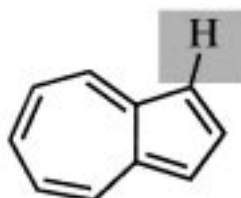
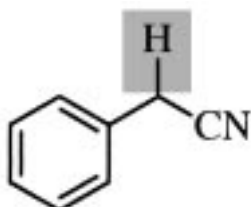
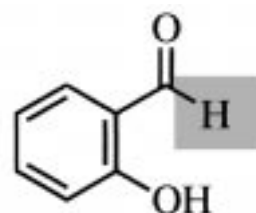
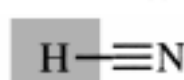
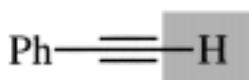
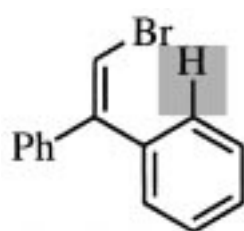
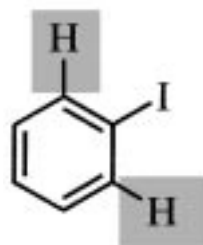
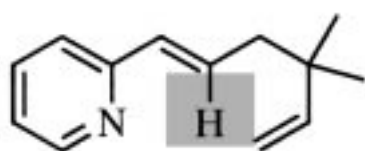
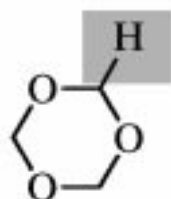


Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd
La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg



A diverse range of C–H activations can be catalyzed by transition metal complexes.

Transition Metal Catalyzed Coupling Reactions under C–H Activation

Gerald Dyker*

The activation of C–H bonds is of the same fundamental importance as the formation of C–C bonds for the construction of organic molecules. Particularly in the last ten years, transition metal catalyzed processes have been developed which achieve both these in a single preparative step and hence combine economy, efficiency, and elegance. Palladium and ruthenium cata-

lysts are currently used most frequently, closely followed by rhodium complexes: For instance, ruthenium has proved to be particularly efficient for the C–H activation of arenes, palladium in catalytic processes with metallacycles as intermediates, and rhodium in coupling reactions under C–H activation at the aldehyde function. Mercury displays catalytic activity on photo-

chemical excitation in the gas phase. Iridium and tungsten complexes likewise show potential for economically valuable reactions, but must first prove their worth in catalytic processes.

Keywords: C–H activation • C–C coupling • homogeneous catalysis • transition metals

*C–H activation means money.
C–H activation begins just below one's own results.
Pointed definitions of C–H activation
(collected at a conference)*

1. Introduction

The development of transition metal catalysts for the activation of C–H bonds is one of the major challenges of modern chemistry. The first of the two above-mentioned definitions stresses the economic importance: a coupling reaction of the “greenhouse gases” methane and carbon dioxide to give acetic acid would be of the same enormous commercial interest as the industrially applicable process for the selective oxidation of simple alkanes to give alcohols and carbonyl compounds. The second bon mot illustrates that there is quite a dispute as to which reactions are part of this diverse area of research. Contrary to the widespread opinion that only particularly inert alkanes require activation, it must be mentioned that also in the case of C–H acidic substrates the change from the usual basic reaction conditions to transition metal catalyzed variants can be of great preparative advantage. Murahashi et al. have shown that ruthenium-catalyzed aldol reactions are an illustrative example (see Section 4).

The application of strong organometallic bases for the activation of C–H groups with low acidity offers quite a

modern and efficient procedure.^[1] Preparative and economic advantages are expected from replacing such stoichiometric metalation reactions by catalytic ones. Particularly for a lack of selectivity under classic conditions or for base sensitivity of the functional groups, transition metal catalysis under mild and neutral conditions offers a promising and superior alternative. Only in recent years have practically applicable examples for transition metal catalyzed coupling reactions under C–H activation become more frequent.

This review focusses on transition metal catalyzed C–H activations that lead to C–C coupling reactions. According to the current understanding of the mechanisms of these reactions, the transition metal catalyst is directly involved in the C–H bond breaking. In Section 2 following this introduction coupling reactions are presented in which the transition metal is precoordinated to a suitable functional group and the C–H bond is cleaved by a cyclometalation step. In the Section 3 sequences are discussed in which the C–H activation occurs intramolecularly by a carbon-bound transition metal atom. This type of reaction is clearly a domain of palladium chemistry. Section 4 introduces the currently still rare examples of “intermolecular” C–H activation, which is taken to cover all reactions of this type that do not require a cyclometalation step. Finally, Section 5 presents an overview of recent stoichiometric C–H activation reactions which are promising candidates for the development of a catalytic reaction.

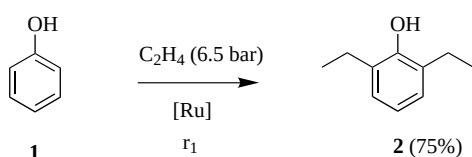
The Heck reaction^[2] and hydrovinylation^[3] are not be considered in this review: in these processes C–H activation occurs only after C–C coupling. C–H activations for C–Heteroatom coupling,^[4] among them oxidation reactions such as the one with Barton's Gif systems are also not discussed in this review.

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2. Intramolecular C–H Activation by a Precoordinated Transition Metal

Cyclometalation reactions under C–H activation have been known for more than thirty years and represent one of the best developed areas of organometallic chemistry.^[5] C–H bond breaking occurs either by oxidative addition at the metal center or by electrophilic substitution; in some cases multi-center reaction paths are discussed.^[5a] Cyclometalated transition metal complexes (often referred to as *ortho*-metalated complexes) participate in numerous coupling reactions; however, attempts to integrate such reactions into a catalysis cycle have proven difficult,^[6, 7] and it is only recently that a number of catalytic processes been discovered that display remarkable turnover numbers and acceptable yields.

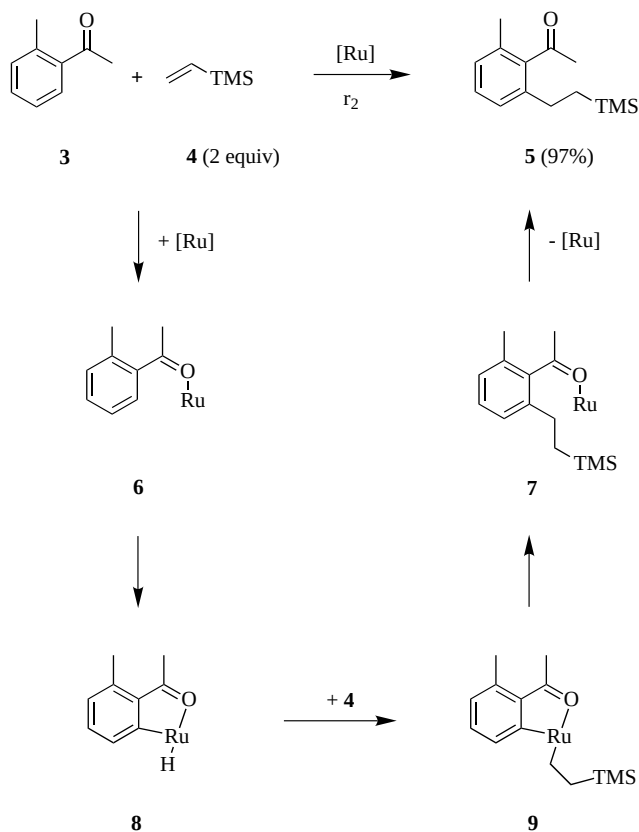
Lewis and Smith achieved an initial success in 1986^[8] with the double alkylation of phenol (**1**) with ethylene selectively in the *ortho* positions (Scheme 1) by using an *ortho*-metalated



Scheme 1. Ru-catalyzed double *ortho* ethylation of phenol; reaction conditions r_1 : 6 mol % cyclometalated Ru-tetrakisphosphite complex, 9 mol % KOPh, 8.6 equiv ethene, 6.5 bar, THF, 177 °C, 3.5 h. Here and in the subsequent schemes the catalyst is denoted by the symbol for the element in square brackets.

ruthenium phosphite complex. A decisive breakthrough was achieved by Murai et al. in 1993, who reported a highly efficient, ruthenium-catalyzed addition of aromatic C–H bonds to olefins,^[6, 9] in which ketones were used as pre-coordinating functional groups (Scheme 2). This coupling process is easy to carry out (2 mol % ruthenium catalyst in toluene at reflux temperature) and in many cases renders practically quantitative yields. For example, the acetophenone **3** is added to the vinyl silane **4** and yields regioselectively the *ortho* product **5**.^[6c] Apparently, the transition metal is pre-coordinated by the carbonyl function of **3** and thus placed in a favorable position for the cleavage of the C–H bond: cyclometalation leads to the ruthenium hydride complex **8**

as the reactive intermediate (additional ligands have been omitted for clarity in Scheme 2). After coordination of the olefin **4** an insertion reaction probably occurs leading to the formation of **9**. The C–C bond is formed by reductive elimination. Subsequent decomplexation of **7** leads to the final product **5** and at the same time liberates the active catalyst.

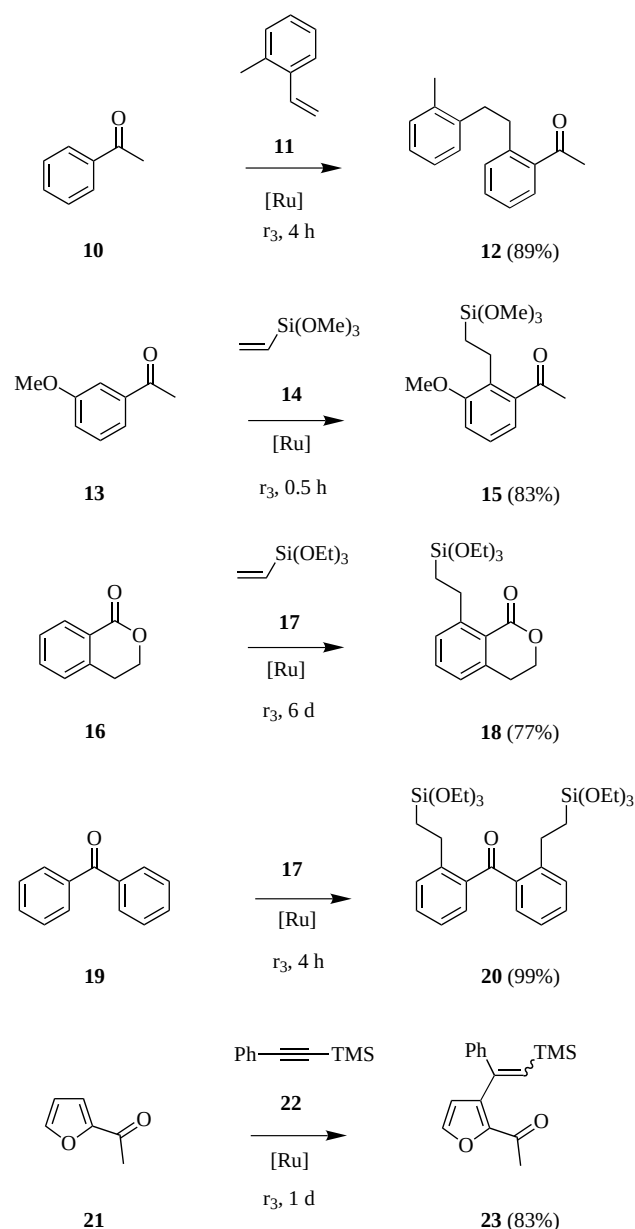


Scheme 2. Reaction conditions r_2 : 2 mol % $[\text{RuH}_2(\text{CO})(\text{PPh}_3)_3]$, toluene, reflux, 2 h.

The Murai reaction is generally applicable with regard to the carbonyl component: aromatic and heteroaromatic ketones such as the compounds **10**, **13**, **19**, and **21**, as well as the six-membered lactone **16** are suitable. (Scheme 3). The coupling reactions with the olefins **11**, **14**, and **17** (also with



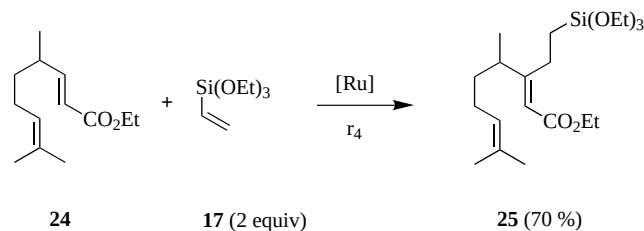
Gerald Dyker was born in 1960 and studied chemistry at the Universität Dortmund, where he received his doctorate under the supervision of R. P. Kreher in 1988 for a thesis on *ortho*-quinoid heteroarenes. After postdoctoral research on ruthenium catalysis at Stanford University with B. M. Trost he worked as a research chemist at the Bayer AG, Monheim, in the field of veterinary pharmaceuticals. He was granted a Liebig fellowship which enabled the completion of his habilitation at the TU Braunschweig in 1994. Since 1995 he has been a Professor for Organic Chemistry at the Universität-Gesamthochschule Duisburg. His current research interests in addition to transition metal catalysis include the synthesis of nanodimensional π systems, new chiral ligands, amino acids as components of syntheses, and the interaction of carbocations with neighboring groups.



Scheme 3. Reaction conditions r_3 : 2 mol % $[\text{RuH}_2(\text{CO})(\text{PPh}_3)_3]$, 2–4 equiv alkene or alkyne, toluene, reflux, 0.5 h to 6 d.

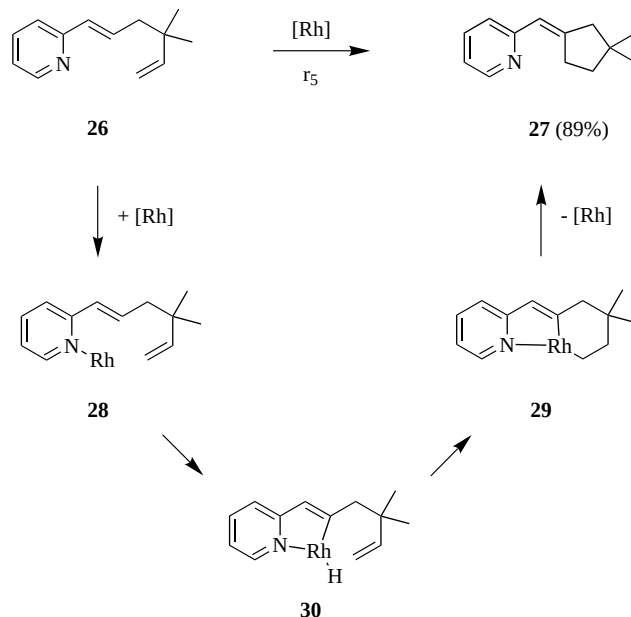
allyl silanes and with norbornene) and with alkynes such as **22**^[9c] gives excellent yields of a broad selection of products such as **12**,^[6c] **15**,^[9b] **18**,^[9d] **20**,^[6c] and **23**.^[9c] Benzoates react in certain cases.^[9d] Aryl-substituted aldimines and ketimines are also applicable.^[9c, 10] Enol ethers and olefins that can undergo double-bond isomerizations as well as electron-poor olefins such as acrylonitrile have so far been unsuitable for coupling reactions;^[6c] a deeper understanding of the mechanistic course and the corresponding modifications of catalyst and reaction conditions should help to overcome these limitations.

The areas of application have been extended by Trost et al.^[11a] and by Murai et al.^[11b,c] to α,β -unsaturated ketones and esters, as illustrated by the addition reaction of **24** to the vinyl silane **17** (Scheme 4); in this reaction a functionalized side chain is introduced, which opens up interesting possibilities for further transformations.



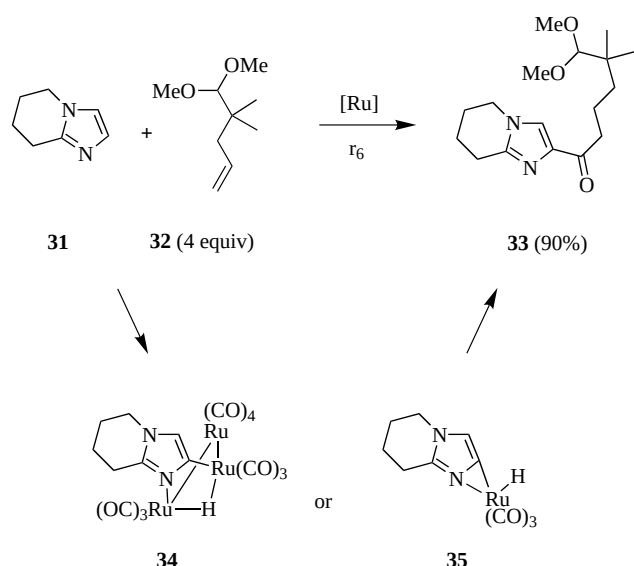
Scheme 4. Reaction conditions r_4 : 2 mol % $[\text{RuH}_2(\text{CO})(\text{PPh}_3)_3]$, toluene/dioxane, reflux, 2 h.

Rhodium seems to be the metal of choice for C–H activation of pyridine derivatives. In this case the pyridyl group is crucial for the precoordination.^[12] According to Kim et al.^[13] 2-phenylpyridine is alkylated in the 2'-position by terminal alkenes when tris(tricyclohexylphosphane)rhodium chloride is used as the catalyst. Murai et al.^[14] reported that Wilkinson's catalyst $[\text{RhCl}(\text{PPh}_3)_3]$ is suitable for C–H activation at pyridyl-substituted 1,5- and 1,6-dienes. Starting from 1,5-diene **26** a formal 5-*endo-trig*-cyclization is observed. The C–H activation at the vinyl position probably occurs through the intermediates **28** and **30**. The intramolecular insertion of the terminal double bond leads to the metalla-cycle **29**, which reacts by reductive elimination to give the final product **27** (Scheme 5).



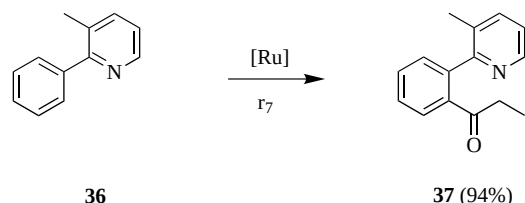
Scheme 5. Reaction conditions r_5 : 10 mol % $[\text{RhCl}(\text{PPh}_3)_3]$, THF, 120 °C, 3 h.

The trinuclear ruthenium cluster $[\text{Ru}_3(\text{CO})_{12}]$ metalates nitrogen-heteroarenes at the *ortho* position. This reaction step was integrated into a catalytic cycle by Moore et al.^[15a] when carbon monoxide and terminal olefins are employed as coupling components, pyridine is acylated in the 2-position. Murai et al. found that imidazole derivatives are significantly more reactive in coupling processes of this type.^[15b] The reaction is highly efficient and selective as illustrated by the synthesis of the functionalized imidazole **33** (Scheme 6). The ruthenium complex **34** with an intact cluster framework is



Scheme 6. Reaction conditions r_6 : 4 mol % $[Ru_3(CO)_{12}]$, 20 atm CO, toluene, 160 °C, 20 h.

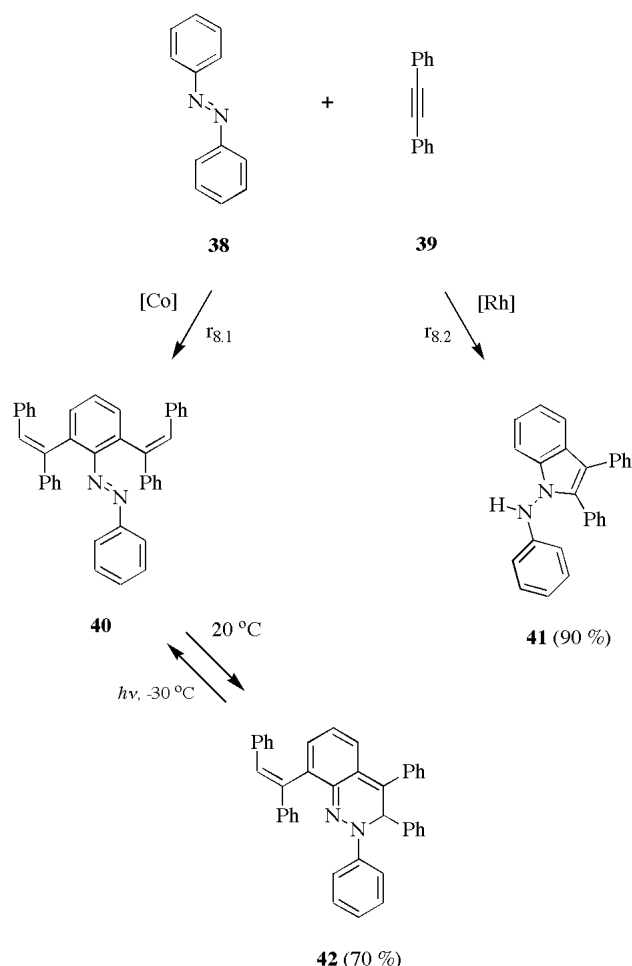
favoured as the key intermediate, although the mononuclear ruthenium carbonyl complex **35** so far cannot be ruled out as a possibility. The acylation of the pyridine derivative **36** at the phenyl substituent with ethene and carbon monoxide as coupling components is closely related to this reaction. (Scheme 7).^[15c]



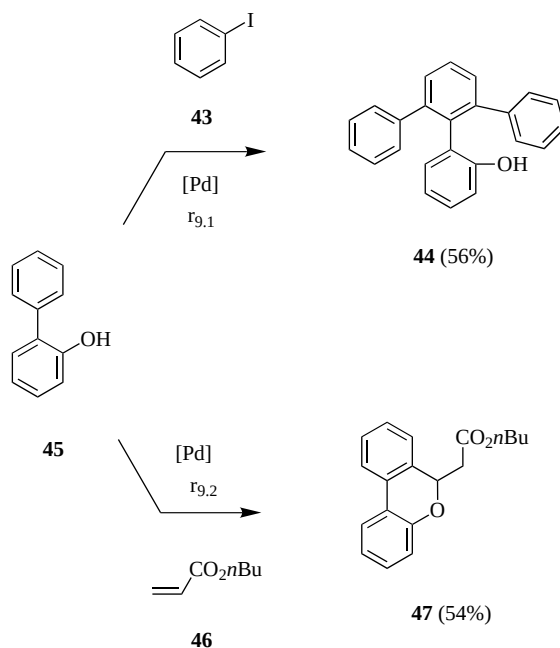
Scheme 7. Reaction conditions r_7 : 2.5 mol % $[Ru_3(CO)_{12}]$, 20 atm CO, 7 atm C_2H_4 , toluene, 160 °C, 20 h.

