustrated in Scheme 2, photolysis of benzyl azidoformate provided for facile insertion reactions with **8** to deliver the 2-amido furanose derivative **9** in 87% yield as the product of hydrolysis of the initially formed oxazoline. High diastereoselectivity (95:5) was imparted, to a large extent, by the steric consequences of the C1 substituent in **8**.^[14] The lactol permitted direct elongation of the carbon chain by Wittig condensation with stabilized ylide **10** producing the *E* isomer of unsaturated ester **11** as a single diastereomer after flash chromatography. Standard conditions for the deprotection, oxidative cleavage, direct reduction, and protection as the Cbz carbamate provided **12**, which was utilized for introduction of the (*S*)-lactic acid residue. This task was accomplished by N-acylation of the *N*-lithium anion of **12** by acid chloride **13**^[15] followed by immediate hydrogenolysis to afford the desired amide **14**. Chemoselective reduction of the α,β -unsaturated ester **14** was smoothly effected upon treatment with L-selectride to yield the primary allylic alcohol **15**. Incorporation of the benzothiazole functionality in **17** proceeded by displacement of the corresponding bromide **16**. Selective cleavage of the *tert*-butyldimethylsilyl ether followed by a sulfur oxidation^[16] gave the fully elaborated C1–C6 sulfone **18**.

In a highly convergent fashion, we constructed the C7–C16 fragment by incorporating two units of L-malic acid to account for the asymmetry at C8 and C14, as shown in Scheme 3. Thus, the optically active primary alcohol **20**^[17]



Scheme 2. a) Benzyl azidoformate, *hν*, 12 W low-pressure lamp, CH₂Cl₂, trace water, RT, 87%, (d.r. 95:5); b) **10**, PhCH₃, reflux, 77%; c) 1. TBAF, THF, 98%; 2. NaIO₄, THF/H₂O; 3. NaBH₄, CH₂Cl₂, MeOH; 4. TBSCl, imidazole, DMF, 95% (3 steps); d) 1. LiHMDS, THF, –78 °C, then acid chloride **13**, 92%; 2. Pd/BaSO₄, EtOAc, 80 psi (5.51 × 10⁵ Pa) H₂, 80%; e) L-selectride, THF, –78 °C → RT, 88%; f) 1. MsCl, 2,6-lutidine, CH₂Cl₂; 2. LiBr, THF, RT, 97%, (2 steps); g) *n*BuLi, 2-sulfanylbzthiazole, THF, –78 °C, then add **16**, –78 °C → RT, 97%; h) 1. PPTs (0.3 equiv), MeOH, cat. water, 85%; 2. (NH₄)₆Mo₇O₂₄·4H₂O (0.4 equiv), 50% H₂O₂ (8 equiv), EtOH, 0 °C → RT, 89%. Cbz = benzyloxycarbonyl, BT = 2-benzothiazole, TBAF = tetrabutylammonium fluoride, TBSCl = *tert*-butyldimethylsilyl chloride, DMF = dimethylformamide, HMDS = 1,1,1,3,3,3-hexamethyldisilazane, L-selectride = lithium tri-*sec*-butyl borohydride, MsCl = methanesulfonyl chloride, PPTs = pyridinium *p*-toluene sulfonate.