Azobenzene (**38**) reacts with numerous transition metal complexes under cyclometalation.^[5] According to Kisch et al. the cobalt-catalyzed coupling reaction of azobenzene with diphenylethyne (**39**) leads to the heterocyclic 1:2 product **42**, which displays photochromic behavior (Scheme 8); the red heterocycle **42** is photochemically transformed at -30°C to give the orange azo compound **40**, which spontaneously recyclizes at room temperature.^[16a] By employing Wilkinson's catalyst under acidic reaction conditions and with equimolar amounts of coupling components, the 1:1 product **41** is obtained in high yields.^[16b]

According to Miura et al. phenolates are also suitable as pre-coordinating group (Scheme 9):^[17] 2-hydroxybiphenyl (**45**) is regioselectively arylated in the 2'-position with iodobenzene (**43**) under palladium catalysis. With an excess of **43** a double arylation occurs to give **44**; for both cases oxapalladacycles can be formulated as reactive intermediates.^[17a] The variant with the acrylate **46** as coupling component deserves particular attention: The catalysis cycle to give dibenzopyran requires reoxidation of Pd^0 to Pd^{II} .^[17b] The aldehydic hydro-

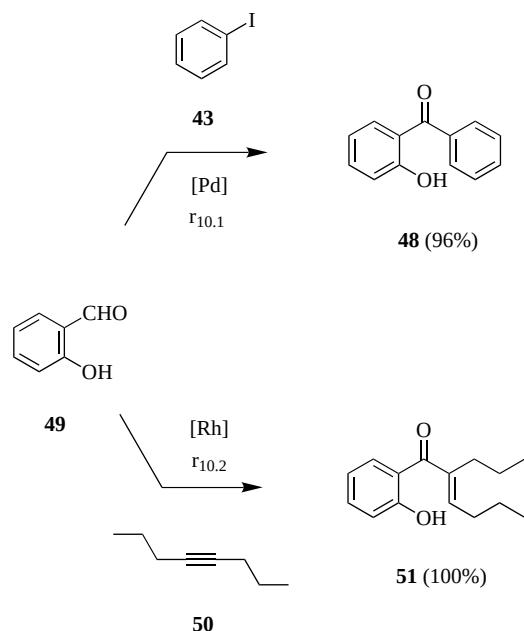


Scheme 8. Reaction conditions; $r_{8.1}$: cat. $[CoH_3(PPh_3)_3]$, 1 equiv **39**, 1-PrOH/HOAc, 110 °C, 24 h; $r_{8.2}$: cat. $[RhCl(PPh_3)_3]$, without solvent, 85 °C, 2 h.



Scheme 9. Reaction conditions; $r_{9.1}$: 5 mol % $Pd(OAc)_2$, 4 equiv **43**, CS_2CO_3 , 4 Å molecular sieves, DMF, 100 °C, 44 h. $r_{9.2}$: 5 mol % $Pd(OAc)_2$, 5 mol % $Cu(OAc)_2 \cdot H_2O$, 4 Å molecular sieves, N_2 /air (5:1), DMF, 120 °C, 9 h.

gen atom of salicylaldehyde (**49**) can be activated both under palladium^[17c] and under rhodium catalysis^[17d] (Scheme 10): cyclometalated complexes can be postulated as reactive intermediates in the formation of **48** and of **51**.

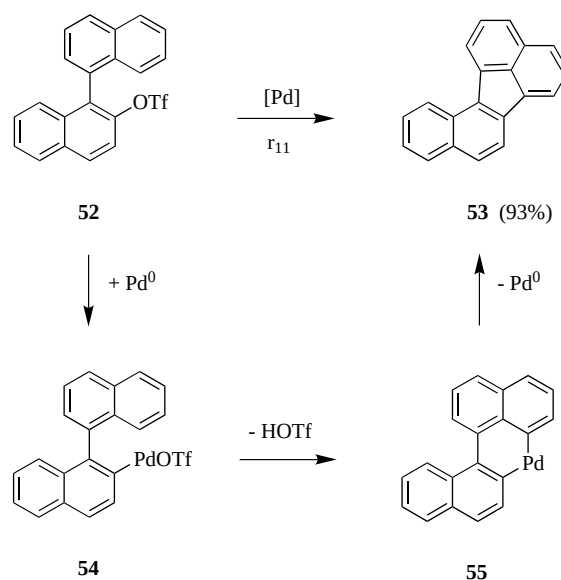


Scheme 10. Reaction conditions; $r_{10.1}$: 5 mol % PdCl_2 , 2 equiv **43**, Na_2CO_3 , DMF, 100°C , 4 h. $r_{10.2}$: 0.5 mol % $[\text{RhCl}(\text{cod})]_2/\text{dppf}$, Na_2CO_3 , toluene, reflux, 0.5 h. cod = cyclooctadiene, dppf = 1,1'-bis(diphenylphosphanyl)ferrocene.

The given examples for transition metal catalyzed coupling reactions under C–H activation, which are controlled by a pre-coordinating functional group, are limited to sp^2 -hybridized C–H groups. The development of analogous catalysis cycles for the C–H activation at alkyl groups is a promising undertaking, especially since corresponding stoichiometric cyclometalation reactions are well known.^[5a]

3. Intramolecular C–H Activation through Carbon-Bound Transition Metals

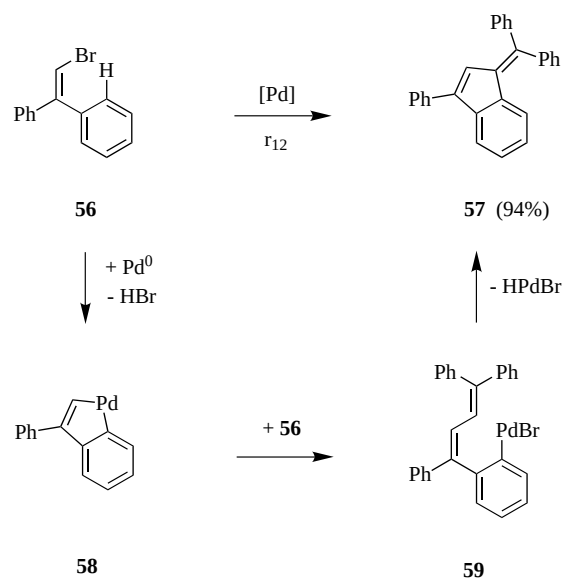
In this section of the review catalytic coupling processes are presented that are initiated by a cyclometalation step. Similar to the examples given in Section 2 the transition metal is directed into the proximity of the C–H bond which is to be broken; however, in these examples by a σ bond between a carbon atom and a transition metal atom. The formation of the fluoroanthene derivative **53** by Rice and Cai illustrates characteristic features of this reaction type (Scheme 11).^[18] In general it is assumed that Pd^{II} compounds used for the catalysis are reduced in situ and that the active catalysts are compounds in the oxidation state 0. The first carbon–palladium σ bond is formed by oxidative addition of the Pd^0 catalyst to the aryl triflate **52**. The electrophilic Pd^{II} species **54** is responsible for the intramolecular C–H activation, during which the second palladium–carbon σ bond is formed. This cyclopalladation step can be interpreted as an electrophilic substitution at the arene rings.^[19] The intermediate **55**



Scheme 11. Reaction conditions r_{11} : 10 mol % $[\text{PdCl}_2(\text{PPh}_3)_2]$, LiCl, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), DMF, 140°C , 10 h.

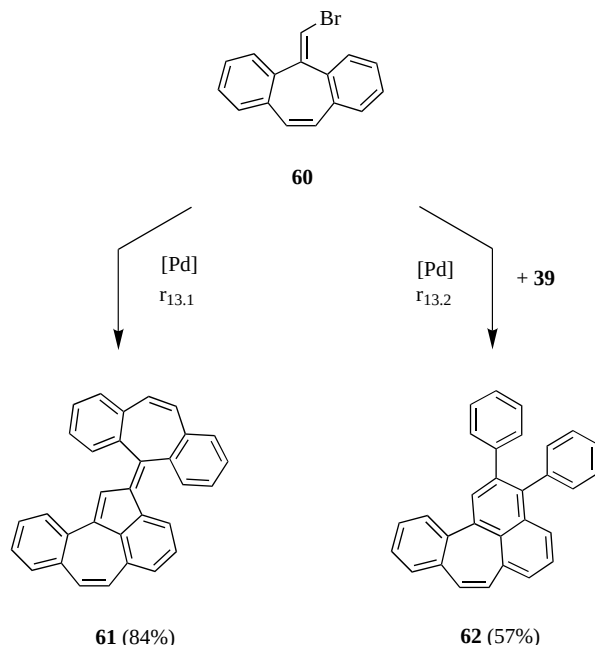
reacts under reductive elimination, a typical reaction step for six-membered and larger palladacycles. In the meantime this type of intramolecular aryl–aryl coupling can be classed as an established method of synthesis and has proven useful for the construction of five- and six-membered carbocyclic and heterocyclic systems.^[20]

Evidently, six-membered palladacycles react readily under reductive elimination to give the five-membered rings, even when this means the build-up of considerable strain within the ring as for fluoroanthene **53**. Five-membered palladacycles usually react differently: in a reaction sequence comprising oxidative addition and C–H activation the phenyl-substituted bromoethene **56** forms the five-membered palladacycle **58** (Scheme 12).^[21a] In this case the reductive elimination of Pd^0 is inhibited because a highly strained and antiaromatic benzo-



Scheme 12. Reaction conditions r_{12} : 1 mol % $\text{Pd}(\text{OAc})_2$, K_2CO_3 , $n\text{Bu}_4\text{NBr}$, DMF, 100°C , 3 d.

cyclobutadiene would be the result. Instead, the palladacycle **58** adds a further equivalent of the starting material **56**, leading to the Pd^{II} intermediate **59**. Ring closure under C–C coupling occurs in the sense of an intramolecular Heck reaction. Tricyclic derivatives of **56** react in the same way; the vinyl bromide **60** with a central seven-membered ring is also condensed to the annelated fulvene **61** (Scheme 13). In the

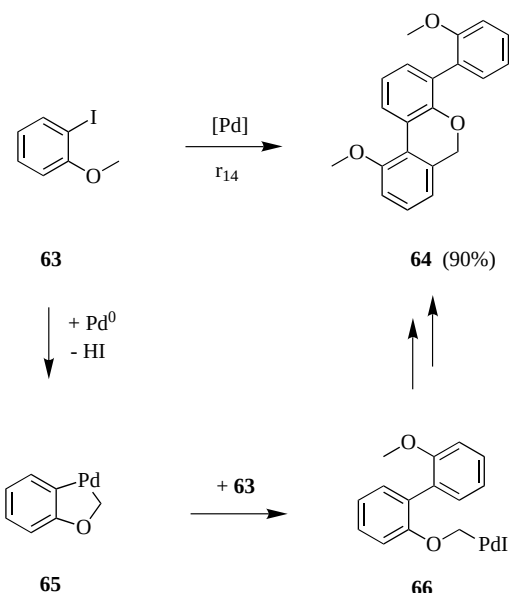


Scheme 13. Reaction conditions; $r_{13.1}$: 1 mol % Pd(OAc)₂, K₂CO₃, *n*Bu₄NBr, DMF, 100 °C, 3 d. $r_{13.2}$: 1 mol % Pd(OAc)₂, 1 equiv diphenylethyne (**39**), K₂CO₃, *n*Bu₄NBr, DMF, 100 °C, 3 d.

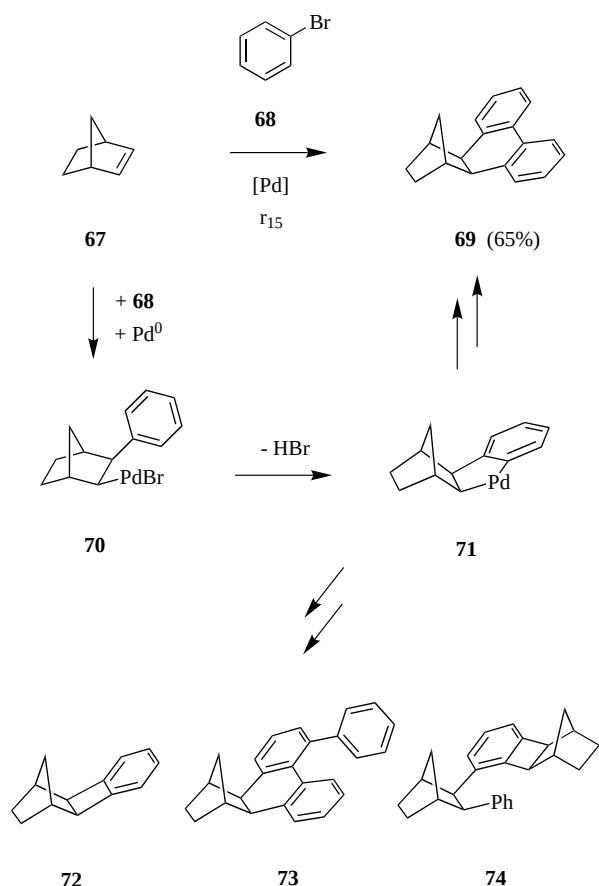
presence of diphenylethyne (**39**) a cross-coupling reaction occurs, leading to the polycyclic hydrocarbon **62**; in this case it can also be assumed that a cyclometalation is crucial for the C–H activation.^[21b]

The coupling reaction of *ortho*-iodoanisole (**63**) to give dibenzopyran **64** is a particularly interesting domino process (Scheme 14).^[22] Evidently, three equivalents of the starting material **63** are combined leading to C–H activation at a sp³-hybridized center, since a methoxy group has been arylated. Once again palladacycles such as **65** are assumed to be the key intermediates. This type of palladium-catalyzed C–H activation at sp³-hybridized centers is by no means limited to methoxy groups. Similar reactions can also be observed for *tert*-butyl groups.^[23]

The substrates in the examples discussed so far are characterized by a structural unit that allows direct cyclopalladation. In the following examples, such a structural unit first needs to be constructed: carbopalladation at an unsaturated C–C bond precedes the C–H activation. This reaction type is illustrated by the extensively researched palladium-catalyzed annelation reaction of norbornene (**67**) with bromobenzene (**68**).^[24] Depending on the reaction conditions the intermediary five-membered palladacycle **71** leads to at least four different types of products (Scheme 15): the 1:2 product **69**,^[24a] the benzocyclobutene derivative **72** as 1:1 product, which is formed directly from the five-membered palladacycle



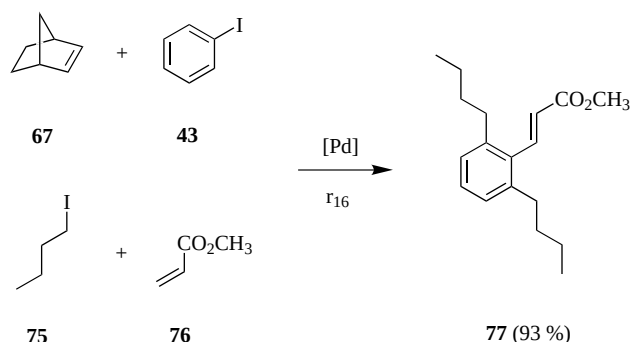
Scheme 14. Reaction conditions r_{14} : 4 mol % Pd(OAc)₂, K₂CO₃, *n*Bu₄NBr, DMF, 100 °C, 3 d.



Scheme 15. Reaction conditions r_{15} : 4 mol % [Pd(PPh₃)₄], KO^{*t*}Bu, anisole, 105 °C, 10 h.

71 by reductive elimination,^[24b] the 1:3 product **73**,^[24c,d] and the 2:2 product **74**.^[24d] Based on stoichiometric reactions the research group of Catellani has recently been able to present conclusive mechanistic concepts on the formation of these products,^[24e] for which the reversibility of the carbopallada-

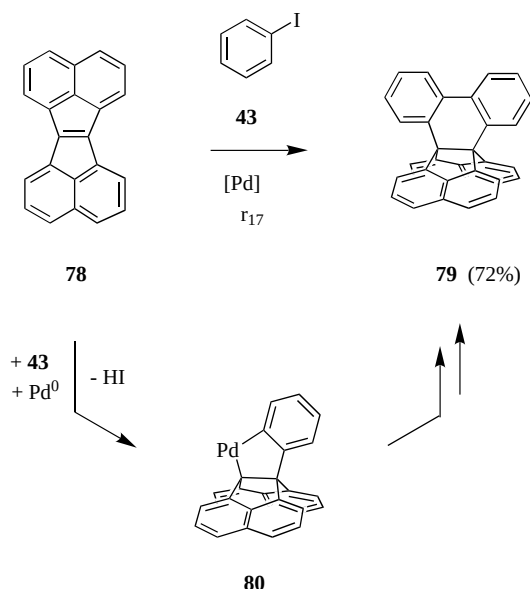
tion of norbornene **67** plays a decisive role. In the case of steric overloading of the corresponding palladium complexes, the reversibility can already occur at room temperature and it is also a key step in the fascinating multicomponent reaction in Scheme 16.^[24f] Norbornene **67** serves as a template to direct



Scheme 16. Reaction conditions r_{16} : 5 mol % Pd catalyst of the isolated structure type **70**, reactants **67**, **43**, **75**, and **76** in the ratio 1:1:4:1.5, K_2CO_3 , dimethylacetamide, 20 °C, 30 h.

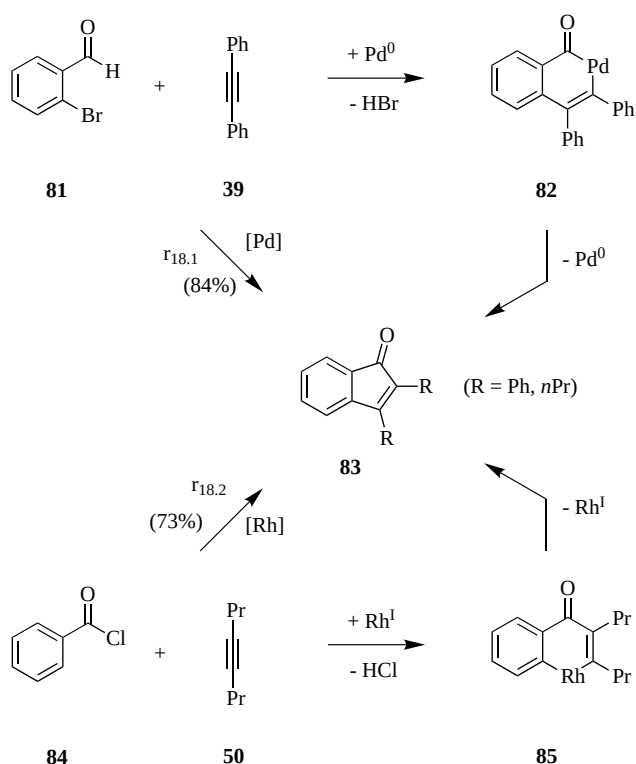
the catalytically active palladium center for the necessary C–H activation steps but is not built into the final product **77** itself.

Similar annelation reactions such as the one at norbornene also occur at disubstituted alkynes^[25] and at the hexacyclic hydrocarbon **78**, a strained, tetrasubstituted olefinic coupling component (Scheme 17);^[26] the reaction with iodobenzene (**43**) results in the propellane **79** with a hexaarylethane structure.



Scheme 17. Reaction conditions r_{17} : 5 mol % $Pd(OAc)_2$, K_2CO_3 , nBu_4NBr , DMF, 100 °C, 14 d.

Larock et al. also postulate a cyclopalladation as the decisive reaction step in the intramolecular C–H activation at the aldehyde function of **81** (Scheme 18).^[27] The coupling of **81** with different disubstituted alkynes provides a general route to indenones **83**. A rhodium-catalyzed coupling reaction starting from aroyl chlorides such as **84** leads to the same



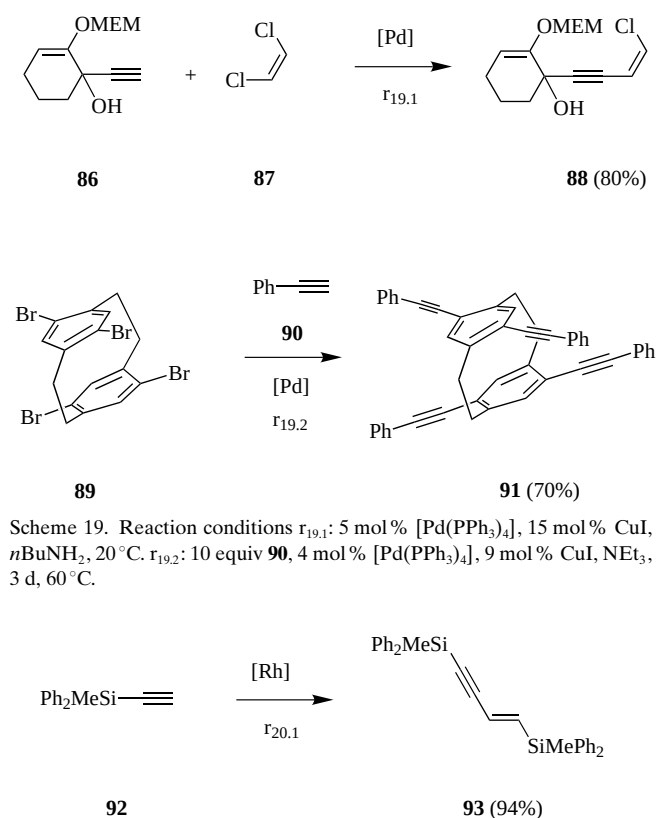
Scheme 18. Reaction conditions; $r_{18.1}$: 1 mol % $Pd(OAc)_2$, NaOAc, nBu_4NCl , DMF, 100 °C, 36 h. $r_{18.2}$: 5 mol % $[RhCl(cod)]_2/PPh_3$, Na_2CO_3 , *o*-xylene, 145 °C, 24 h.

result.^[28] According to Miura et al. rhodacycles such as **85** can be assumed as intermediates in this process. In comparison to the palladium-catalyzed variant, this method seems to have the advantage that the starting materials are more readily accessible and it illustrates that intramolecular C–H activation through carbon-bound transition metal complexes is by no means limited to palladium.

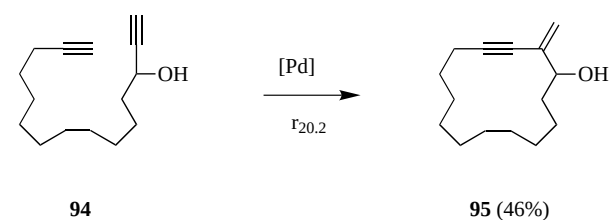
4. C–H Activation without Cyclometalation

Numerous examples for transition metal catalyzed C–C couplings with the participation of relatively acidic C–H groups are well known and intensively used. The transition metal catalyst can be considered as an organometallic base responsible for the C–H activation. For the Stevens–Castro–Sonogashira reaction^[29] (e. g. coupling of **86** with **87** to give **88**, Scheme 19)^[30a], additional bases such as amines or carbonates are always present; however, their main purpose is the regeneration of the active Pd^0 catalyst from intermediate hydridopalladium halide complexes. These bases are not absolutely necessary for the C–H activation step. The Sonogashira reaction is of particular interest for the construction of large, conjugated π systems. The fourfold reaction at the paracyclophane **89** provides an indication of the potential synthetic applications.^[30b]

The transition metal is undoubtedly responsible for the C–H activation in the rhodium-catalyzed head-to-head dimerization of the alkynyl silane **92** because in this case no further bases are necessary (Scheme 20).^[31] For Ene–yne syntheses^[32]

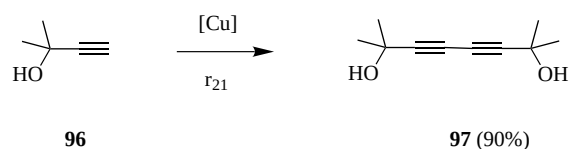


Scheme 19. Reaction conditions $r_{19.1}$: 5 mol % $[Pd(PPh_3)_4]$, 15 mol % CuI, $nBuNH_2$, 20 °C. $r_{19.2}$: 10 equiv **90**, 4 mol % $[Pd(PPh_3)_4]$, 9 mol % CuI, NEt_3 , 3 d, 60 °C.



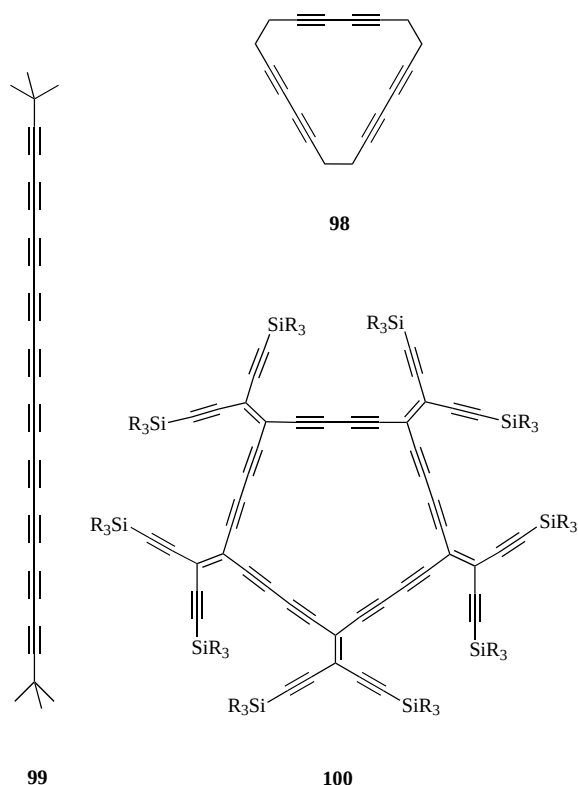
Scheme 20. Reaction conditions $r_{20.1}$: 5 mol % $[RhCl(PPh_3)_3]$, toluene, room temperature, 30 h. $r_{20.2}$: 10 mol % $Pd(OAc)_2$, tris-(2,6-dimethoxyphenyl)phosphane, benzene, reflux.

of this kind the head-to-tail dimerization such as that for the macrocycle **95** is the rule (Scheme 20).^[33] In this context a classic among C–H activation reactions, the Glaser coupling,^[34] in which terminal acetylenes are coupled under copper catalysis and oxidation to give butadiynes, cannot go unmentioned; the efficient conversion of the butynol **96** serves as a good example (Scheme 21).^[34b] A selection of particularly



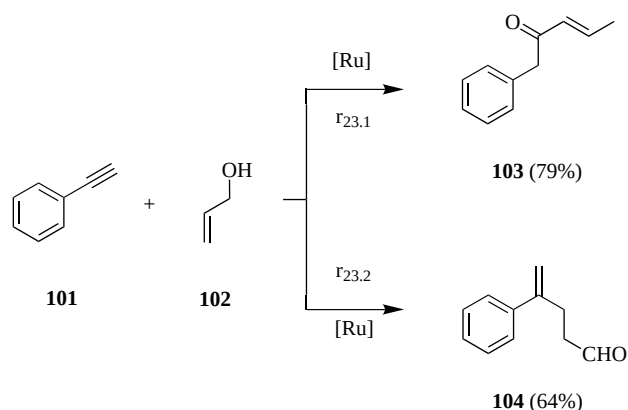
Scheme 21. Glaser coupling; reaction conditions r_{21} : 1.2 mol % Cu_2Cl_2 , pyridine/MeOH, O_2 , 35 °C, 2.5 h.

carbon-rich compounds that are accessible by the Glaser coupling are presented in Scheme 22. The potential applications range from macrocycles such as **98**^[34c] through polynes such as **99**^[34d] to building blocks for carbon networks **100**.^[34e]



Scheme 22. Selection of structures accessible by Glaser coupling. R = *i*Pr.

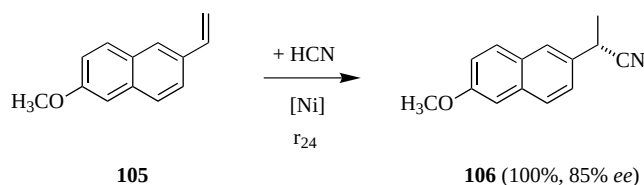
Ruthenium catalysts open up new reaction pathways for terminal alkynes since C–H activation can be used for the formation of cationic vinylidene complexes: with allyl alcohols as the coupling partner a great number of products is selectively accessible.^[35] Depending on reaction conditions, substitution pattern, and catalyst, β,γ -unsaturated ketones and α,β -unsaturated ketones such as **103**^[35a] or even γ,δ -unsaturated carbonyl compounds^[35b] such as **104**^[35d,e] are obtained selectively (Scheme 23).



Scheme 23. Reaction conditions $r_{23.1}$: 1. 10 mol % $[CpRuCl(PPh_3)_2]$, NH_4PF_6 , 100 °C, 10 h; 2. $RhCl_3$, THF, reflux. $r_{23.2}$: 5 mol % $[RuCl(cod)(C_5Me_5)]$, water as solvent, 90 °C, 1 h; in addition to the major product **104**, the regioisomer 5-phenyl-4-pentenal was obtained in 21 % yield.

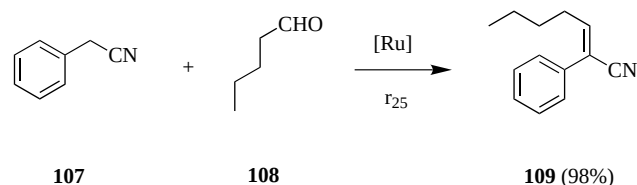
Some reactions which fall into the category of transition metal catalyzed C–C couplings with acidic C–H groups are already of commercial interest: the copper acetylide catalyzed

synthesis of butynediol from acetylene and formaldehyde^[36] as well as hydrocyanation should be emphasized: the enantioselective synthesis of the naproxene-nitrile **106** is a well-known example (Scheme 24).^[37] Related coupling reactions of



Scheme 24. Reaction conditions r_{24} : 0.1 mol % $[\text{Ni}(\text{cod})_2]$, chiral bisphosphite ligand, toluene, 25 °C.

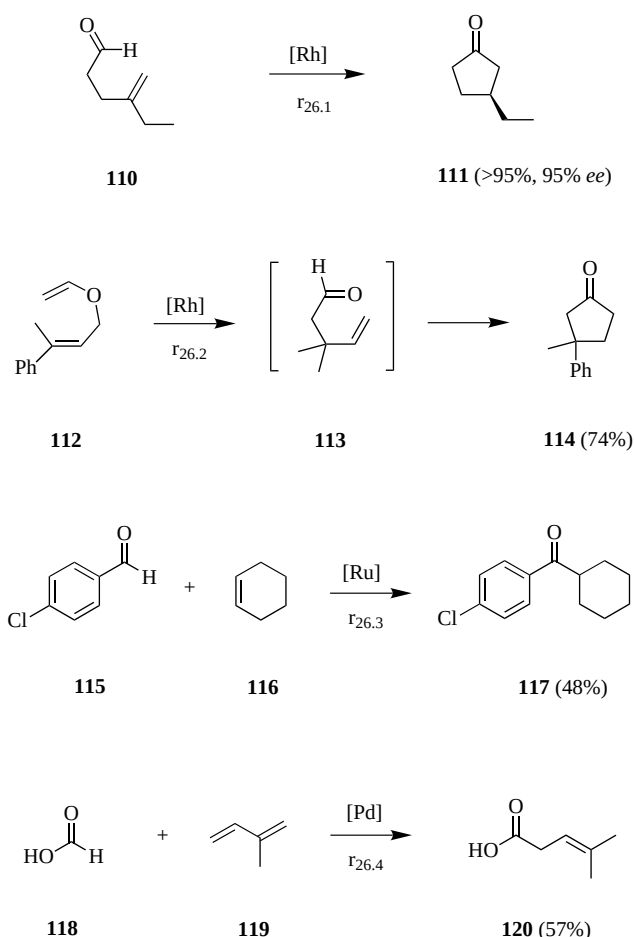
C–H acidic compounds, such as malonates and ketones, with alkenes and alkynes are of particular interest since they are atom-economic addition reactions;^[38] however, in many cases the additional bases and not the transition metal catalyst are responsible for the C–H activation. According to Murahashi and Naota the advantages of carrying out a reaction under clearly neutral conditions become particularly apparent for ruthenium-catalyzed aldol reactions (Scheme 25):^[39, 40] C–H



Scheme 25. Reaction conditions r_{25} : 3 mol % $[\text{RuH}_2(\text{PPh}_3)_4]$, THF, room temperature, 24 h.

acidic nitriles such as **107** can be chemoselectively condensed with even stronger C–H acidic aldehydes. In contrast, under classic basic reaction conditions the homocondensation of the aldehydes is the determining reaction path.

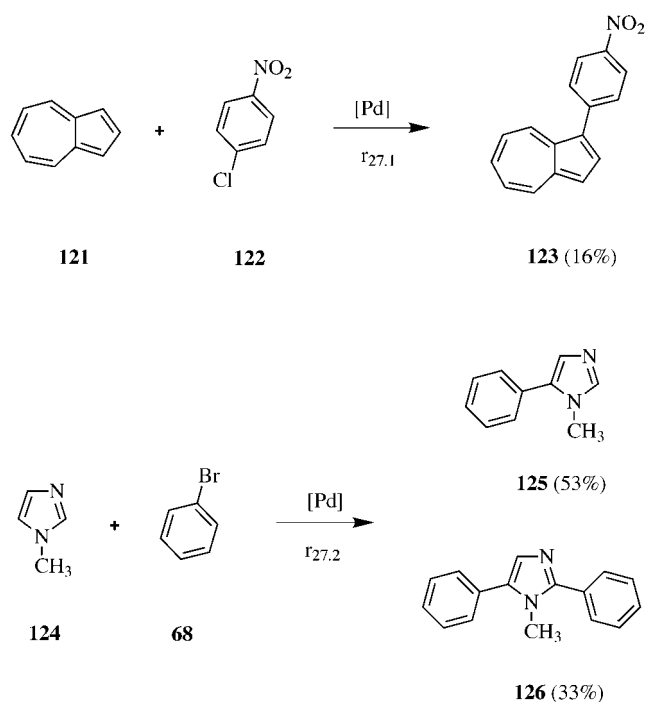
Aldehydic hydrogen atoms are classed as more chemically inert with regard to C–H activation, nevertheless ruthenium and rhodium catalysts are particularly successful in this case: Wilkinson's catalyst can efficiently decarbonylate primary aldehydes at room temperature as a result of C–H activation.^[41] Under suitable reaction conditions the decarbonylation can be avoided; if necessary the intermediate hydridorhodium acyl complexes can add to alkenes and alkynes. The resulting hydroacylation^[42] is a versatile reaction type (Scheme 26): an impressive enantiomeric excess is achieved in the intramolecular hydroacylation of the γ,δ -unsaturated aldehyde **110** by using a rhodium catalyst with chiral bidentate phosphane ligand.^[42a] In the synthesis of the cyclopentanone **114** a Claisen rearrangement precedes the intramolecular hydroacylation.^[42c, d] Intermolecular hydroacylations^[42e–i] are more difficult to achieve and their yields are usually significantly lower (e.g. synthesis of **117**).^[42e] An increase in yields is, for example, achieved by the intermediary formation of pyridylimines on addition of a catalytic amount of 2-amino-3-picoline. Evidently, in this case both the resulting intramolecular C–H activation by cyclometalation and the stabilization of the acyl complexes by the pyridyl ligand have a



Scheme 26. Variants of the hydroacylation reaction; reaction conditions $r_{26.1}$: 5 mol % $[\text{Rh}(\text{S,S-Me-duphos})(\text{acetone})_2]\text{PF}_6$, acetone, 25 °C, < 5 min. $r_{26.2}$: 3 mol % $[\text{RhCl}(\text{cod})(\text{dppe})]$, DMF, 140 °C, 20 h. $r_{26.3}$: 1 mol % $[\text{Ru}_3(\text{CO})_{12}]$, 8 equiv **116**, CO, pressure, 200 °C, 48 h. $r_{26.4}$: 10 mol % Pd/C, dppp, PPh_3 , DME, CO, pressure, 110 °C, 24 h. S,S-Me-duphos see ref. [42a], dppe = 1,2-bis(diphenylphosphanyl)ethane, dppp = 1,3-bis(diphenylphosphanyl)propane.

favorable effect.^[42f] The hydroacylation of dienes with formic acid (**118**) is a preparatively valuable special case. In spite of the acidic acid function a palladium-catalyzed C–H activation occurs at the formyl hydrogen atom.^[42g]

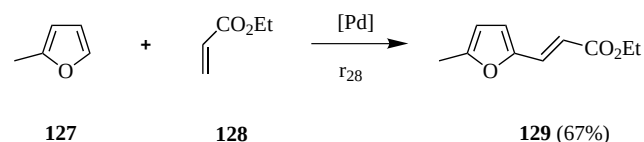
C–C coupling reactions that are based on “intermolecular” catalytic C–H activation on otherwise nonactivated sp^2 - and especially sp^3 -hybridized centers continue to represent a challenge. Such coupling processes are rare. The few examples known are based on the electrophilicity of Pd^{II} complexes. Whereas the intramolecular coupling reaction of aryltriflates and of aryl halides with a second aryl partial structure has found a considerable number of applications to date^[18] (for example the synthesis of **53** as explained in Section 2), the corresponding intermolecular process seems to be limited to specific cases with electron-rich aromatic hydrocarbons and with hetarenes as coupling components. Azulene (**121**) is a suitable substrate for the palladium-catalyzed arylation with 1-chloro-4-nitrobenzene (**122**) and with iodobenzene (**43**) (Scheme 27).^[43] The C–C coupling occurs regioselectively at the electron-rich 1-position of azulene (**121**) and is, for example, catalyzed by palladium clusters.^[44] Possibly, the



Scheme 27. Reaction conditions, $r_{27.1}$: 5 mol % $Pd(OAc)_2$, 5 equiv **122**, DMF, K_2CO_3 , nBu_4NBr , $140^\circ C$, 3 d. $r_{27.2}$: 10 mol % $Pd(OAc)_2$, PPh_3 , 2 equiv **68**, DMF, K_2CO_3 , $140^\circ C$, 20 h.

C–H activation occurs according to the pattern of the electrophilic aromatic substitution with an arylpalladium halide as the electrophilic agent. By employing different hetarenes, Miura et al. obtained considerably higher yields in intermolecular aryl–aryl coupling reactions.^[45, 46] In the case of the imidazole **124** the first arylation occurs at the electron-rich 5-position; however, a second arylation at the more electron-poor 2-position is evidently also possible (Scheme 27).

These Pd^0 -catalyzed coupling reactions must be differentiated from the Pd^{II} -catalyzed biaryl formation under C–H activation: a palladium acetate induced coupling reaction converts benzene oxidatively to give biphenyl. In these cases, reoxidation of Pd^0 intermediates is necessary to close the catalytic cycle. Under an oxygen atmosphere or in the presence of oxidants such as Cu^{II} salts or peroxides, this reaction occurs to a certain extent catalytically.^[47] A peracid is used as the oxidant in the related arylation of the acrylate **128** with the furan **127** (Scheme 28).^[48] The key step in this

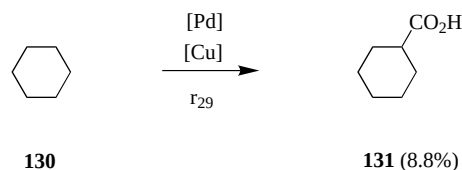


Scheme 28. Reaction conditions r_{28} : 5 mol % $Pd(OCOPh)_2$, AcOH, $PhCO_3tBu$, $100^\circ C$.

reaction is the metalation of **127** at the electron-rich 5-position by the electrophilic palladium benzoate, which finally leads to the Heck-like product **129**.

Fujiwara et al. developed a palladium-catalyzed carboxylation of aromatic hydrocarbons and alkanes with carbon

monoxide; under suitable reaction conditions turnover numbers of up to 200 are achieved for the formation of cyclohexane carboxylic acid (**131**) (Scheme 29).^[49] The analogous reaction proceeds slightly more efficiently with toluene and with *para*-xylene, however slightly worse with methane.