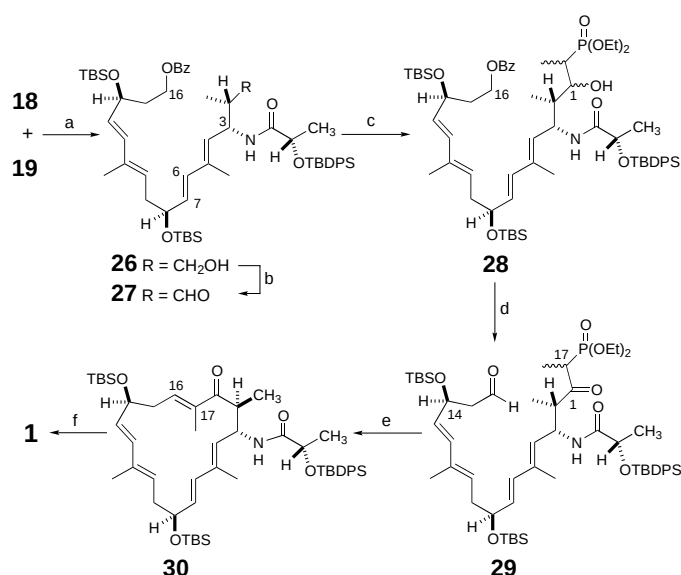


Scheme 3. a) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, –78 °C, then add **10**, 88%; b) 1. DIBAL, CH₂Cl₂, –78 °C; 2. DEAD, Ph₃P, 2-sulfanylbzthiazole, DMF, 96%, (2 steps); c) PPTs (0.3 equiv), 95% EtOH, 78%; d) (NH₄)₆Mo₇O₂₄·4H₂O (0.4 equiv), 50% H₂O₂ (8 equiv), EtOH, 0 °C → RT, 91%; e) BzCl, Et₃N, CH₂Cl₂, 92%; f) 1. 48% HF, CH₃CN, 53%; 2. (COCl)₂, DMSO, Et₃N, CH₂Cl₂, –78 °C, 91%; g) LDA (2.0 equiv) **23**, THF, –78 °C, 5 min, then add **24**, –78 °C → RT, 3 h, 57%; h) Dess–Martin periodinane, NaHCO₃, CH₂Cl₂, 76%. DIBAL = diisobutylaluminum hydride, DEAD = diethyl azodicarboxylate, Bz = benzoyl, LDA = lithium diisopropylamide.

served a dual purpose. The oxidation and Wittig reaction starting from **20** were followed by hydride reduction and displacement to produce the benzothiazole sulfide **21**. In straightforward fashion, a three-step conversion of **20** led to aldehyde **24** without evidence of epimerization at C14 during the low-temperature Swern oxidation (91% yield).

A Julia olefination process was implemented for the coupling of fragments **23** and **24**.^[18] Low-temperature deprotonation of sulfone **23** generated a reactive α -sulfonyl anion^[19] which produced a single diastereomer **25** when the condensation reaction was allowed to warm to ambient temperature immediately after introduction of **24**. Flash chromatography purification afforded the *E,E* diene **25** in 57% yield, and Dess–Martin oxidation^[20] gave aldehyde **19** with no evidence for epimerization at C8. However, the chemical instability of this system raised serious issues for devising our synthesis plan and manipulating advanced intermediates from this point forward.

The assembly of the functionalized C4–C8 pentadienyl system of **1**, and closure of the large-ring carbocycle led to completion of the total synthesis as shown in Scheme 4. Expedient use of the modified Julia olefination^[21] required formation of the dianion of sulfone **18** and condensation with optically pure aldehyde **19**. Notably, low-temperature deprotonation, to generate the α -sulfonyl carbanion of **18**, does not produce fragmentation by loss of the allylic C3-amido side chain, and undesired products due to N → O acyl migration were not observed. In the event, the addition of aldehyde **19** led to the *E,E* diene **26** as a single isomer in 72% yield following flash



Scheme 4. a) LDA (2.05 equiv), **18**, THF, -78°C (5 min), then add **19**, $-78^{\circ}\text{C} \rightarrow \text{RT}$, 72 %; b) Dess–Martin periodinane, NaHCO_3 , CH_2Cl_2 , RT, 98 %; c) 1. LDA, ethyl diethylphosphonate, THF, -78°C , 1 h; 2. CeCl_3 , THF, -78°C , 1 h, add **27**, $-78^{\circ}\text{C} \rightarrow \text{RT}$, 61 %; d) 1. MeLi, Et_2O , $-10^{\circ}\text{C} \rightarrow \text{RT}$, quench with $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$, 77 %; 2. Dess–Martin periodinane, NaHCO_3 , CH_2Cl_2 , 74 %; e) $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ ($4 \times 10^{-4}\text{M}$), THF/ H_2O (40/1), 59 %; f) TBAF (6.0 equiv), NH_4Cl (12.0 equiv), THF, 71 %.

chromatography. The Horner–Wadsworth–Emmons (HWE) process appeared to offer a mild technique for macrocyclization^[22] and was pursued by the addition of the organocerium anion,^[23] prepared from ethyl diethylphosphonate, to the sensitive aldehyde **27** to yield the β -hydroxyphosphonate **28** as a single diastereomer.^[24] Our use of the more basic lithiophosphonate anion produced elimination of the C3-amido group to afford the corresponding conjugated triene from **27**. Nucleophilic cleavage of the C16 benzoate of **28** and a double oxidation using freshly prepared Dess–Martin reagent delivered the fragile ketoaldehyde **29** with undefined, albeit high, diastereomeric purity with regard to asymmetry at C17 ($\geq 98:2$ d.r.).