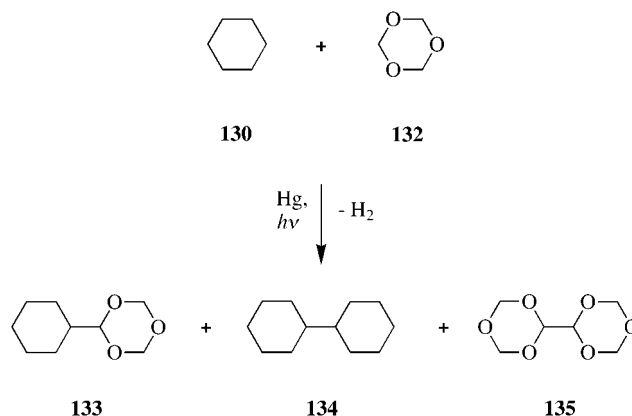


Scheme 29. Reaction conditions r_{29} : 5 mL cyclohexane, 0.02 mmol $Pd(OAc)_2$, 1.0 mmol $Cu(OAc)_2$, 9.0 mmol $K_2S_2O_8$, 3.3 mL CF_3CO_2H , 20 atm CO, $80^\circ C$, 20 h (turnover number ca. 200).

Further investigations into this type of reaction should focus their attention on the role of the cocatalyst copper acetate. Surprisingly, according to Sen and Lin the carboxylation of methane with carbon monoxide and peroxodisulfate can also be achieved without a transition metal catalyst.^[50]

Corresponding carbonylations of aliphatic hydrocarbons with carbon monoxide under nonoxidizing conditions are of particular industrial interest since such processes might be able to compete with hydroformylation. Since the early work of Tanaka et al., radical reactions,^[51] which in addition to the transition metal catalysis require photochemical activation, have been favored for this. Whereas Tanaka et al.^[52] use rhodium–phosphane complexes^[53] for the photochemical carbonylation, Hill et al. introduced polytungstates as alternative catalysts.^[54, 55] Recent results have shown that the latter are also suitable for the photochemical cyanation of alkanes with cyanofornate.^[54c]

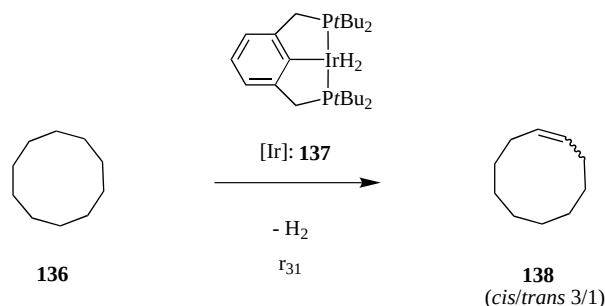
Although these examples of C–C coupling reactions with alkanes are very promising, it is still a long way to industrial applications. One exception is the Mercat process, mainly developed by Crabtree et al.^[56] This unique process combines photochemistry with transition metal catalysis in the gas phase, as illustrated by the coupling reaction of cyclohexane (**130**) with the formaldehyde trimer **132** (Scheme 30).^[56b] A mixture of the organic substrates and a drop mercury are



Scheme 30. Hg-catalyzed and photochemically induced oxidative dimerization in the gas phase.

heated under reflux and irradiated by UV light: mercury atoms in the excited triplet state cause the homolysis of the C–H bonds. The resulting alkyl radicals can dimerize and the higher boiling coupling products accumulate in the condensed phase, in which they are protected from multiple reactions. The cross-coupling product **133** can easily be separated from the homocoupling products **134** and **135**.

Finally, the transition metal catalyzed dehydrogenation of alkanes as a special case of C–C coupling under C–H activation is discussed briefly.^[57] A large number of different transition metal complexes has proven active for this process, including complexes of iridium, rhenium, rhodium, ruthenium, and tungsten as well as heterogeneous catalysts such as palladium on active charcoal. Since the reactions are usually endothermal, sacrificial alkenes are often added to prevent the reverse reaction. The decomposition of the ligands at the often quite high reaction temperatures also presents a problem. Recently the research groups of Kaska, Jensen, and Goldman reported that the cyclometalated iridium complex **137** is sufficiently stable under the required reaction conditions (Scheme 31);^[58] for the dehydrogenation of cyclodecane (**136**) no less than 360 catalysis cycles are completed in 24 h.

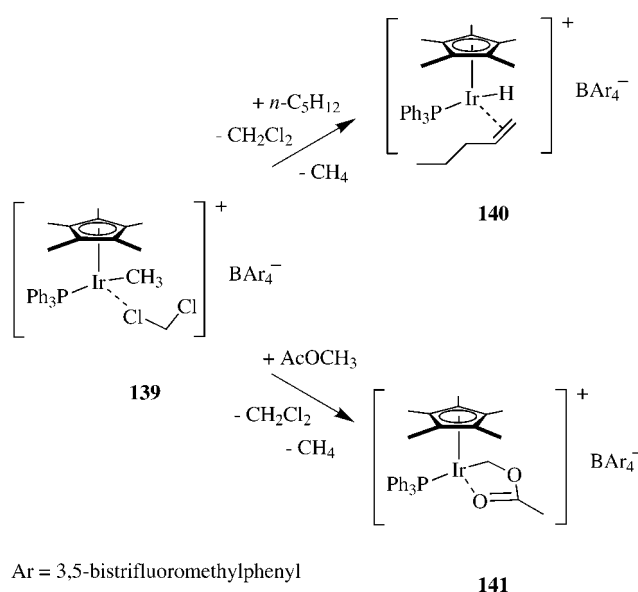


Scheme 31. Reaction conditions r_{31} : 1.0 mM solution of **137** in **136**, reflux (201 °C).

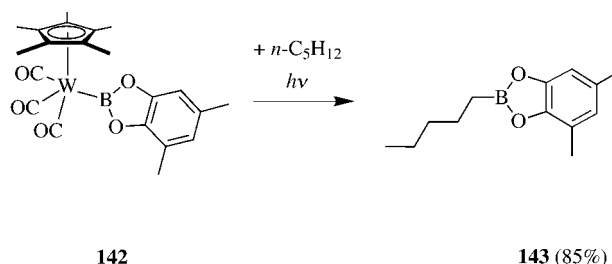
Shock wave experiments and more recent calculations^[59] show that methane disproportionates into hydrogen and higher hydrocarbons at 100 GPa and 4000 K until under pressures of greater than 300 GPa and 5000 K it is finally converted into diamond and hydrogen. Of course, these results are without any industrial relevance because they occur under extreme conditions as anticipated inside the large planets Uranus and Neptune. However, transition metal catalysis can evidently facilitate similar reactions under more moderate conditions: according to Choudhary et al. methane can be converted to higher alkanes below 600 °C, if a special galloaluminosilicate zeolite is used as the catalyst and a sacrificial alkene is employed to couple the hydrogen.^[60] Basset et al.^[61a] recently reported an alkane metathesis that is catalyzed by tantalum hydrides on a silica gel surface. For example, under normal pressure and at 150 °C ethane disproportionates to methane^[62] and propane; however, this is a very slow process (turnover number 46 after more than 50 h reaction time). The C–H and C–C activation steps are part of the catalysis cycle. Corresponding gallium hydrides on silica gel are capable of degrading polyethylene to aromatic hydrocarbons at temperatures of above 500 °C.^[61b]

5. From Stoichiometric C–H Activation to Catalytic Reactions?

Since the early 1980s transition metal complexes have been known that are capable of the oxidative addition to alkane C–H bonds.^[63] In addition to rhodium, osmium, rhenium, iron, palladium, and platinum complexes, iridium complexes have received particular attention.^[63a] Fundamentally, these are electronically unsaturated complexes with vacant coordination sites.^[62b] Bergman et al. have recently made significant progress in achieving C–H activation under moderate conditions. The cationic iridium complex **139** proved particularly reactive (Scheme 32).^[63c] With benzene, C–H activation already occurs at –30 °C, with *n*-pentane it happens within a



Ar = 3,5-bistrifluoromethylphenyl



Scheme 32. Stoichiometric C–H activation with cyclopentadienyl complexes of the late transition metals.

few minutes at room temperature, leading to the alkene complex **140**. The selectivity of the C–H activation becomes especially significant during the reaction with methyl acetate: the C–H activation occurs exclusively at the methoxy group and leads to the cyclometalated complex **141**; however, the more acidic acetyl group is not attacked. The development of possibilities for C–H activation under moderate conditions is the first step on the way to useful catalysis cycles. Further investigations should be aimed at searching for reagents which

regenerate an active transition metal species from complexes such as **140** and **141**, while at the same time liberating a valuable final product.

In the development of new active transition metal complexes Hartwig et al. focussed attention on compounds containing a bond between a transition metal and boron. With alkanes such boryl complexes are capable of forming photochemically induced alkylboronates such as **143** (Scheme 32).^[64] In this reaction the boryl group is selectively incorporated in the terminal position. So far, the best results have been achieved with the tungsten complex **142**, however similar iron and ruthenium complexes are also active. The photochemical activation probably eliminates a CO ligand, leading to the formation of a reactive 16-electron complex; an alternative thermal activation would be desirable. The driving force of the reaction is the formation of relatively stable carbon–boron bonds, which makes the development of a catalytic variant look promising.

6. Summary

The presented selection of results illustrates that coupling reactions under C–H activation are an active and current area of research. Most of the literature mentioned is from the 1990s. Noteworthy progress has been made and the first industrial applications are in sight. Thus, the development of new catalytic systems, which improve the selectivity or even open up new reaction paths, is an even more fascinating and rewarding challenge for future research.

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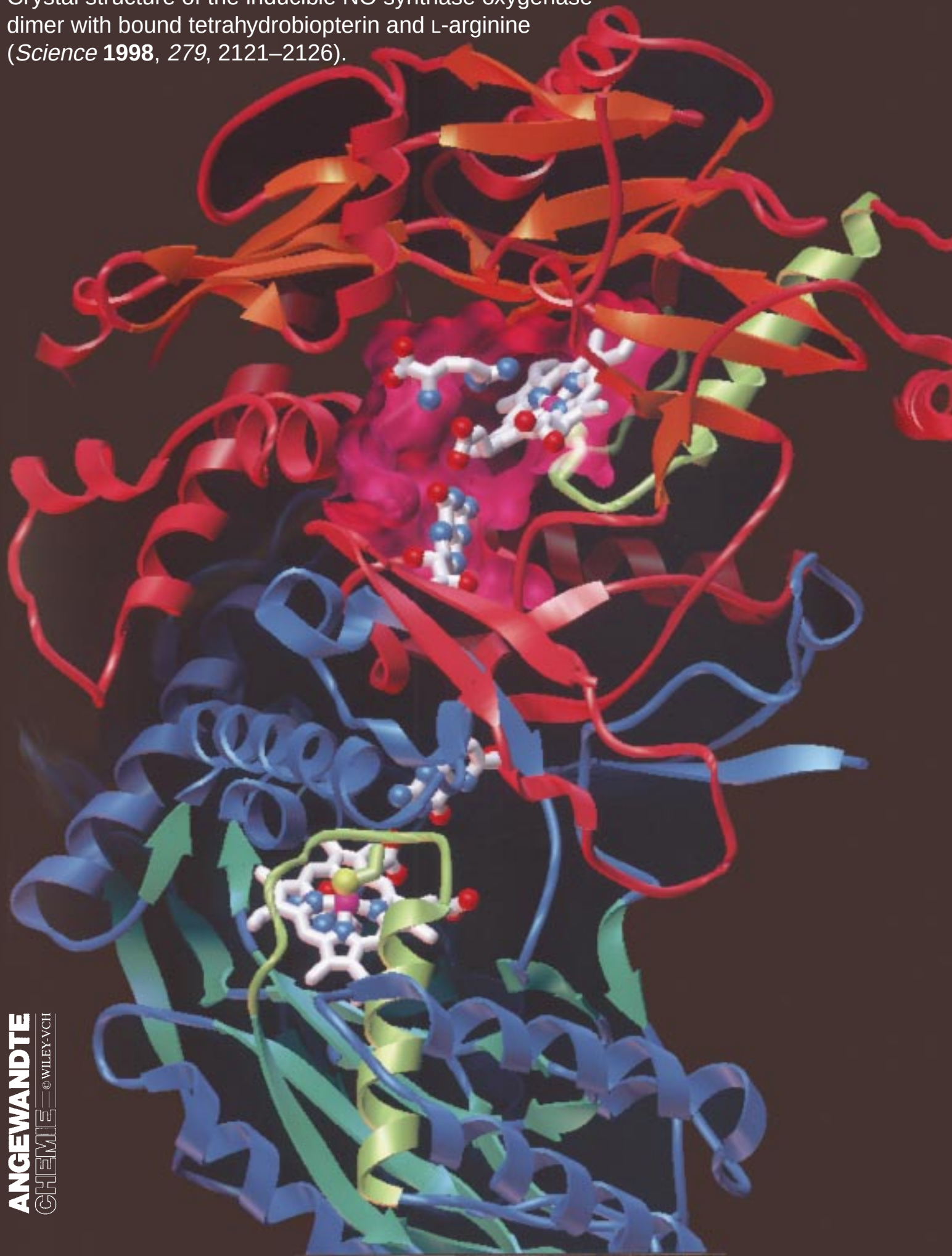
Deposition of Data from X-Ray Structure Analyses

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Prior to submitting a manuscript please deposit the data for your compound(s) **electronically** at the appropriate data base, that is, at the CCDC for organic and organometallic compounds and at the FIZ for inorganic compounds. Both data bases will be pleased to provide help (see our *Notice to Authors* in the first issue of this year). In general, you will receive a depository number from the data base within two working days after electronic deposition; please include this number with the appropriate standard text (see our *Notice to Authors*) in your manuscript. This will enable the referees to retrieve the structure data quickly and efficiently if they need this information to reach their decision.

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Crystal structure of the inducible NO synthase oxygenase dimer with bound tetrahydrobiopterin and L-arginine (*Science* **1998**, 279, 2121–2126).



Nitric Oxide: Chemical Puzzles Posed by a Biological Messenger**

Silvia Pfeiffer, Bernd Mayer,* and Benjamin Hemmens

About ten years ago, the inorganic gas nitric oxide (NO) was discovered to have a biological role as a signaling molecule in the cardiovascular system. It is produced by the cells that line blood vessels, and promotes relaxation of the muscle cells in the wall of the vessel, thus lowering blood pressure. Since then, other signaling functions have been discovered. In some muscles that are directly controlled by nerves, NO is the transmitter released by the nerve terminal that causes relaxation.

NO is also produced in the brain, where it functions as a regulator of synaptic activity and may be important for development of memory. Furthermore, macrophages—cells of the immune system—produce a variety of toxic compounds, including NO, at the scene of an infection. Research on NO has thrown up a variety of challenging chemical problems. How can it function as a specific and reversible signaling molecule? How can it survive long enough in biological fluids to exert its

physiological effects? Which of its many reactions with biological molecules contribute to its useful or harmful effects? How is it produced, and how is its production regulated? In this review, we present some of the current discussions about these issues.

Keywords: heme proteins • nitric oxide • peroxynitrite • radicals • signal transduction

1. Introduction

Nitric oxide (NO) is currently one of the most studied molecules in the biomedical sciences. This interest is driven by the multiplicity of roles that NO plays and by the prospect of developing new drugs to tackle important disease states. The cardiovascular role of NO^[1] is already exploited by drugs that release NO, thus improving blood supply to the heart muscle. Another signaling role of NO—control of blood flow into the corpus cavernosum of the penis—is exploited by the anti-impotence drug Viagra. In this case NO functions as a peripheral neurotransmitter.^[2]

NO can also have damaging, even fatal effects. In the often fatal condition of septic shock, excessive NO synthesis is induced in blood vessels, leading to vascular leakage and a disastrous drop in blood pressure. In chronic diseases such as rheumatoid arthritis, NO contributes to the tissue damage caused by an inappropriate inflammatory response. In stroke, NO may contribute to brain damage. The race is on to find specific inhibitors of NO synthesis that may limit this harmful overproduction.^[3, 4]

In the first part of this review, we show that the physiological effects of NO can only be understood by studying its basic chemistry—which has turned out to be surprisingly complex. For a radical like NO, biological fluids present a wide choice of substances with which to react. Some of the products of these reactions may be biologically inert; some may have similar activity to NO itself; some may be poisonous. The partitioning of NO between these different fates is made all the more complex because the decisive steps may involve highly reactive intermediates that are hard to detect.

In the second part, we present current discussions on the enzymes responsible for NO biosynthesis. The catalytic machinery of these enzymes offers many challenges for basic biochemical research. The essential catalytic puzzle, in our opinion, centers on the division of roles between the heme and the tetrahydrobiopterin cofactor. Much of the hope of new drugs centers on discovery of specific inhibitors of one of the three isozymes that are involved in different functions of NO. Some substrate analogues show some specificity; the basis of this is only partly understood.

Although many of the topics we discuss here are of direct physiological relevance, we have tried to focus on the progress on understanding underlying chemical events. Readers interested in pursuing the physiological aspects in more detail are referred to excellent reviews by others on the role of NO in the peripheral nervous system,^[2] in the brain,^[5] and in the cardiovascular system.^[1] A visit to the NO homepage (<http://www.apnet.com/no>) is also to be recommended.

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[**] A list of the abbreviations used is presented in Section 5.

2. Biological Chemistry of NO

2.1. Reactions of NO

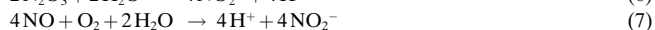
2.1.1. Reaction with Oxygen

NO reacts with O₂ both in the gas phase and aqueous solution to form NO₂. The reaction is second order with respect to NO and first order with respect to O₂ [Eq. (1)].^[6]



Consequently, lower concentrations of NO react much more slowly than higher concentrations: In air, for example, the time required for half of a given concentration of NO to react is 24 s for 10000 ppm of NO, but 7 h for 10 ppm. In aqueous solution, the stoichiometry of the reaction between NO and O₂ is different from the gas phase, although the form of the rate law is the same.^[7, 8] The rate constant in aqueous solution has been calculated to be $6.3 \times 10^6 \text{ M}^{-2} \text{ s}^{-1}$.^[8, 9]

Whereas in the gas phase NO₂ is the stable oxidation product of NO, in aqueous solution NO₂ gives stoichiometric amounts of NO₂⁻ and NO₃⁻ [Eqs. (2) and (3)]. In oxygen-containing aqueous solution NO reacts to yield exclusively NO₂⁻.^[10] The difference could be explained if during NO autoxidation NO₂ does not accumulate in quantities sufficient to dimerize to N₂O₄, but reacts with another molecule of NO to give N₂O₃. Hydrolysis of N₂O₃ in aqueous solution would then give NO₂⁻ [Eqs. (4)–(6)]. The sum equation [Eq. (7)]



agrees well with the overall stoichiometry of NO autoxidation in aqueous solution.^[10, 11] The formation of N₂O₃ as an intermediate in this process is controversial, but would account not only for the lack of NO₃⁻ formation, but also for the nitrosative activity of NO (see Section 2.1.3).^[12, 13]

2.1.2. Reaction with Superoxide

As a radical, NO is capable of reacting with other radicals. An important reaction of NO in biological media is a direct bimolecular reaction with O₂⁻, yielding peroxynitrite [oxoperoxonitrate(1-)].^[14] Peroxynitrite is not a free radical because the unpaired electrons on NO and O₂⁻ have combined to form a new N–O bond. Formation of peroxynitrite from NO and O₂⁻ occurs at nearly diffusion controlled rates (4.3×10^9 – $6.7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$).^[15, 16] The rate constant is about three times higher than the rate constant for dismutation of O₂⁻ by superoxide dismutase ($2.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$).^[17] Therefore, NO outcompetes the reaction of O₂⁻ with superoxide dismutase at steady-state concentrations that are likely to exist in vivo.^[18]

Peroxynitrite is a potent oxidant ($E^\circ(\text{ONOO}^-, 2\text{H}^+/\text{NO}_2, \text{H}_2\text{O}) = 1.6 \text{ V}$ at pH 7.0) and has been shown to react with virtually all classes of biomolecules in vitro.^[19] Table 1 summarizes the rate constants of the most relevant reactions

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B. Mayer



S. Pfeiffer



B. Hemmens

In 1989 he was awarded a Humboldt Fellowship to work with Prof. Eycke Böhme and Prof. Günther Schultz at the Free University of Berlin. Since his return to Graz in 1991 he has built up a research group working on a variety of biochemical and pharmacological projects. Since January 1998 he has been interim head of the institute.

Silvia Pfeiffer was born in Waidhofen an der Thaya, Austria. She obtained a degree in biotechnology at the Universität für Bodenkultur in Vienna. Her PhD thesis focussed on effects of NO on cGMP formation in plants. She joined Bernd Mayer's research group in 1995 and works mainly on the biological chemistry of NO and peroxynitrite.