Our initial attempts to perform an intramolecular HWE reaction using the usual Masamune–Roush conditions^[25] led only to β -elimination of the C14-silyl group in **29**. Fortunately, our adaptation of the use of activated barium hydroxide hydrate, as described in a number of intermolecular HWE precedents^[26] led, under high dilution conditions, to the formation of the seventeen-membered enone **30** in 59 % yield. A buffered, fluoride-induced deprotection produced lankacyclinol (**1**) as a white crystalline solid (mp 220°C). Our synthetic sample was identical, in all respects, by direct comparison with a sample of naturally occurring **1**. Our efforts have established the assignment of relative stereochemistry and absolute configuration as shown in **1**.^[27]

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[24] Best results for this coupling were obtained when high-quality CeCl_3 (Aldrich, 99.9 %) was pulverized to a fine powder with the aid of a mortar and pestle, and activated by heating to 120°C under full vacuum for 1 hour, followed by cooling to ambient temperature under an inert atmosphere. The resulting white solid was suspended in THF for 30 minutes at ambient temperature and then cooled to -78°C prior to addition of the lithium anion. For our purposes, the relative stereochemistry at C17 and C1 of β -hydroxyphosphonate **28** was inconsequential. However, ^1H and ^{13}C NMR data clearly indicated a single pure compound.
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[27] Lankacyclinol (**1**): $R_f = 0.25$ in acetone/benzene (1/1); 0.21 in methyl ethyl ketone/ethyl acetate (2/8); mp 220 °C (uncorrected); synthetic **1**: $[\alpha]_D^{25} = -163^\circ$ ($c = 0.3$, EtOH), natural **1**: $[\alpha]_D^{25} = -165^\circ$ ($c = 0.35$, EtOH); IR (EtOH) $\tilde{\nu}_{\max}$ 3422, 3096, 2996, 1661, 1624, 1105, 1078 cm^{-1} ; ^1H NMR (500 MHz, $[\text{d}_6]$ acetone): $\delta = 7.18$ (d, $J = 9.5$ Hz, NH), 6.56 (t, $J = 7.8$ Hz, 1H), 5.98 (d, $J = 15.6$ Hz, 1H), 5.70 (d, $J = 15.9$ Hz, 1H), 5.38 (dd, $J = 15.6$, 7.87 Hz, 1H), 5.32 (dd, $J = 15.9$, 7.87 Hz, 1H), 5.22–5.18 (m, 2H), 5.05 (q, $J = 9.9$ Hz, 1H), 4.28–4.20 (m, 1H), 4.10–4.00 (m, 3H), 3.66–3.60 (m, 1H), 2.60–2.54 (m, 2H), 2.42–2.36 (m, 1H), 2.28–2.19 (m, 1H), 1.70 (s, 3H), 1.64 (s, 3H), 1.52 (s, 3H), 1.29 (d, $J = 6.8$ Hz, 3H), 0.95 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR (125 MHz, $[\text{d}_6]$ acetone): $\delta = 203.2$, 174.64, 139.13, 137.88, 136.29, 135.73, 134.73, 131.76, 131.72, 130.04, 129.07, 74.48, 72.65, 68.97, 49.67, 43.79, 42.44, 38.54, 37.42, 21.74, 16.16, 13.23, 12.66, 12.55; MS (DCI/ CH_4), m/z (%) 418 (4) [M^+], 400 (6) [$M^+ - \text{H}_2\text{O}$]; HR-MS (DCI/ CH_4): calcd for $\text{C}_{24}\text{H}_{36}\text{NO}_5$ [M^+]: 418.2593, found: 418.2573.

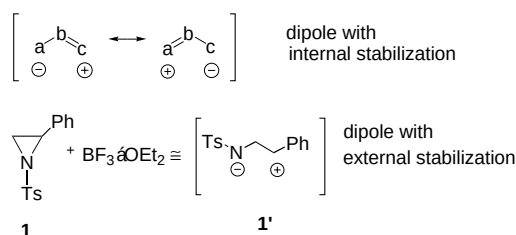
Phenylaziridine as a Masked 1,3 Dipole in Reactions with Nonactivated Alkenes**

Ioana Ungureanu, Philippe Klotz, and André Mann*

In memory of Toshiro Ibuka

Aziridines are known to react with a wide variety of nucleophiles, and their ability to undergo regioselective ring-opening reactions contributes largely to their synthetic value.^[1] In our previous work, we disclosed a new type of reactivity for phenylaziridine **1**, a formal [3+2] dipolar cycloaddition on *activated* double bonds (Ts = tosyl = *p*-toluenesulfonyl). Phenylaziridine **1** reacts with allylsilanes^[2] or dihydropyran (DHP)^[3] in the presence of a Lewis acid to produce highly substituted pyrrolidines. This type of reactivity for aziridines has been noticed before, but has never been systematically investigated.^[4]

We concluded that in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ at -78°C , **1** reacts via **1'**, a rather uncommon 1,3 dipole (Scheme 1). The two charges of **1'** are isolated by an sp^3 -hybridized carbon atom, and therefore, according to Huisgen, an internal stabilization by delocalization, as found in classical 1,3 dipoles,



Scheme 1. Possible modes of dipole stabilization.^[5]

[*] Dr. A. Mann, I. Ungureanu, P. Klotz
Laboratoire de Pharmacochimie de la Communication Cellulaire
UMR 7081, Faculté de Pharmacie
74 route du Rhin, BP 24, 67401 Illkirch (France)
Fax: (+33) 3-88-67-47-94
E-mail: andre.mann@pharma.u-strasbg.fr

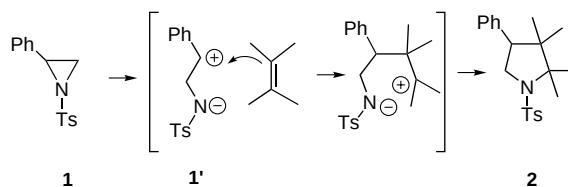
[**] We thank Professor C. G. Wermuth for stimulating discussions.

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is not possible.^[5] In contrast, **1'** is stabilized externally by the double contributions of the aromatic ring and the arylsulfonyl group. Thus, following the Huisgen classification **1'** can be considered as a zwitterionic 1,3 dipole.^[5] More interestingly, **1'** is electron deficient and should thus react with electron-rich partners. Therefore we wondered if **1** reacts with *nonactivated* olefinic double bonds.

Herein we present our results on the reactivity of **1** with alkenes. This new use of **1** as a 1,3-dipole precursor is an advance on our recent work,^[3] and of importance, not only from its theoretical, but also from its preparative significance in providing direct access to substituted pyrrolidines.

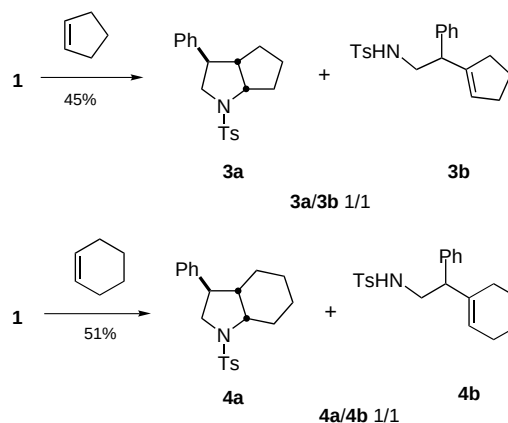
To avoid undesirable problems with regioselectivity we first considered a symmetrically substituted alkene.^[6] Therefore we chose to react tetramethylethylene with **1** in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ in CH_2Cl_2 at -78°C (the same experimental conditions were used for all reactions with **1**). A fast reaction took place and the only isolated product was the pyrrolidine **2** in 92 % yield (Scheme 2).



Scheme 2. Reaction of **1** with tetramethylethylene.

This result shows that **1'** is electron deficient enough to react even with a *nonactivated* alkene. The dipole **1'** is produced at -78°C , the olefinic π system attacks at the benzylic position giving rise to a stable tertiary carbocation, ready for a ring closure with the adjacent amide. This process constitutes a formal [3+2] cycloaddition that is useful for the synthesis of 2,2,3,3-tetrasubstituted pyrrolidines.