Benjamin Hemmens was born in Dublin, Ireland. He studied Biochemistry at Trinity College, Dublin. He obtained his PhD from the University of Dundee, Scotland, for work on enzymes of pyrimidine biosynthesis. He spent two years in the research group of Dr. Irmgard Ziegler at the GSF-Forschungszentrum in Munich, working on pteridine biosynthesis. In 1995 he moved to Graz to join the research group of Bernd Mayer, and works on the enzymology of NO synthases.

Table 1. Rate constants of peroxynitrite reactions with enzymes, biomolecules, and some antioxidants.^[a]

Reactant	k [M ⁻¹ s ⁻¹]	Ref.
myeloperoxidase	6.2×10^6 ^[c]	[229]
horseradish peroxidase	3.2×10^6 ^[c]	[229]
lactoperoxidase	3.3×10^5 ^[c]	[229]
glutathione peroxidase	4.5×10^4 ^[b]	[230]
alcohol dehydrogenase	2.6×10^5 ^[d]	[231]
aconitase	1.4×10^5 ^[c]	[232]
oxyHb	2.0×10^4 ^[b]	[76]
cytochrome c	1.4×10^4 ^[b]	[76]
CO ₂	3.0×10^4 ^[b]	[74]
ebselen	2.0×10^6 ^[c]	[86]
cysteine	5.0×10^3 ^[b]	[80]
glutathione	1.5×10^6 ^[b]	[233]
methionine	1.8×10^2 ^[c]	[234]
ascorbate	2.3×10^2 ^[c]	[235, 236]
NO	9.1×10^4 ^[b]	[72]

[a] Reported rate constants were obtained from the literature and represent the apparent values from pH 7.2–7.6. [b] $T = 37^\circ\text{C}$. [c] $T = 25^\circ\text{C}$. [d] $T = 23^\circ\text{C}$. [e] $T = 12^\circ\text{C}$.

of peroxynitrite with enzymes, antioxidants, and some other biologically relevant molecules.

2.1.3. Nitrosation Reactions

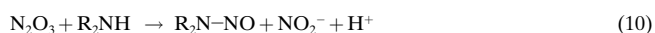
Under physiological conditions, the reaction of NO with O₂ is complicated by the presence of a variety of other possible reactants. In particular reduced thiols are more or less ubiquitous in vivo. At physiological pH, O₂, NO, and thiols react to form *S*-nitrosothiols.^[12] Two possible mechanisms for this reaction are in discussion.^[20] The first of these involves electrophilic nitrosation, that is, transfer of a nitrosonium (NO⁺) equivalent. Nitrous acid, alkyl nitrite, nitrosyl halide, N₂O₃, and N₂O₄ are all able to act as carriers of NO⁺. Possibly, in the presence of oxygen, N₂O₃ is formed, which can react in this way [Eq. (8)].^[21, 22] The other proposal is a radical mechanism based on formation of a thiyl radical [Eq. (9)].



In vivo, thiols such as glutathione are used as antioxidants, and thus a low steady-state concentration of thiyl radicals is to be expected—sufficient, perhaps, to sustain this type of nitrosation reaction. *S*-Nitrosothiols have in fact been shown to occur endogenously in various tissues, including human plasma,^[23] airways,^[24] white blood cells,^[25] and rat cerebellum.^[26] These compounds act as NO donors and can thus mediate the usual spectrum of effects expected for NO.^[27] Release of NO from *S*-nitrosothiols is rather slow, but can be greatly accelerated by Cu^I in vitro.^[28] They survive longer than NO in blood because they are not subject to the same inactivation reactions, and in principle they could transmit NO signals through the circulation—particularly if the target cells contained a catalyst of their decomposition. Stamler and co-workers discovered one example of such signaling.^[29] Cysteine 93 of the β subunit of hemoglobin (Hb) can be rapidly nitrosated when the protein is in the R (oxy) state, but not in the T (deoxy) state. *S*-nitroso-Hb was detected

in arterial but not in venous blood in rats; moreover, it caused vasodilation in perfused blood vessels if glutathione was present in the perfusion medium. The sum of these reactions would be a net transport of NO from the lungs to the tissues.

In contrast to *S*-nitrosation, which seems to have fairly benign effects, *N*-nitrosation reactions of NO have deleterious consequences. An intermediate of NO autoxidation, presumably N₂O₃, can directly nitrate primary amines on DNA bases. In the presence of oxygen, NO may damage DNA also by nitrosation of various secondary amines to form *N*-nitrosamines [Eq. (10)], which can then be metabolized to form



strongly alkylating electrophiles that react with DNA.^[30, 31] Synthetic nitrosamine derivatives (NONOates), however, are useful tools in NO research because their rates of NO release can be engineered.^[32, 33]

2.1.4. Reaction with Hemoglobin

NO reacts with oxyhemoglobin (oxyHb) with a second-order rate constant of about $3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ to form metHb and NO₃⁻ [Eq. (11)].^[34] The first-order dependence on NO



concentration and the lack of a limiting rate at high NO concentrations show that NO reacts directly with bound O₂ without first displacing it from the heme.^[35] Stopped-flow spectroscopy studies recently showed that this reaction occurs by the intermediate formation of peroxynitrite.^[36] NO also interacts with deoxyHb and metHb, binding to the heme iron center. In the case of metHb the binding is hindered by a water molecule coordinated to the heme Fe^{III} atom, and the association rate constant is about 100-fold smaller than for ferrous deoxyHb.^[35, 37]

The reaction with oxyHb strictly limits the transport of NO in blood, so that free NO, whether produced endogenously or released from NO-donating drugs, does not have systemic effects. As an NO sink, it is probably important for deactivation of NO-stimulated sGC, at least in vascular smooth muscle (see Section 2.1.6). The impact of the reaction with oxyHb on outward diffusion of NO from vascular endothelium has been addressed by mathematical modeling.^[38, 39] Neural NO signaling may be less subject to these effects because of greater distances to the nearest blood vessels.

2.1.5. Reaction with Other Metal Centers

Cells contain a variety of other metalloproteins that could bind or react with NO, such as cytochromes P450 and cytochrome c oxidase.^[40] NO competes with oxygen for binding to cytochrome c oxidase: The resulting inhibition of mitochondrial respiration may be a significant toxic effect of NO, particularly under inflammatory conditions with increased NO production or in ischemic states with depressed

oxygen tension.^[41, 42] The discovery of an NO synthase in mitochondria^[43] raises the prospect that the balance of NO versus O₂ binding to cytochrome c oxidase may also be used to regulate respiration under normal physiological conditions. Some reactions of NO with zinc- and copper-containing proteins have been reported.^[44–46]

NO has been shown to bind to iron–sulfur clusters, leading to the formation of iron–nitrosyl complexes.^[47, 48] Recent studies have shown that NO inactivates aconitase due to a direct reaction of NO with the iron–sulfur cluster resulting in the reversible formation of an iron–nitrosyl complex. Cytosolic aconitase has a second function as the iron-regulatory protein (IRP). The NO-inhibited form appears to have increased IRP activity; this activity inhibits translation of genes responsible for iron storage and utilization, while stabilizing the mRNA for the transferrin receptor, which is responsible for iron uptake.^[49] Thus NO is proposed to be a signal that counteracts iron deficiency.

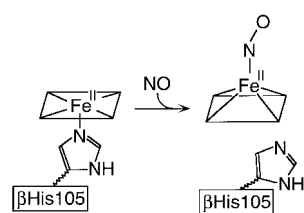
2.1.6. Activation of Soluble Guanylyl Cyclase

The effects of NO as a physiological signaling molecule in smooth muscle cells and neurons are due to its activation of soluble guanylyl cyclase (sGC) by up to 200-fold above the basal activity.^[50–52] sGC catalyzes, in the presence of Mg²⁺ or Mn²⁺ ions, the conversion of guanosine 5'-triphosphate (GTP) to guanosine 3',5'-monophosphate (cGMP). cGMP is an intracellular messenger that connects the NO signal to the cellular response by activating specific protein kinases, phosphodiesterases and ion channels. sGC is a heterodimer of α and β subunits. The N-terminal half of each subunit is not needed for basal catalytic activity, but is essential for the response to NO. NO exerts its effect by binding to a heme group bound in the N-terminal portion of the β subunit.

Spectral studies of sGC in the absence of exogenous heme ligands suggest a pentacoordinate, high-spin Fe^{II} state, with a Soret maximum at 431 nm and a single broad α/β peak at 555 nm,^[53] consistent with histidine as a proximal ligand. Site-directed mutagenesis showed that NO-dependent activation was completely eliminated by the mutation H105F in the β subunit.^[54, 55]

Binding of NO to the heme results in a pentacoordinate complex: The bond to the proximal histidine is lost (Scheme 1).^[56, 57]

This dissociation of the proximal ligand is believed to be the trigger that activates the enzyme. This is supported by a variety of results. sGC can be activated by protoporphyrin IX (PPIX) as well as by Fe^{II}PPIX(NO).^[58] The enzyme is only marginally activated by CO, which binds to the heme without causing the proximal histidine to dissociate.^[59] An Mn^{II}PPIX-substituted enzyme fails to be activated by NO, and also retains the proximal ligand.^[60] Co^{II}PPIX-substituted sGC was fully activatable by NO, and the activation was accompanied by loss of the proximal ligand.^[60]



Scheme 1. Binding of NO to soluble guanylyl cyclase. β His105 = His 105 of the β subunit.

Surprisingly, sGC binds oxygen very poorly. Also, the dissociation of NO from the heme is unusually fast. NO dissociates from sGC with a k_{obs} of about $6 \times 10^{-4} - 8 \times 10^{-4} \text{ s}^{-1}$, several times faster than from myoglobin ($k_{\text{obs}} = 10^{-4} \text{ s}^{-1}$; both at 20 °C).^[61] The dissociation from sGC was found to be faster still ($k_{\text{obs}} = 0.04 \text{ s}^{-1}$) in the presence of substrate and Mg²⁺: Extrapolated to 37 °C, this would give a half-life of about 5 s.^[62] One element of the effect may be the negative *trans* effect of the proximal histidine moiety. In pentacoordinate nitrosyl heme, the iron atom is displaced by 0.14 Å out of the plane of the porphyrin, whereas in hexacoordinate complexes this displacement is reduced by half, which brings the NO nitrogen atom up against the four porphyrinato nitrogen ligands.^[61] Imidazole speeds up NO dissociation by 1000-fold without a significant amount of hexacoordinate complex appearing in the absorbance spectrum: Presumably a small steady-state concentration of the hexacoordinate intermediate is sufficient.^[61] Taken together, these results all point to a special protein environment around the heme in sGC, the nature of which will probably be resolved only by crystal structures of the enzyme.

Finally, it should be noted that the dissociation rates just discussed were measured in the presence of large concentrations of NO scavengers (oxyHb or dithionite) and thus represent the maximal rates that can be reached.^[61, 62] Therefore the effectiveness of physiological NO sinks is highly relevant to the kinetics of NOS-sGC signaling. Oxidation of the heme^[63] would also cause NO dissociation: This is the mechanism of action of the specific sGC inhibitor 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ).^[64] There is no evidence yet that controlled heme oxidation of sGC is exploited under normal physiological conditions. Deactivation mechanisms that override the effect of bound NO are also conceivable.

2.2. Peroxynitrite, Nitration, and Disease

In many disease states, tyrosine residues in tissues are found to be nitrated. From studies on tyrosine nitration of some proteins in vitro, it seems that this modification can have considerable effects on protein and enzyme function; thus it seems likely to be a contributing factor to pathological dysfunction. The prime suspect for the cause of nitration is peroxynitrite; however, as the following discussion shows, this small molecule has unexpectedly complex chemistry, and the link between it and pathological tissue damage is still a matter for lively debate. Table 1 lists rate constants for a variety of reactions of peroxynitrite.

2.2.1. Decomposition of Peroxynitrite in Aqueous Solution

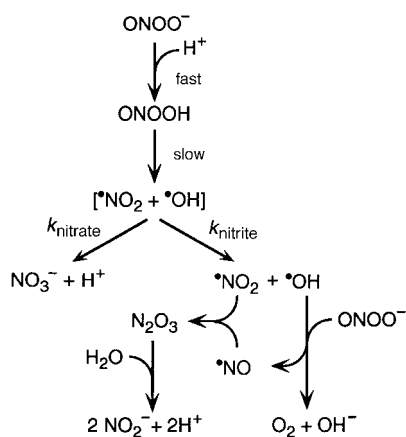
At alkaline pH, peroxynitrite is quite stable (it can be stored at pH 13 for weeks). This stability is ascribed to the adoption of the *cis* conformation under alkaline conditions.^[65] Ab initio calculations suggest that a barrier of 21–24 kcal mol^{−1} limits isomerization between the *cis* and the *trans* anion.^[66] When protonated (peroxynitrous acid), isomerization to the *trans* conformation is facilitated by a substantially lower energy barrier of 10–12 kcal mol^{−1}.^[65]

Stopped-flow and pulse radiolysis studies revealed that the decay of peroxyxynitrite follows first- (or lower) order kinetics between pH 1–11, and that peroxyxynitrite exhibits a pK_a of 6.8 at 37 °C.^[19] Independently, the decomposition of peroxyxynitrite was also studied in the presence of the 'OH scavenger 2,2'-azino-bis-(3-ethyl-1,2-dihydroxybenzothiazoline-6-sulfonate) (ABTS). From the pH-dependent formation of ABTS^{•+} radicals during the decomposition reaction, a pK_a of 8.0 was derived.^[67, 68] The different pK_a values were ascribed to the *cis* and *trans* isomers of peroxyxynitrite.

The mechanism of the decomposition reaction is still not well understood.^[68] Under acidic conditions peroxyxynitrous acid isomerizes to NO_3^- .^[19, 69] The mechanism of this transformation is the subject of debate. Proposed intermediates include a vibrationally excited form of peroxyxynitrous acid (ONOOH^*) on the reaction pathway and a complex of 'NO₂ and 'OH radicals, although it is not certain whether a specially activated intermediate is necessary. Whether homolytic cleavage of peroxyxynitrous acid into 'NO₂ and 'OH radicals is thermodynamically possible is under investigation.^[70, 71]

In addition to NO_3^- , O₂ and NO_2^- are formed in a 1:2 stoichiometry during peroxyxynitrite decomposition in neutral to alkaline solutions.^[72] The two reactions yielding NO_2^- and NO_3^- have different temperature dependences and a difference in free energy of activation of about 26 kJ mol⁻¹. The mechanism leading to NO_2^- and O₂ formation is still unclear, but a bimolecular reaction between peroxyxynitrite and peroxyxynitrous acid as the rate-determining step can be excluded^[73] since decomposition of peroxyxynitrite does not follow second-order kinetics and the partitioning of the two pathways does not depend on the concentration of peroxyxynitrite.^[72]

Scheme 2 shows a hypothetical mechanism of peroxyxynitrite decomposition to NO_2^- and NO_3^- , involving a conversion of ONOOH into an activated intermediate as the rate-limiting



Scheme 2. Decomposition of peroxyxynitrite.

step in both reactions. According to this mechanism, the caged 'NO₂ and 'OH radicals can recombine to NO_3^- , or at alkaline pH, 'OH may react with peroxyxynitrite anion to form O₂, NO, and OH⁻. NO could then react with 'NO₂ radicals to yield N₂O₃ and finally NO_2^- . It may be that intermediates of this pathway of peroxyxynitrite decomposition have significant biological activities as well as peroxyxynitrite itself.

2.2.2. Reaction with CO₂

Peroxyxynitrite anion reacts rapidly with carbon dioxide ($k = 3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7.4 and 37 °C) to yield the adduct nitroso peroxycarbonate, which decomposes to NO_3^- and CO₂ in the absence of other reactive molecules [Eqs. (12) and (13)].^[74, 75]

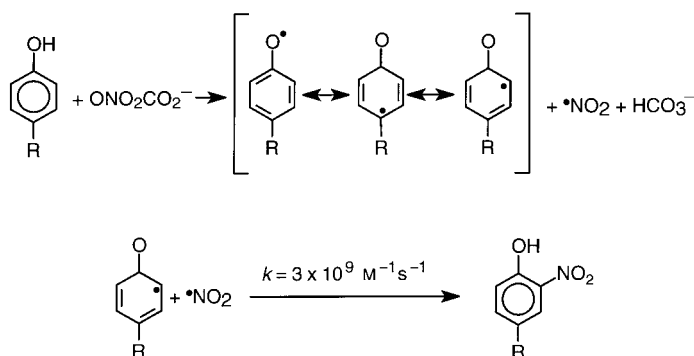


The rate constant of this reaction is large enough that it could be the predominant route of peroxyxynitrite disappearance in normal biological fluids, where the total carbonate concentration is between 1 and 25 mM. $\text{ONO}_2\text{CO}_2^-$ exhibits a different reactivity than peroxyxynitrite.^[75] For instance, in the presence of CO₂ nitration yields are usually increased by two- to four-fold,^[76–78] whereas thiol oxidation is dramatically reduced.^[76]

The mechanism of $\text{ONO}_2\text{CO}_2^-$ decomposition is not well characterized, but homolysis of the weak oxygen–oxygen bond is favored, leading to the formation of 'CO₃⁻ and 'NO₂. These radicals ($k = 1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) are postulated to recombine to form NO_3^- and CO₂ [Eqs. (14) and (15)].^[79] The



enhanced nitration promoted by peroxyxynitrite in the presence of CO₂ could be explained by one-electron oxidation of a phenolic ring by 'CO₃⁻ in concert with a termination reaction of the phenoxyl radical with 'NO₂ (Scheme 3).^[79]



Scheme 3. Tyrosine nitration by peroxyxynitrite/CO₂.

2.2.3. Reaction with Thiols

A particularly important reaction is the oxidation of protein-bound and low molecular mass thiols such as glutathione or cysteine.^[80] The reactions occur with moderate second-order rate constants at physiological pH and 37 °C, but the high intracellular concentration of thiols (1–10 mM) makes this a relevant route for the cellular detoxification of peroxyxynitrite. One- and two-electron oxidations of thiols have been reported, mainly resulting in the formation of disulfides (> 90 %).^[19, 80] Small amounts ($\leq 1\%$) of *S*-nitrosothiols,^[81, 82] which release NO in a Cu⁺-catalyzed reaction,^[28] are also formed by a direct nucleophilic nitrosation mechanism.^[83] This accounts for the apparent ability of peroxyxynitrite to activate soluble guanylyl cyclase,^[81] to induce vasodilation,^[84] and to inhibit platelet aggregation and leukocyte adhesion.^[82]

Another related reaction is the oxidation of selenium, both in naturally occurring proteins such as glutathione peroxidase and synthetic complexes such as ebselen.^[85, 86] These have been found to reduce peroxynitrite to NO_2^- with formation of a selenoxide, which can be reduced back by thiols. It has been proposed that this reaction plays a major role in cellular detoxification of peroxynitrite.

2.2.4. Tyrosine Nitration and Disease

3-Nitrotyrosine has been detected in injured tissues for a wide variety of disease states. These include neurodegenerative diseases,^[87, 88] acute lung injury,^[89] atherosclerosis,^[90, 91] bacterial and viral infections,^[92, 93] and chronic inflammation.^[94] Tyrosine nitration appears to represent a prominent pathway of pathophysiological protein modification under inflammatory conditions with increased expression and/or activity of NO synthases.^[69, 95] These diseases often involve increased plasma levels of cytokines and endotoxin, which increase the expression of inducible NO synthase in many different cell types, causing a high output of NO over relatively long periods of time.^[96]

Nitration of tyrosine residues has been shown to alter the functional properties of a variety of proteins, and it is proposed to be causally involved in the pathogenesis of certain diseases.^[69, 97] Nitration of cardiac actin may impair contractile function of the heart in myocarditis.^[98] Nitration of human neurofilament L has been shown to interfere with filament polymerization in amyotrophic lateral sclerosis.^[69] Nitration of surfactant protein A impairs its ability to aggregate lipids and to bind mannose, two functions that are essential for building a functional surfactant monolayer at the air–water interface in the lung.^[89, 99] In addition to these structural proteins, tyrosine nitration may affect the function of enzymes, including Mn-SOD^[100, 101] and glutamine synthetase.^[102, 103] Nitration of tyrosine residues can also disrupt signal transduction cascades that depend on reversible phosphorylation of tyrosine.^[104–106] Potentially, many more proteins could be affected: 140 mammalian proteins are known whose activity is dependent upon tyrosine residues.^[107] The main culprit in these phenomena has been believed to be peroxynitrite, the product of the reaction of NO with superoxide. As a basis for discussion of these ideas, we first need to consider the basic chemistry of peroxynitrite.