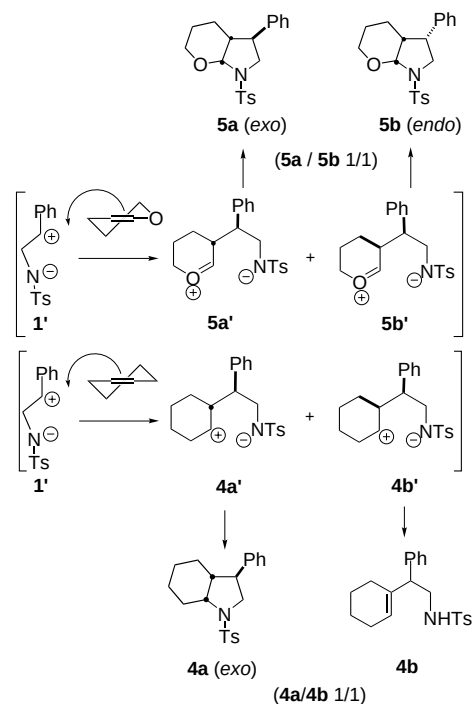
To explore the generality of this heterocyclization, we examined the reactivity of **1** towards cyclopentene and cyclohexene. We identified for each alkene a pair of product compounds **3a/3b** and **4a/4b**, formed in a 1/1 ratio in a total yield around 50 % (Scheme 3). The bicyclic adducts **3a** and **4a** are isolated as single diastereomers, and their relative



Scheme 3. Reaction of **1** with cycloalkenes.

configurations found to be *exo* by single-crystal X-ray analysis.^[7]

To investigate the diastereoselectivity of this reaction, we recall that **1** reacts with DHP giving **5a** and **5b** in a 1/1 ratio (Scheme 4).^[3] As the steric hindrance at C1 of cyclohexene and C3 of DHP are alike, we can consider that similar stereoelectronic factors govern the two processes, that is, **4a'** and **4b'** should be formed in the same ratio as **5a'** and **5b'**.

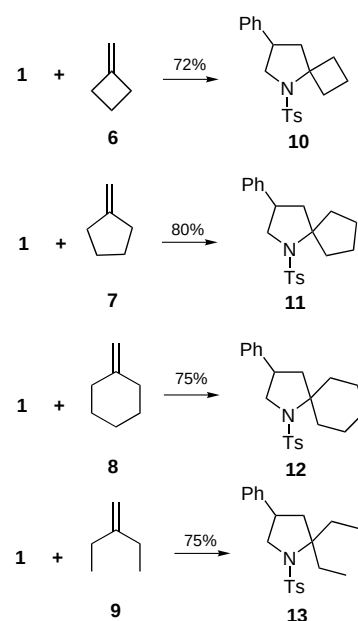


Scheme 4. Diastereoselectivity of the reaction of **1** with dihydropyran and cyclohexene.

The transient carbocations in **4a'**/**4b'** are not stabilized as they are in **5a'**/**5b'**, and side reactions can occur, competing with the formation of **4a**/**4b**. The intermediate dipole **4b'** prefers to eliminate, the ring closure is disfavored, probably for steric reasons. The formation of **3a**/**3b** and **4a**/**4b** in a 1/1 ratio, supports our argumentation. The same explanation can be evoked to account for the formation of **3a** and **3b** in a 1/1 ratio.

Since the nature and the yield of the products obtained in the reaction of **1** with olefins depend on the stability of the carbocation in the 1,5-zwitterionic intermediates, we decided to use geminal disubstituted alkenes. If the reaction pathway is conserved, a more stable, tertiary carbocation is expected in the transition state, and the heterocyclization should be favored. Indeed the olefins **6–8**, containing an exocyclic double bond, and the structurally similar open-chain compound **9**, react cleanly with **1**, under the usual conditions, to yield the spiropyrrolidines **10–12** and the geminal disubstituted pyrrolidine **13** as the only isolated products (Scheme 5).

To the best of our knowledge, this type of spiroannulation is without precedent. The good yields obtained support our hypothesis that heterocyclization is favored over elimination if the lifetime of the 1,5-zwitterionic intermediate is long



Scheme 5. Reaction of geminal disubstituted alkenes.

enough. Clearly the tertiary carbocations generated by the reaction of **1'** with methylenecycloalkanes have the required lifetime (in contrast to the secondary carbocations produced with cycloalkenes) and are able to cyclize. Elimination products are not observed.