2.2.5. What Nitrates Tyrosine?

In vitro, 3-nitrotyrosine can be formed by the reaction of tyrosine with a variety of nitrogen oxide species (NO_x), such as peroxynitrite, nitrogen dioxide (NO_2), nitrous acid (HNO_2), nitronium ion (NO_2^+), and nitryl chloride (NO_2Cl).^[97] NO by itself does not directly nitrate tyrosine,^[69, 97] but can cause nitration in the presence of additional oxidants that generate tyrosyl radicals. The second-order reaction of NO with tyrosyl radicals ($k > 10^9 \text{ M}^{-1} \text{ s}^{-1}$)^[108] leads to formation of *C*-nitroso and/or *O*-nitrosotyrosine addition products, which can be converted into 3-nitrotyrosine in a two-electron oxidation reaction.^[109, 110] NO_2 formed by the autoxidation of NO has been shown to nitrate tyrosine

residues in proteins in vitro.^[111] At micromolar concentrations of NO, the third-order autoxidation reaction^[7] is certainly not fast enough to generate sufficient amounts of NO_2 to explain 3-nitrotyrosine formation in cells. Also, small amounts of ascorbate or thiols have been shown to strongly inhibit tyrosine nitration by NO_2 .^[111] However, recent data indicate that the reaction of NO with O_2 is about 300-fold faster in the hydrophobic phase of biological membranes, so that these may be important sites for formation of NO-derived reactive species.^[112] Under acidic conditions ($\text{pH} < 2$) the stable end product of NO autoxidation, NO_2^- , forms nitrous acid (HNO_2), which is also capable of nitrating tyrosine residues. Such a reaction might occur at the low pH values present in the stomach,^[113] but in most other tissues the pH probably never becomes sufficiently low to promote significant protonation of NO_2^- .

Of these nitrogen oxide species, peroxynitrite is postulated to make the largest contribution to the pathogenesis of inflammatory and infectious disorders.^[97] Peroxynitrite-dependent nitration has been extensively studied in vitro by bolus addition of synthetic peroxynitrite from alkaline stock solutions.^[97, 114, 115] It has been pointed out that this approach may be a poor approximation to in vivo situations, in which the peroxynitrite is formed by continuous reaction of NO and $\text{O}_2^{\cdot-}$ at physiological pH.^[116, 117] Recently, methods have been developed that should simulate these conditions more closely, by combining NO-generating and superoxide-generating systems. Surprisingly, tyrosine nitration by NO and $\text{O}_2^{\cdot-}$ generated simultaneously at equal rates was about 100-fold less efficient than the reaction triggered by synthetic peroxynitrite.^[117] These results may reopen the question whether peroxynitrite is really the main mediator of tyrosine nitration in vivo.

Some alternative mechanisms of tyrosine nitration involve myeloperoxidase, which is secreted by monocytes and polymorphonuclear neutrophils under inflammatory conditions. Myeloperoxidase generates tyrosyl radicals^[118] which can be trapped by NO, leading to formation of 3-nitrotyrosine in the presence of additional oxidants, for example H_2O_2 .^[110] This mechanism could account for the extensive tyrosine nitration observed in atherosclerotic lesions of blood vessels.^[91, 119] In the presence of H_2O_2 , myeloperoxidase may also cause nitration by oxidation of NO_2^- to NO_2 .^[120] Finally, myeloperoxidase catalyzes formation of hypochlorous acid (HOCl), which reacts non-enzymatically with NO_2^- to form the potent nitrating agent nitryl chloride (NO_2Cl).^[121, 122] Thus, tyrosine nitration may become significant at sites where NO synthase (NOS) is activated together with oxidative pathways generating tyrosyl radicals and H_2O_2 or other oxidants. The presence of myeloperoxidase appears to be important, but would not be obligatory if other enzymatic pathways acting in a similar manner were activated together with NO synthase in tissue inflammation. The origins of tyrosine nitration and its relevance to particular disease states seem likely to occupy researchers for some time to come.

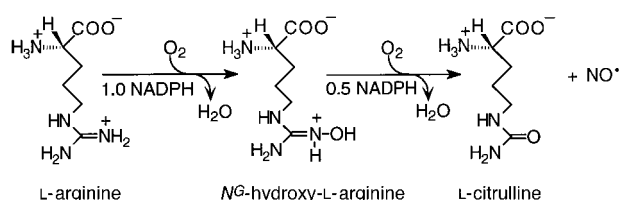
2.2.6. Other Deleterious Effects of Peroxynitrite

In addition to tyrosine nitration, some other deleterious reactions have been attributed to peroxynitrite. Being a strong

oxidant, it can cause DNA strand breaks. In response, cells activate the nuclear enzyme poly(ADP-ribose) synthetase (PARS; E.C. 2.4.2.30).^[123] However, this can deplete the intracellular pool of reduced nicotinamide adenine dinucleotide, causing breakdown of energy metabolism and ultimately cell death. Peroxynitrite-mediated activation of PARS has been implicated to importantly contribute to the cellular injury in endotoxic shock, inflammatory pancreatic islet cell destruction, and central nervous system ischemia. Another damaging effect of peroxynitrite is its ability to oxidize hemoproteins.^[48]

3. Enzymology of NO Synthases

Scheme 4 shows the biosynthesis of NO catalyzed by the three isozymes of NO synthase (NOS) listed in Table 2. The structure and mechanism of the three isozymes are basically similar, but they display adaptations that allow different modes of regulation.



Scheme 4. The NO synthase reaction.

Neuronal NOS (nNOS) is expressed in postsynaptic terminals of neurons in the brain, where it is activated by Ca^{2+} influxes caused by binding of the neurotransmitter glutamate to receptors in the cell membrane: Here, the NO produced contributes to long-term potentiation, a phenomenon that may underlie learning and memory formation. In peripheral nerves, nNOS is also activated in response to Ca^{2+} influx, this time through voltage-gated Ca^{2+} channels opened by a membrane depolarization. In these nitrergic neurons, NO is the neurotransmitter that triggers the response in the target organ (blood vessels, gastrointestinal tract, corpus cavernosum of the penis, etc.); these responses are mediated by NO activating sGC (see Section 2.1.6).

Table 2. The isozymes of NO synthase.

NOS isozyme	Alternative descriptions	M_r [kDa]	Distinctive properties	Subcellular localization	Tissue expression
neuronal	type I, nNOS, ncNOS, bNOS	160	Ca^{2+} -dependent, constitutively expressed	binds to specific membrane proteins through an N-terminal PDZ domain ^[237]	neuronal cells, skeletal muscle
endothelial	type III, eNOS, ecNOS	134	Ca^{2+} -dependent, constitutively expressed	targets to the Golgi and to caveoli through N-terminal myristoylation and palmitoylation	endothelial cells, epithelial cells, cardiomyocytes, some neurons
inducible	type II, iNOS, macNOS	130	Ca^{2+} -independent, induced by inflammatory stimuli (cytokines, LPS ^[a])	soluble?	macrophages, hepatocytes, astrocytes, smooth muscle cells, and many more

[a] LPS = lipopolysaccharides.

Endothelial NOS (eNOS) is expressed in endothelial cells lining blood vessels and produces the endothelium-derived relaxing factor (EDRF). NO diffuses into the smooth muscle cells of the blood vessel wall and elicits a cGMP-dependent relaxation, facilitating increased blood flow through the vessel. This was the first signaling role of NO to be discovered.^[124–126]

Inducible NOS (iNOS) is expressed in macrophages in response to inflammatory cytokines and other stimuli associated with infection. In this case, NO is produced as one of a palette of toxic compounds used to inactivate pathogens.

This rough sketch of the main roles and locations of the NO synthases is helpful for basic orientation, but is somewhat oversimplified. Thus it should be remembered that eNOS is present in blood vessels in the brain and can therefore influence events in the adjacent neurons; it has also been discovered inside some neurons^[127] and in cardiomyocytes.^[128] iNOS is not only present in activated macrophages, but its synthesis can be induced in smooth muscle,^[129, 130] cardiac muscle,^[131, 132] liver,^[133] and glial cells.^[134]

3.1. Domain Architecture

The active form of each NO synthase isozyme is a homodimer. Each subunit consists of a C-terminal reductase domain and an N-terminal oxygenase domain (Figure 1);

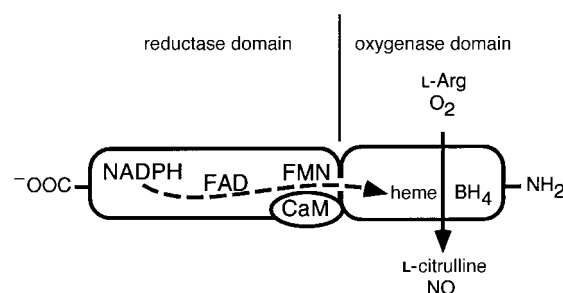


Figure 1. Domains and cofactors of NO synthase. The dashed arrow shows the path of electron transfer.

these domains remain folded independently of each other. The isolated oxygenase domain remains homodimeric whereas the isolated reductase domain is monomeric, showing that

the dimeric interface of the enzyme is formed by the oxygenase domain.^[135, 136] The reductase contains one molecule each of bound FAD and FMN, and has a binding site for NADPH.^[137] The oxygenase contains a heme group and one binding site each for the pteridine cofactor tetrahydrobiopterin and the substrate L-arginine. Between the reductase and oxygenase domains is a binding site for calmodulin.^[137, 138] On the N-terminal side of the oxygenase domain, each isozyne has an extension that is not essential for the enzyme activity, but may have a role in positioning the enzyme correctly within the cell.

3.1.1. The Reductase Domain

Like the homologous protein cytochrome P450 reductase, the NOS reductase domain is made up of nucleotide-binding modules that bind NADPH, FAD, and FMN.^[137, 139] The only break in the homology to P450 reductase is an insert of about 45 amino acid residues within the FMN-binding region; this insert is present in eNOS and nNOS, but absent in iNOS.^[140] The function of the reductase domain is to shuttle electrons from NADPH to the oxygenase domain. This electron transfer activity requires the binding of calmodulin to its site between the oxygenase and reductase domains.

3.1.2. The Reductase–Oxygenase Interface

NOS is the only P450 heme containing enzyme in animals that has a dedicated reductase as an integral part of the protein (another, cytochrome P450 BM-3, occurs in bacteria^[141, 142]), suggesting that a special oxygenase–reductase interface is crucial to its function. Indeed, attempts to reconstitute NOS using isolated oxygenase and reductase domains resulted in low activity.^[143] Both EPR^[144] and time-resolved fluorescence experiments^[145] indicated a structural linkage of the heme to the flavins. No crystal structure of a full-length NOS has been published yet, so that the three-dimensional orientation of the domains is still a matter of conjecture. However, a pair of recent papers^[146, 147] indicated that the dimeric structure of the enzyme is important for reductase–oxygenase communication. Both depended on the construction of heterodimers of iNOS in which one subunit was full-length, whereas the other consisted only of the oxygenase domain. In the first study it was shown that maximally one heme per heterodimer could be reduced, suggesting that the single reductase domain could only reduce one of the two heme groups.^[146] In the second,^[147] a mutation^[148] in the oxygenase domain was used to disrupt L-arginine binding to one of the subunits (Figure 2). If the mutation was in the full-length subunit (Figure 2A), the activity of the heterodimer was unaffected. If it was in the oxygenase-only subunit (Figure 2B), the activity was abolished. Thus it was concluded that the reductase domain can only reduce the heme of the opposite subunit.

3.1.3. The Oxygenase Domain

The CO complex of reduced NOS has a strong absorbance band at 446 nm, like the cytochromes P450, indicative of a

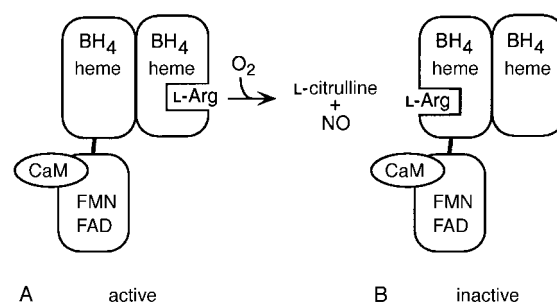


Figure 2. Domain swapping in NO synthase. A) When the L-arginine binding site and the reductase domain are on opposite subunits, NOS activity is observed. B) When L-arginine can only bind to the subunit that contains a reductase domain, no NOS activity is observed.

proximal thiolate ligand.^[149–151] The ligation is somewhat modified (as in chloroperoxidase) by extra hydrogen bonds to the proximal ligand, as revealed by resonance Raman spectroscopy^[152] and the recent crystal structures.^[153, 154] A view of the crystal structure of the iNOS oxygenase dimer is shown in the frontispiece on page 1714 (more images can be seen at <http://www.scripps.edu/~jat/nos.html>). The heme is cupped within an assembly that is comparable to a left-handed baseball catcher's mitt. The “thumb” (bright green) is formed by an α helix and loop carrying the heme proximal ligand cysteine. The distal side of the heme is shielded by a winged β sheet corresponding to the palm and fingers (cyan in the lower subunit, orange in the upper one).

The large content of β sheets is very unusual among hemoproteins. This restricts the scope for movements based on sliding of secondary structural elements, often exploited in α -helical proteins, for example in substrate binding by P450s.^[155] While this article was in preparation, a crystal structure of the eNOS oxygenase was published, confirming that the NOS isoenzymes share the same fold.^[156]

3.1.4. Heme and Dimerization

The native form of NOS is a homodimer. However, under conditions of heme deficiency, monomeric forms of the enzyme have been observed.^[157, 158] They contained no heme and could bind neither H₄biopterin (see Section 3.2.3) nor L-arginine,^[158] but their flavin content and cytochrome c reductase activity (see Section 3.1.1) were normal. In the case of iNOS, dimerization was observed on incubating the subunits simultaneously with haem, H₄biopterin, and L-arginine;^[157] for nNOS, heme alone seemed to be sufficient.^[158] Both isozyms could be reactivated.^[157, 159] Heme binding did not drastically affect the UV CD spectrum (a measure of secondary structure content),^[158] but did cause strong quenching of tryptophan fluorescence.^[159] Similar quenching was observed with PPIX, Mn-PPIX, or Co-PPIX. Of these, only Mn-PPIX stimulated dimerization. It was also the only one for which absorbance spectra (by comparison with metal-substituted P450_{cam}) suggested the acquisition of a thiolate ligand from the enzyme. It was proposed that the picking-up of the thiolate ligand by the heme metal could be the trigger for dimerization.^[159]

3.2. Ligand Binding and Allosteric Effects

3.2.1. $\text{Ca}^{2+}/\text{CaM}$

In nNOS and eNOS, only the Ca^{2+} -bound form of calmodulin can bind and activate the enzyme.^[160, 161] In contrast, iNOS already binds CaM, and is fully active at such low calcium concentrations that its activity *in vivo* can never be limited by Ca^{2+} .^[162] This difference between iNOS and the other isoforms has been ascribed both to the presence of the 40-residue insert in the FMN domains of nNOS and eNOS and to different characteristics of the CaM-binding sequences. Synthetic peptides corresponding to the insert in the FMN domain inhibited the enzyme and displaced CaM from its binding site.^[140] Several other CaM-dependent enzymes contain a flexible loop that occupies the CaM-binding site in the absence of $\text{Ca}^{2+}/\text{CaM}$, and must be displaced in order for the enzyme to be activated. It was suggested that the insert may play a similar role in NOS.

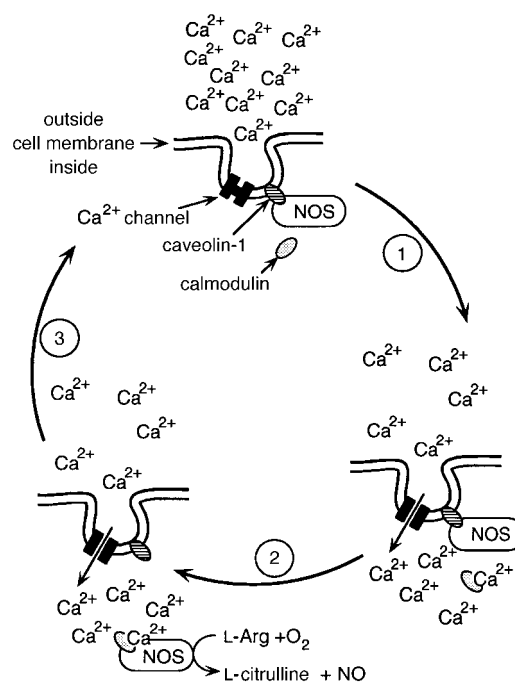
Another study showed, however, that the Ca^{2+} -independent binding of CaM to iNOS cannot be explained merely by the absence of this inhibitory loop.^[163] A series of chimeric enzymes were made in which the CaM-binding sequences were exchanged between isoforms. The modified eNOS containing the CaM-binding site from iNOS bound CaM Ca^{2+} -independently, but still required Ca^{2+} to become active. This suggests that the sequence of the CaM-binding site determines the nature of CaM binding, whereas the inhibitory insert confers Ca^{2+} -dependent activation.

Binding of calmodulin improves electron flow from NADPH to the flavins.^[164] It also facilitates electron transfer from FMN to the heme,^[165] though in the native enzyme this does not seem to be the rate-limiting step.^[166] The reductase can also catalyze reduction of external electron acceptors such as oxidized cytochrome *c*;^[167] this reaction is also largely dependent on calmodulin binding. The nature of the change induced by CaM that allows electron transfer is still poorly understood.

3.2.2. Caveolin

Another mode of regulation of NOS activity has come to prominence in the last couple of years. Caveolae are specialized invaginations of the plasma membrane found in many cell types.^[168] They are organized by the protein caveolin, of which there are three subtypes, caveolin-1, caveolin-2, and caveolin-3.^[169] Caveolin is proposed to be a generalized negative regulator of signal transduction that sequesters signaling proteins in an inactive form at the cell membrane, ready to respond to an incoming signal. In endothelial cells, a fraction of the eNOS is found in the caveolae.^[170] Treatment of the cells with agents that cause a calcium influx into the cells leads to dissociation of the eNOS from the caveolae and its activation (Scheme 5). The association with the caveolae is also largely dependent on fatty acylation of the eNOS at sites near its N terminus.^[171]

Studies on purified eNOS have found effective inhibition by a few micromolar caveolin that was reversed by addition of $\text{Ca}^{2+}/\text{CaM}$.^[172] A peptide corresponding to residues 82–101

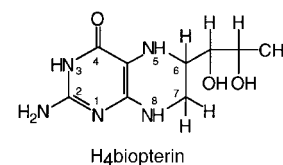


Scheme 5. Regulation of NO synthase by caveolin and calmodulin. 1) A Ca^{2+} channel in the membrane opens. 2) The rising initial concentration of Ca^{2+} allows calmodulin to compete with caveolin for NOS; NOS dissociates from caveolin and becomes active. 3) When the Ca^{2+} signal subsides, the equilibrium shifts back in favor of NOS binding to caveolin.

of caveolin-1 inhibited eNOS as effectively as the full-length protein. The peptide inhibited the cytochrome *c* reductase activity (though not ferricyanide reductase activity) with an IC_{50} of $3\ \mu\text{M}$, identical to that for the complete NOS reaction.^[173] Hydrogen peroxide dependent conversion of NOHLA into L-citrulline, a reaction that can be catalyzed by the oxygenase alone,^[174] was not inhibited. Immunoblotting showed that both the isolated reductase and oxygenase domains bound to immobilized caveolin-1, although the binding of the oxygenase domain was weaker. Thus caveolin-1 appears to inhibit NOS by interfering with electron transfer within the reductase domain. It is not yet clear whether the additional, apparently noninhibitory, binding to the oxygenase is functionally significant.

3.2.3. Negative Cooperativity of H_4 biopterin Binding

Purified NOS enzymes usually contain one molecule of tightly bound H_4 biopterin per heme, yet in the presence of exogenously added H_4 biopterin, they can be activated a further twofold, suggesting the presence of a second H_4 biopterin binding site.^[175] This was clarified using a H_4 biopterin-free version of the enzyme, prepared by expressing nNOS under conditions of low pteridine availability.^[176] Binding studies with radiolabeled H_4 biopterin and L-NNA (an L-arginine analogue) provided clear indications that H_4 biopterin binding occurs in two steps with about a 1000-fold difference in



affinity. The first molecule of H₄biopterin binds with an affinity in the subnanomolar range, whereas the second molecule binds with an affinity of around 1 μ M.

This negative cooperativity of H₄biopterin binding suggested a strong structural communication between the subunits. This was probed further in a study using 1,4-dithiothreitol (DTT).^[177] Inhibition of nNOS containing one equivalent of H₄biopterin per dimer was monophasic and competitive with respect to L-arginine, with a K_i of about 10 mM. In contrast, binding of DTT (as measured by the absorbance of the bithiolate complex, absorbing at 456 nm) had an additional, L-arginine-independent component at higher affinity. H₄biopterin-free NOS bound DTT monophasically at the higher affinity. Thus, in NOS containing one molecule of bound H₄biopterin per dimer, NO synthesis at the H₄biopterin-containing subunit was not affected by DTT binding to the opposite subunit. In this sense, the hemes of the two subunits function independently of one another.

3.2.4. H₄biopterin, L-Arginine, and the Heme Spin State

Like the P450s, the heme of NOS can exist in either high-spin, pentacoordinate or low-spin hexacoordinate states.^[151, 176, 178] In contrast to most P450s, in which the high-spin state only appears upon substrate binding,^[155] the haem of NOS as isolated is usually already mainly in the high-spin form. The reason for this was revealed by studies on H₄biopterin-free nNOS, which was found to have a substantially higher low-spin content but converted into high spin after addition of H₄biopterin.^[176] The conversion of nNOS from low to high spin was rather slow (about 0.1 min⁻¹ at 25 °C), allowing its comparison with enzyme activity. The gain of activity after addition of H₄biopterin to pteridine-free nNOS correlated exactly with the shift to high spin. Interestingly, the rate of conversion was independent of the concentration of added L-arginine or H₄biopterin, suggesting a slow spin-state equilibrium that determines substrate and cofactor binding. In other words, dissociation of the distal ligand (probably water, by analogy to P450s^[179]) is a precondition of L-arginine or H₄biopterin binding. This also seems to hold for eNOS^[180] and iNOS (though pteridine-free iNOS has a higher high spin content than nNOS).^[181]

Surprisingly, imidazole can bind to the heme of nNOS to form a low-spin complex in the presence of H₄biopterin, although its binding is competitive with respect to L-arginine.^[182] The crystal structures have provided an explanation, in that H₄biopterin binds on the proximal side of the porphyrin plane, whereas L-arginine as expected binds on the distal side (see the frontispiece on page 1714).^[154] Thus, the loss of the distal ligand is more directly coupled to L-arginine than to H₄biopterin binding. Indeed, the recent crystal structure of the eNOS oxygenase domain revealed no significant conformational differences between the H₄biopterin-free and H₄biopterin-bound forms.^[156] Thus the linkage of H₄biopterin binding to spin state may be electrostatic rather than steric in nature.

In both isoenzymes hydrogen bonds were identified from N3 (directly) and the carbonyl O atom at C4 (via a water molecule) of H₄biopterin to a heme propionate, which in turn

is hydrogen bonded to the α -amino group of the substrate L-arginine.^[154, 156] Also, the carbonyl O atom at C4 and N5 of the pteridine have hydrogen bonds to the helix that defines the substrate-binding channel.^[154] This network of interactions may account for effects of H₄biopterin on both the coordination behavior of the heme and on L-arginine binding without a large movement of the protein.

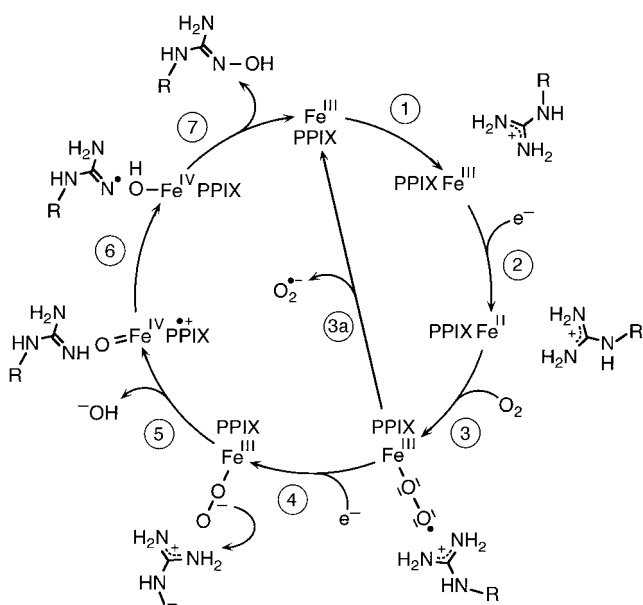
3.2.5. Dimer Stabilization

Another structural correlate of H₄biopterin and L-arginine binding is the stabilization of the NOS homodimer. In sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis, NOS migrates according to the relative molecular mass (M_r) of the monomeric subunits—as would be expected in this method, which normally involves heating the samples briefly to 95 °C to ensure full denaturation in the detergent. However, if the enzyme is preincubated with L-arginine and/or H₄biopterin, and the heating step before electrophoresis is omitted, a substantial fraction of the enzyme resists dissociation by the SDS.^[180, 181, 183] This unusual stabilization may be accounted for by the large buried surface area in the subunit interface (2800 Å²),^[154] and by the fact that each H₄biopterin molecule makes some contacts with the opposite subunit. A new factor to be considered in NOS dimer stability is suggested by the discovery in the eNOS oxygenase structure of a zinc ion coordinated by two cysteine residues from each subunit.^[156]

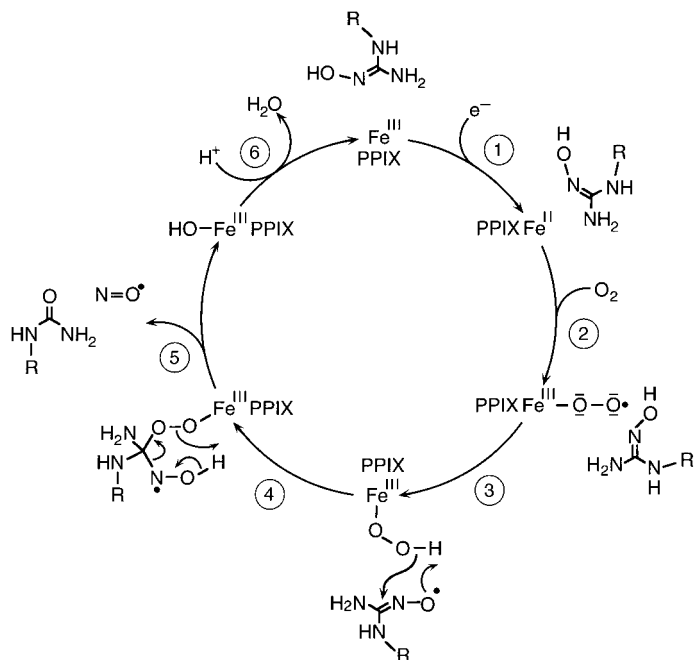
3.3. NOS Catalysis

NOS is the only known heme iron dependent H₄biopterin enzyme. However, apart from NOS, four enzymes are known that use H₄biopterin as a cofactor: phenylalanine monooxygenase, tyrosine 3-monooxygenase, tryptophan 5-monooxygenase, and glycerylether monooxygenase. In these enzymes H₄biopterin is directly involved in the hydroxylation of the substrate, being oxidized in the process to H₂biopterin, which is recycled to H₄biopterin by dihydropteridine reductase.^[184, 185] In contrast the NOS reaction does not result in net oxidation of H₄biopterin.^[186] Also, H₄biopterin binds too far away, and on the wrong side of the porphyrin, to participate as a hydroxylating cofactor at the distal side of the heme iron center.^[154]

Current discussion of the NOS mechanism is often based on concepts derived from P450 research.^[187–189] This is justified partly by the involvement of the thiolate-ligated heme of NOS in both stages of the reaction,^[149, 190] and partly by results on analogous reactions of guanidines and *N*-hydroxyguanidines catalyzed by classical P450s. However, mechanisms such as we present in Schemes 6 and 7 should be handled with caution. Even for P450s, after many years of sophisticated investigations, crucial steps of the reaction are still the subject of much debate.^[155] For NOS, though we can report rapid progress, the direct evidence on details of the reaction is still fragmentary. Nonetheless, recent work has begun to provide intriguing hints, particularly about the unconventional role of the pteridine cofactor.



Scheme 6. Hydroxylation of L-arginine. See text for details.



Scheme 7. Oxidation of NOHLA to L-citrulline and NO. See text for details.

3.3.1. What Does H₄Biopterin Do?

H₄biopterin is certainly an allosteric activator of NOS, with conformational effects reported by L-arginine binding, the heme spin conversion, and dimer stabilization (see Sections 3.2.3 and 3.2.4). Functionally, it is needed for coupling of NADPH oxidation to NO synthesis. The H₄biopterin-free rat nNOS catalyzes efficiently oxidation of NADPH and production of H₂O₂ (step 3a in Scheme 6). This reaction continues in the presence of L-arginine. When the enzyme in the presence of a constant L-arginine concentration (100 μ M) is titrated with H₄biopterin, there is a progressive and quantitative shift from H₂O₂ production to citrulline production by the enzyme (Figure 3);^[176, 191] Thus, H₄biopterin is truly indispensable for the correct outcome of the reaction: In terms of Scheme 6,

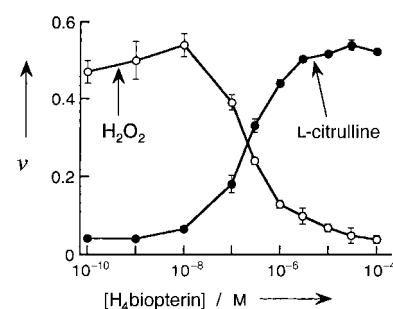
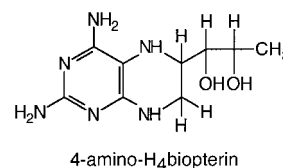


Figure 3. Effect of H₄biopterin concentration on coupled and uncoupled reactions of NO synthase (reproduced from reference [176]). Pteridine-free rat nNOS was titrated with H₄biopterin in the presence of 100 μ M L-arginine, Ca²⁺, CaM, and NADPH. Formation of H₂O₂ was monitored by absorbance at 492 nm and was corrected for CaM-independent rates. The coupled NOS reaction was assayed by measuring the conversion of [³H]L-arginine into [³H]L-citrulline. The rate of formation of H₂O₂ or L-citrulline (v) is given in μ mol min⁻¹ mg⁻¹.

H₄biopterin is needed for step 4 to outcompete step 3a. Probably the hottest issue in NOS catalysis currently is whether this shift is the result of a chemical involvement in the reaction—perhaps as a transient electron donor—or just another aspect of the allosteric role of H₄biopterin, perhaps adding a crucial final adjustment to the spatial juxtaposition of the substrate, the heme, and the protein.

One approach to solving this question is the study of analogues of H₄biopterin and their effects on the NOS reaction. For example, H₂biopterin binds with considerably lower affinity, and even at saturating concentrations does not activate the enzyme.^[192, 193]

Thus the tetrahydro form seems to be necessary. One analogue, 4-amino-H₄biopterin (for the numbering of atoms in H₄biopterin, see Section 3.2.3), has been found that binds with even higher affinity than H₄biopterin, and brings about, as far as current assays can measure, the same allosteric changes.^[181, 194, 195] Although it exists in the tetrahydro state (and can be oxidized) 4-amino-H₄biopterin does not support NOS activity; in fact, it inhibits both L-arginine hydroxylation and the conversion of NOHLA into L-citrulline and NO. Among the possible reasons why it does not support the NOS reaction are 1) subtle structural changes; 2) that although it can undergo reversible oxidation, some aspect of its redox mechanism is incompatible with the NOS reaction (for example, it might be less amenable to reversible one-electron oxidation than H₄biopterin); and 3) that the charge distribution on the pteridine is important in fine-tuning the heme redox potential, which is coupled to the protonation state of the propionates and is likely to be sensitive to the hydrogen bonds from N3 and carbonyl O atom at C4 of the pteridine.^[154]



3.3.2. Regulation of Heme Reduction

Reduction of the heme (step 2 in Scheme 6) is controlled by the supply of electrons from the flavins (see Section 3.2.1), but also by adjustment of the heme redox potential. P450s

generally use the binding of substrate to raise the heme midpoint potential into the range where reduction becomes possible.^[196] This avoids running down the cellular stores of reducing equivalents only to produce harmful reduced oxygen species. iNOS similarly links heme reduction to binding of L-arginine and H₄biopterin.^[197] In contrast, the heme midpoint potential of nNOS is higher in the absence of L-arginine, and is not significantly altered by L-arginine and H₄biopterin binding.^[197] These findings would explain the previous observation that of all three isozymes, nNOS has by far the highest NADPH oxidase activity in the absence of substrate and cofactor.

3.3.3. Oxygen Activation and L-Arginine Hydroxylation

Two groups have observed spectral intermediates formed after addition of oxygen to ferrous nNOS (steps 3–5 of Scheme 6); Abu-Soud et al.^[198] observed a complex absorbing at 427 nm by the stopped-flow technique at 10 °C, whereas Bec et al.,^[199] in a study done at –30 °C, found an intermediate with the Soret maximum at 416 nm. The reason for the discrepancy is not known; most ferrous-oxy P450s absorb maximally at around 418–420 nm. In the stopped-flow study, L-arginine inhibited the decay of the 427-nm complex, whereas H₄biopterin accelerated it by 70-fold.

In the low-temperature study, a further complex absorbing at 405 nm was observed, but only in the presence of both L-arginine and H₄biopterin as well as dithionite-reduced heme. Hydroxylation of L-arginine was also observed only under these conditions, suggesting that this complex might be an intermediate on the reaction pathway. It was suggested to be an oxo-ferryl complex (product of step 5 in Scheme 6), but further spectral analysis is necessary for a firm assignment.

The supply of protons to the active site may also be crucial to fission of the dioxygen bond (step 5 in Scheme 6): In the structure with bound L-arginine, the guanidino group is well shielded from solvent. It was suggested that one of the protons may be donated by the substrate itself.^[154]

Though the hydroxylation of L-arginine is formally similar to that of P450—in that it requires one equivalent of NADPH and results in the incorporation of one oxygen atom from O₂ into the product—it has never been found to be catalyzed by a classical P450.^[200, 201] Interestingly, P450-dependent hydroxylation of at least one non-physiological guanidine has been observed, showing that this type of reaction is chemically feasible without H₄biopterin, though apparently at very low rates.^[202] Again, one could suppose that H₄biopterin helps poise a heme–oxygen intermediate at a more favorable redox potential for attack on the L-arginine, but a more radical deviation from a P450 paradigm cannot be ruled out.

3.3.4. From N^G-Hydroxy-L-arginine to L-Citrulline and NO

We must emphasize at the beginning of this section that the second stage of the NOS reaction, from NOHLA to L-citrulline and NO (see Scheme 7) is also completely dependent on H₄biopterin.^[195] That similar reactions can occur non-enzymatically^[203, 204] or be (very inefficiently) catalyzed by H₄biopterin-independent enzymes^[200, 201] should not obscure this.

This stage requires the oxidation of only 0.5 equivalents of NADPH.^[189, 205] This contrasts with the hydroxylation of L-arginine by NOS and with P450 reactions, in which a further reduction of the heme is needed to achieve oxygen fission (steps 4 and 5 of Scheme 6). However, O₂^{•–} has been shown to oxidize some *N*-hydroxyguanidine model compounds to urea products with release of NO, and the Fe^{III}-O-O[•] complex that would be formed after one-electron reduction of the heme represents a nearly equivalent redox state of oxygen to O₂^{•–}.

NOHLA itself does not reduce the ferric iron of NOS;^[206] therefore the reaction is likely to be initiated by NADPH-dependent reduction of the iron center (step 1 of Scheme 7) followed by O₂ binding (step 2 of Scheme 7). It was suggested that an electron transfer from NOHLA to the Fe^{III}-O-O[•] complex might then occur, leading to the formation of Fe^{III}-O-O[–] and the radical cation NOHLA^{•+}.^[187, 207] Countering this proposal, it was pointed out, with the aid of an electrochemical experiment on NOHLA, that the formation of the radical cation would be very unlikely, and that a hydrogen-atom abstraction would be more feasible (step 3 in Scheme 7).^[188]

3.3.5. Recognition of L-Arginine Analogues

In the iNOS crystal structure,^[154] specific binding of the guanidino group of L-arginine, involving hydrogen bonds with the carboxylate group of E371, can be seen. This is in agreement with the earlier result that the mutation E371A disrupts L-arginine binding. However, several modifications to the guanidino group are tolerated by the enzyme and even increase the binding affinity, giving rise to a series of effective inhibitors that are competitive with L-arginine (Figure 4).

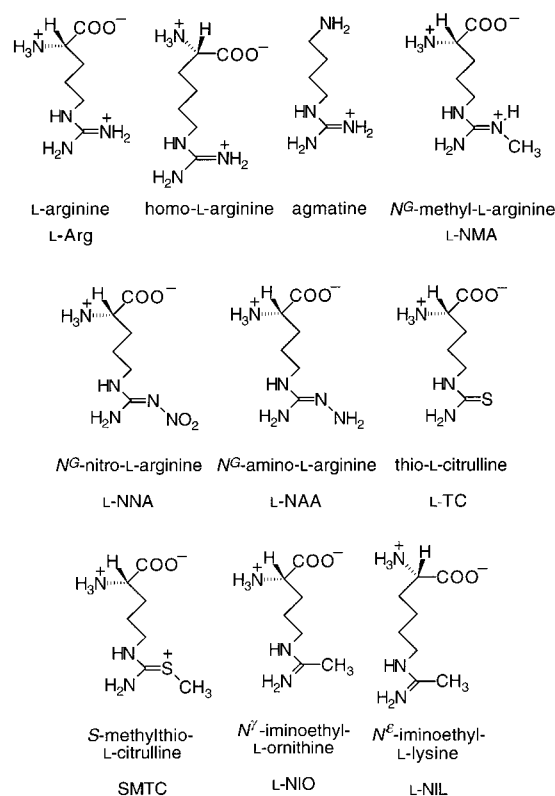


Figure 4. Schematic representations of L-arginine and analogues that are competitive with L-arginine.

Recognition of the α -aminopentanoic acid part of the substrate is also important: Besides L-arginine, only homo-L-arginine and agmatine are detectably active as NOS substrates, whereas neither guanidine nor hydroxyguanidine is a substrate.^[208–210] Removal of the α -aminopentanoic acid part of L-NMA, L-NAA, and L-NNA to give the corresponding guanidine derivative also resulted in a loss of binding affinity.^[208, 209] Another series of inhibitors is based on L-thiocitrulline. In this case, the removal of the α -aminopentanoic acid portion to give the corresponding isothiurea derivative increases the affinity.^[211, 212] An iNOS crystal structure with bound L-TC has been determined,^[154] but structures with isothiurea derivatives will be needed before this change in affinity can be understood.

Apart from the competition with L-arginine, these inhibitors have diverse additional effects. Some analogues (L-NMA,^[213, 214] L-NIL,^[215] L-NIO^[216]) exhibit mechanism-based inhibition, causing irreversible inactivation when both oxygen and NADPH are present.^[210] L-NMA^[213, 214, 217] and L-NIL allow NADPH oxidation (albeit with progressive inactivation), whereas the others inhibit it. The same pattern was observed with the corresponding guanidine analogues, without the α -aminopentanoic acid part of the substrate.^[209, 211] Inhibition of NADPH oxidation has been suggested to be due to an electrostatic effect of an electron-rich substituent on the guanidine/isothiurea group on the heme redox potential. Another factor that may be relevant is the access of oxygen to the heme: Binding of CO, for example, is slowed by the presence of L-arginine in its binding site.^[218] Analogues that inhibit H₂O₂ formation do not necessarily prevent reduction of the heme: Aminoguanidine allows NADPH-dependent heme reduction, whereas L-NNA blocks heme reduction even by dithionite.^[197]

3.3.6. Isozyme-Specific Inhibitors

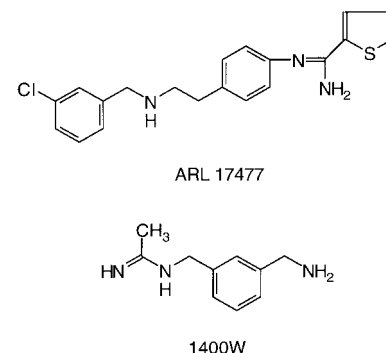
A major difficulty of all NO-related drugs is to avoid crossover between different physiological functions of NO. It is hoped that isozyme-specific NOS inhibitors will offer a way to avoid analogous problems with drugs aimed at suppressing NO biosynthesis. Selective nNOS inhibitors might help to alleviate ischemic brain damage: Mice lacking nNOS were more resistant to experimentally induced stroke.^[219] Selective iNOS inhibitors would have a wider range of possible indications, including, for example, the severe hypotension in septic shock, chronic inflammatory diseases such as rheumatoid arthritis, and adverse immune reactions associated with transplantation. As a rule of thumb, lead compounds should have 100-fold isozyme selectivity and a submicromolar IC₅₀.

Most of the L-arginine analogues mentioned in Section 3.3.5 exhibit only marginal selectivity between isozymes. One exception is L-NIL, which is about 30-fold more selective for iNOS^[215] and was effective in both adjuvant-dependent arthritis and leishmaniasis models in mice.^[220, 221] The substituted isothiureas are also mostly nonselective,^[211] though *S*-methylisothiurea is about 20-fold selective for iNOS,^[222] whereas 1-amino-*S*-methylisothiurea is selective for nNOS (8-fold versus iNOS and 34-fold versus eNOS) in vitro.^[223]

7-Nitroindazole is selective for nNOS in vivo, and is protective in experimental stroke^[224] as well as having antinociceptive activity. However, it inhibits the purified NOS isozymes with about equal potency. Its selectivity may be due to differences in its uptake or metabolism in different tissues, or to its unusual mode of inhibition: It competes with both H₄biopterin and L-arginine binding.^[225] Thus the nNOS selectivity could be due to relatively low concentrations of L-arginine and H₄biopterin in certain neurons.