The reactions described above have some similarities with the Prins reaction, where water plays the role of the nucleophile in quenching the carbocation derived from the olefin.^[8] In our case the nucleophile is delivered intramolecularly. If we consider the simultaneous formation of a C–C and C–N bond, this reaction could be termed “carboamination” of olefins. Few examples of this type are reported in the literature, in spite of considerable synthetic value for such a reaction.^[9] Noteworthy is that the replacement of the tosyl by the nosyl group (nosyl = *p*-nitrobenzenesulfonyl) in **1** did not alter the reactivity of the corresponding aziridine. Finally our results are in line with a recent example of a Lewis acid catalyzed reaction of oxiranes with alkenes.^[10]

The new chemical behavior of *N*-tosyl-2-phenylaziridine **1** is of interest from several points of view 1) in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ the reactivity **1** with *nonactivated* olefins is equivalent to that of a 1,3 dipole, 2) molecular complexity is achieved in a single step, and 3) the obtained adducts are important precursors of biologically active molecules. Indeed the azabicycloadducts **3a**/**4a** are valuable as strained phenyl-ethylamines,^[11] and are easily accessible from cheap starting materials, furthermore the production of spiropyrrolidines **10–12** is of importance as this structural motive forms the core of some biologically active alkaloids.^[12]

Experimental Section

General procedure for the reaction of **1** with alkenes: A solution of $\text{BF}_3 \cdot \text{OEt}_2$ (0.06 mL, 0.5 mmol, 1 equiv) in CH_2Cl_2 (0.3 mL) was added dropwise under argon to a solution of **1** (140 mg, 0.51 mmol) and alkene (1.4 equiv) in CH_2Cl_2 (5 mL) cooled at -78°C . After 20 min at -78°C , the solution was quenched with water (2 mL). The aqueous phase was extracted twice

with CH₂Cl₂ (5 mL) and dried over Na₂SO₄. The crude material was purified by column chromatography on silica gel eluting with hexane/diethyl ether.

Physical data for selected compounds (full details are in the Supporting Information):

4a: R_f = 0.37 (hexane/diethyl ether 1/1); ¹H NMR (300 MHz, CDCl₃, 25 °C, TMS) δ = 7.79 (d, J = 8.4 Hz), 7.36 (d, J = 7.8 Hz), 7.34–7.19 (m, 3H), 7.02–6.93 (m, 2H), 3.97–3.78 (m, 2H), 3.43–3.31 (m, 1H), 3.22 (dd, J = 9.7, 10 Hz, 1H), 2.46 (s, 3H), 2.19–2.05 (m, 1H), 1.98–1.83 (m, 1H), 1.75–1.69 (m, 1H), 1.63–1.09 (m, 6H); ¹³C NMR (75 MHz, CDCl₃, 25 °C, TMS): δ = 143.4, 139.6, 135.8, 129.8, 128.8, 127.8, 127.5, 127.3, 60.4, 54.4, 44.9, 44.7, 30.9, 24, 21.7, 20.6; MS (IE) m/z : 355.

10: R_f = 0.44 (hexane/diethyl ether 1/1); ¹H NMR (300 MHz, CDCl₃, 25 °C, TMS): δ = 7.76 (d, J = 8.4 Hz, 2H), 7.4–7.15 (m, 7H), 3.87 (m, 1H), 3.41–3.26 (m, 2H), 3.21 (dd, J = 8.4, 10.3 Hz, 1H), 2.89 (dd, J = 10, 20.3 Hz, 1H), 2.57 (dd, J = 5, 11.9 Hz, 1H), 2.43 (s, 3H), 2.11–1.74 (m, 4H), 1.71–1.49 (m, 1H); ¹³C NMR (50 MHz, CDCl₃, 25 °C, TMS): δ = 143, 139.9, 138.5, 129.8, 128.8, 127.2, 127.1, 66.9, 55.7, 47.3, 40.5, 37.4, 34.6, 21.6, 14.5; MS (ESI) m/z : 342 [$M+H$]⁺.

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 [7] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-142893 (**4a**) and -142894 (**3a**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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