4-Amino-H₄biopterin (see Section 3.3.1) exhibits little isozyme selectivity with the purified enzymes,^[181, 194, 195] but appears to be selective for iNOS in cultured cells.^[226] It was speculated that this may be because 4-amino-H₄biopterin does not readily replace tightly bound endogenous H₄biopterin from the enzyme,^[194] so that NOS would be most sensitive to the inhibitor during de novo protein synthesis. This effect would cause more efficient inhibition of the iNOS, because this isozyme is synthesized rapidly on induction, whereas the other isozymes are subject to much slower protein turnover.^[3, 195]

The most selective nNOS inhibitor available at present (100-fold) is the Astra Arcus compound ARL 17477. It substantially reduced the volume of infarcts caused by



temporary occlusion of the middle cerebral artery in rats. The doses used caused nearly complete inhibition of cortical NOS activity, but did not affect mean arterial blood pressure.^[227]

The most selective iNOS inhibitor reported to date is the Glaxo Wellcome compound 1400W (*N*-(3-aminomethyl)benzyl)acetamidine).^[228] It exhibits rapidly reversible, low-potency ($K_i \approx 2\text{--}50\text{ }\mu\text{M}$) inhibition of all NOS isozymes, but an additional slow phase of binding to iNOS has an estimated binding constant of 7 nM. This slow phase required the presence of NADPH, but did not appear to involve chemical conversion of the inhibitor. In endothelium-intact rat aortic rings, it caused only a slight contraction at 300 μM , reflecting a lack of inhibition of eNOS. In contrast, in endothelium-removed aorta from LPS-treated animals, 1400W caused contraction with an EC₅₀ of 0.8 μM , reflecting inhibition of iNOS. In LPS-treated rats it was shown to protect against microvascular damage as reflected by vascular leakage into the ileum. It could be administered simultaneously with the LPS without adverse effects, unlike nonspecific NOS inhibitors that actually cause increased vascular leakage in this model. While continuous infusion at 120 mg kg⁻¹ per day was tolerated, a bolus of 50 mg kg⁻¹ given intravenously was immediately lethal. This toxicity is lower than that of

isothioureas. It remains to be seen whether the toxicity of such compounds can be lowered sufficiently to make them clinically useful. In the meantime, 1400W should allow much better definition of experimental disease models in which iNOS inhibition can produce a therapeutic benefit.

4. Summary and Outlook

The most important signaling functions of NO are certainly mediated by activation of sGC and the consequent increases in intracellular cGMP. The reversible binding of NO to the heme of sGC is in striking contrast to its irreversible reaction with oxyHb, leading to formation of metHb and nitrate, which can be considered a major inactivation mechanism for NO. The causes of these differences are to be found in the special heme environment of sGC and can probably only be assessed exactly when a crystal structure is solved.

Reactions with superoxide and thiols can also determine the biological effects of NO. The reaction with oxygen leads to formation of reactive intermediates that can (unlike free NO) nitrosate amines and thiols. Some of the resulting nitroso compounds—particularly *S*-nitrosothiols—probably have important functions in extending the range of NO signals or as stores of NO. Reaction of NO with $O_2^{\cdot -}$ yields peroxynitrite, whose oxidative activity contributes substantially to tissue damage in inflammatory and infective diseases. Resolving the question of which reactions of peroxynitrite are causing the most damage is likely to lead to new therapeutic strategies.

The biosynthesis of NO is catalyzed by NO synthases. These are complex homodimeric enzymes in which each subunit contains a thiolate-bound heme group that binds molecular O_2 and catalyzes, in the presence of the cofactor H_4 biopterin, the oxidation of L-arginine to L-citrulline and NO. The reducing equivalents needed for the reaction are supplied by NADPH through the FMN- and FAD-containing reductase domain of the enzyme. This electron transport is gated by the binding of calmodulin. The constitutively expressed neuronal and endothelial isozymes bind calmodulin only in the presence of $0.1 - 1 \mu M$ Ca^{2+} , whereas the inducible isozyme is practically independent of Ca^{2+} concentration. To understand the reaction mechanism it will be decisive to establish the role of H_4 biopterin. In addition to its known allosteric effects it seems likely to have an as yet undefined redox function. These questions seem likely to be answered in the near future by a combination of biophysical and functional experiments, and by crystal structure analysis of specific NOS mutants and NOS-pteridine complexes. Finally, the development of isozyme-selective NOS inhibitors is expected to open new perspectives in the treatment of infective and inflammatory diseases and ischemic conditions.

5. Abbreviations

4-amino- 2,4-diamino-5,6,7,8-tetrahydro-6-(L-erythro-1,2-
 H_4 biopterin dihydroxypropyl)pteridine
 H_4 biopterin 2-amino-4-oxo-(6*R*)-5,6,7,8-tetrahydro-6-(L-
 erythro-1,2-dihydroxypropyl)pteridine

H_2 biopterin 2-amino-4-oxo-(6*R*)-7,8-dihydro-6-(L-erythro-
 1,2-dihydroxypropyl)pteridine
 CaM calmodulin
 FAD flavin adenine dinucleotide
 FMN flavin mononucleotide
 Hb hemoglobin
 IC₅₀ concentration causing 50 % inhibition
 IRP iron regulatory protein
 L-NAA *N*^G-amino-L-arginine
 L-NNA *N*^G-nitro-L-arginine
 L-NMA *N*^G-methyl-L-arginine
 L-NIL *N*^ε-iminoethyl-L-lysine
 L-NIO *N*^δ-iminoethyl-L-ornithine
 L-TC thio-L-citrulline
 SMTc S-methylthio-L-citrulline
 Mn-SOD manganese superoxide dismutase
 NADPH nicotinamide adenine dinucleotide phosphate
 (reduced)
 NMDA *N*-methyl-D-aspartate
 NOHLA *N*^G-hydroxy-L-arginine
 NOS nitric oxide synthase (EC 1.14.13.39)
 eNOS endothelial nitric oxide synthase
 iNOS inducible nitric oxide synthase
 nNOS neuronal nitric oxide synthase
 PARS poly(ADP-ribose) synthetase
 PPIX protoporphyrin IX
 sGC soluble guanylyl cyclase (GTP pyrophosphate-
 lyase (cyclizing), EC 4.6.1.2.)

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Silicates and Perovskites: Two Themes with Variations

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Silicates with the general formula $\mathbf{A}_m\text{Si}_n\text{O}_p$ [***] are the third largest class of chemical compounds after the carbon compounds and the oxides. The number of silicate minerals that occur in nature is very large: At present approximately 1000 natural silicates are known and, on average, about ten new ones are discovered each year. In addition, there are also several hundred synthetic silicates. The large number of synthetic silicates can be attributed to the fact that the experimental chemist is not restricted to compositions of raw materials that appear in nature when carrying out the synthesis in the laboratory. This wide range in chemical composition, therefore, goes hand-in-hand with an exceptional diversity of different structures. Long and intensive investigations have led to a classification system of silicates on a structural chemistry basis which has contributed to a greater understanding of these important compounds.^[2, 3]

Recently, silicate research has gained a strong impetus from various groups who have replaced the oxygen partially or completely by nitrogen in the $\mathbf{A}_m\text{Si}_n\text{O}_p$ silicates, and have thus achieved the transition from the oxosilicates to the oxonitridosilicates $\mathbf{A}_m\text{Si}_n(\text{O},\text{N})_p$ ^[4, 5] and the nitridosilicates $\mathbf{A}_m\text{Si}_n\text{N}_p$.^[6] Here the principles governing the linkage of tetrahedra, which were relatively simple and clear for the oxosilicates, have been considerably extended. The recent discovery of an octahedrally coordinated silicon atom surrounded by six nitrogen atoms in the synthetic oxonitridosilicate $\text{Ce}_{16}\text{Si}_{15}\text{O}_6\text{N}_{32}$ ^[5] serves as an opportunity for a comparison between these three types of silicates. The structure of

this compound, which has been described as a derivative of the perovskite type by its discoverers, highlights the ability of nature to vary a simple, highly symmetrical structure type in a diversity of ways.

Oxosilicates and Silicon Oxo Salts

The exceptionally wide variety of $\mathbf{A}_m\text{Si}_n\text{O}_p$ compounds can be principally attributed to the central position of silicon in Group 14 of the periodic system of the elements. Silicon is therefore amphoteric; that is, its oxide acts as an acid towards oxides of metallic elements with lower electronegativity than silicon ($\chi_{\text{Si}} = 1.74$ ^[7]), but as a base towards oxides of strongly electronegative nonmetals (e.g. P_2O_5 and As_2O_5). The first of the two groups of $\mathbf{A}_m\text{Si}_n\text{O}_p$ compounds are the true oxosilicates. They represent the largest group by far of silicate compounds. Representatives of the second, much smaller group are more correctly described as silicon oxo salts, for example, the silicon oxophosphate, SiP_2O_7 , usually abbreviated to silicon phosphate.

Oxosilicates: In the oxosilicates, every silicon atom is tetrahedrally coordinated by four oxygen atoms, so that the coordination number of silicon with respect to oxygen is $\text{CN}(\text{Si}/\text{O}) = 4$. The $[\text{SiO}_4]$ tetrahedra can be linked together through shared corners ($L = 1$)^[8] to form multiple tetrahedra, rings, chains, layers, and frameworks.^[2] Linkage of the $[\text{SiO}_4]$ tetrahedra over shared edges ($L = 2$) or even faces ($L = 3$) is very unfavorable energetically because of the involved reduction of the distance between the silicon atoms (Figure 1) and the resulting increase in repulsive forces (Pauling's third rule), and has not yet been established with any certainty.^[***] If the **A** cations are disregarded and only the silicon atoms on neighboring oxygen atoms are considered, the coordination number $\text{CN}(\text{O}/\text{Si})$ of the O atoms takes on the values 0 ($\text{O}^{[0]}$ not bound to Si), 1 (terminal $\text{O}^{[1]}$ bound to one Si atom), or 2 (bridging $\text{O}^{[2]}$ atom bound to two Si atoms). From the given restrictions for $\text{CN}(\text{Si}/\text{O})$, $\text{CN}(\text{O}/\text{Si})$, and L in Table 1, the degree of condensation of oxosilicate anions can vary between $\text{Si}:\text{O} = 0.25$ for monosilicates with isolated $[\text{SiO}_4]^{4-}$ anions and $\text{Si}:\text{O} = 0.5$ for silicon dioxide, SiO_2 , with its three-dimensionally infinite frameworks of tetrahedra.

[***] The structure of fibrous SiO_2 described in 1954, said to contain chains of edge-sharing $[\text{SiO}_4]$ tetrahedra,^[9] has not been verified up to now.

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[**] In accordance with a proposal from Smith, Roberts, Bayliss, and Liebau,^[1] a differentiation is made in the structure formula between the usual symbols for the chemical elements B, C, F, H, I, K, N, O, P, S, U, V, W, and Y and the symbols accentuated in bold type for mono- or polyatomic species which occupy specific crystallographic positions (structure sites). These structure-site symbols are for cations: **A** (independent of coordination), **D** (coordination number $\text{CN} \geq 9$), **E** ($\text{CN} = 8, 7$), **G** ($\text{CN} = 6$), **J** ($\text{CN} = 5$), **Q** ($\text{CN} = 4$, planar, or 2, linear), **T** ($\text{CN} = 4$, tetrahedral), **L** (cations with a lone electron pair), and **M** (for molecules), **X** (for monatomic anions), and **Z** (polyatomic anions). Structural parameters such as CN for coordination number, L for linkedness (linkage type), and others are written in italic and bold type.^[2]

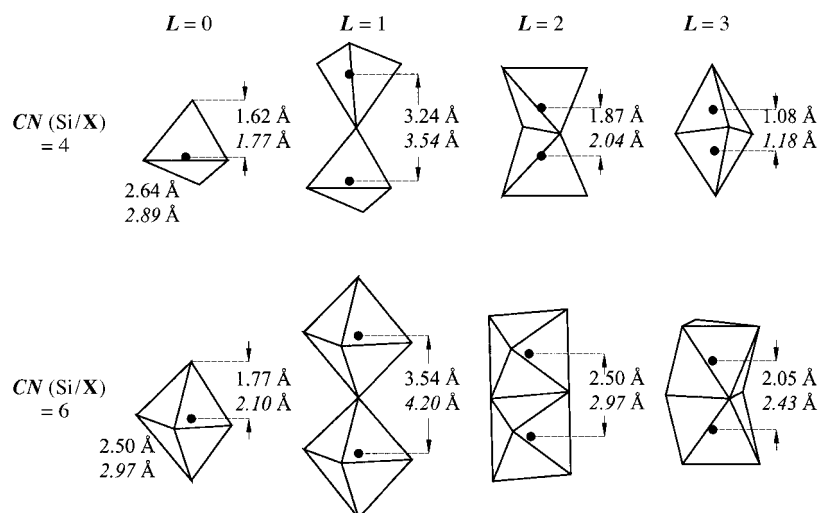


Figure 1. Mean atomic distances $d(\text{Si}-\text{X})$ and $d(\text{X}\cdots\text{X})$ of $[\text{SiX}_4]$ tetrahedra and $[\text{SiX}_6]$ octahedra and maximum distances $d(\text{Si}\cdots\text{Si})$ for corner-, edge-, and face-sharing polyhedra of these types. Normal type: $\text{X} = \text{O}$; italic type: $\text{X} = \text{N}$.

Table 1. Comparison of the coordination relationships in oxo- and nitridosilicates and the resulting theoretically possible molar $\text{Si}:\text{X}$ ratios of their $[\text{Si}_i\text{X}_s]$ polyhedral structures.^[a]

		$\text{X} = \text{O}$	$\text{X} = \text{N}$
$\text{CN}(\text{Si}/\text{X}) = 4$	L	0, ^[b] 1, (2)	(0), ^[b] 1, 2
	$\text{CN}(\text{X}/\text{Si})$	0, ^[b] 1, 2	(0), ^[b] 1, 2, 3, 4
	$(\text{Si}:\text{X})_{\text{theor.}}$	0.25 to 0.5	0.25 to 0.75
	$(\text{Si}:\text{X})_{\text{obs.}}$	0.25 to 0.5	0.333 to 0.75
$\text{CN}(\text{Si}/\text{X}) = 6$	L	0, ^[b] 1, 2, (3)	(0), ^[b] (1), (2), (3)
	$\text{CN}(\text{X}/\text{Si})$	0, ^[b] 1, 2, 3	(0), ^[b] (1), (2), (3), (4)
	$(\text{Si}:\text{X})_{\text{theor.}}$	0.1667 to 0.5	0.1667 to 0.75
	$(\text{Si}:\text{X})_{\text{obs.}}$	0.1667 to 0.5	–

[a] The values in parentheses are theoretically possible but have not yet been definitely established. [b] Oxygen atom (“free”) not bound to silicon ($\text{O}^{[0]}$).

Silicon oxo salts: Typical compounds of this group, such as SiP_2O_7 ,^[10] contain silicon in octahedral coordination. The tendency of silicon to form $[\text{SiO}_6]$ octahedra instead of $[\text{SiO}_4]$ tetrahedra is stronger with increasing electronegativity of the A atoms.^[11] Since the $\text{O}-\text{O}$ bond distance of about 2.50 Å in $[\text{SiO}_6]$ octahedra is considerably smaller than the corresponding distance of about 2.64 Å in $[\text{SiO}_4]$ tetrahedra, the transition of the coordination number, $\text{CN}(\text{Si}/\text{O})$, from 4 to 6 is associated with a decrease in volume and can therefore be initiated by an increase in pressure. In fact, the majority of currently known phases that contain $[\text{SiO}_6]$ octahedra have been formed at pressures greater than 8 GPa.

Since the $\text{Si}-\text{O}$ bond distances in the $[\text{SiO}_6]$ octahedra are longer than those in the $[\text{SiO}_4]$ tetrahedra, it follows that the distances between the Si atoms of linked $[\text{SiO}_6]$ octahedra are also longer, and so the repulsive forces between them are weaker than those between linked tetrahedra (Figure 1). In agreement with this, edge sharing ($L=2$) between $[\text{SiO}_6]$ octahedra is not uncommon, and even a linkage over the common faces of two $[\text{SiO}_6]$ groups appears to be energetically somewhat more favorable than the linkage of two $[\text{SiO}_4]$ tetrahedra over a common edge.

An increase in the coordination number $\text{CN}(\text{Si}/\text{O})$ from 4 to 6 involves an increase in the mean $\text{CN}(\text{O}/\text{Si})$ coordination

number. In fact, $\text{O}^{[3]}$ atoms occur in structures with linked $[\text{SiO}_6]$ octahedra—in addition to the oxygen species $\text{O}^{[0]}$, $\text{O}^{[1]}$ and $\text{O}^{[2]}$ —that have not been observed so far in structures with linked $[\text{SiO}_4]$ tetrahedra (Table 1). The molar atomic ratio $\text{Si}:\text{O}$ of polyhedral structures which consist exclusively of $[\text{SiO}_6]$ octahedra can therefore range between 0.1667 (e.g. in SiP_2O_7) and 0.5 (in stishovite, a high-pressure phase of SiO_2).

In addition to the pure oxosilicates and the pure silicon oxo salts, there is a range of compounds that contain both $[\text{SiO}_4]$ tetrahedra and $[\text{SiO}_6]$ octahedra.^[12] After spectroscopic investigations indicated the existence of fivefold-coordinated silicon in glasses made by quenching silicate melts previously held at a few GPa of pressure,^[13a,b] $[\text{SiO}_5]$ polyhedra, together with $[\text{SiO}_6]$ octahedra, have been established by a crystal structure determina-

tion of high-pressure CaSi_2O_5 .^[13c] Such $[\text{SiO}_5]$ polyhedra have long been assumed to exist as the transition state in condensation and hydrolysis reactions in aqueous solution and in rheological and diffusion processes in silicate melts.^[14] Compounds containing $[\text{SiO}_5]$ polyhedra and compounds with mixed $\text{CN}(\text{Si}/\text{O})$ coordination numbers that are stable at intermediate pressures occupy an intermediate position between the oxosilicates and the silicon oxo salts.

Nitridosilicates and Oxonitridosilicates

Although, up to now, the structures of only a couple of dozen $\text{A}_m\text{Si}_n\text{N}_p$ nitridosilicates and four $\text{A}_m\text{Si}_n\text{O}_p\text{N}_q$ oxonitridosilicates have been sufficiently well established, it is possible to derive principles for crystal chemical analysis. These principles are compared in Table 1 with those of the $\text{A}_m\text{Si}_n\text{O}_p$ oxo compounds. Table 2 contains values of several

Table 2. Several parameters that are useful for the interpretation of silicate structures.

	Si	$\text{X} = \text{O}$	$\text{X} = \text{N}$
$r^{[d]}$ [Å] ^[a]	0.26	1.38	1.46
$\chi^{[b]}$	1.74	3.50	3.07
$\Delta\chi^{[c]}$	–	1.76	1.33
$I_{\text{Si-X}} [\%]$ ^[d]	–	53	36

[a] Effective radius of the fourfold coordinated ions.^[15] [b] Electronegativity.^[7] [c] $\Delta\chi = \chi_{\text{X}} - \chi_{\text{Si}}$. [d] $I_{\text{Si-X}} = 1 - \exp[-0.25(\chi_{\text{X}} - \chi_{\text{Si}})^2]$ (ionicity).^[16]

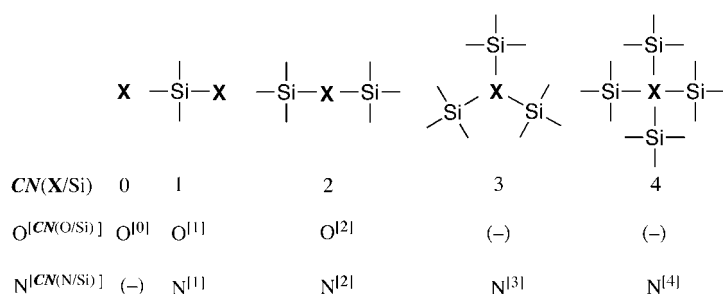
parameters of the elements silicon, oxygen, and nitrogen that are useful for a rough interpretation.

Nitridosilicates: In all the $\text{A}_m\text{Si}_n\text{N}_p$ structures known so far, silicon is, without exception, tetrahedrally coordinated; these compounds are therefore true nitridosilicates. No indications were found for $[\text{SiN}_6]$ octahedra, even in phases that were synthesized at high pressures. This means that silicon nitrido salts corresponding to the silicon oxo salts are, until now, not known. This reflects the predicted lower tendency of silicon to sixfold coordination with the larger nitride ion N^{3-} than with the smaller oxide ion O^{2-} , in accordance with the radius–ratio

rule (Pauling's first rule). The low tendency to form $[\text{SiN}_6]$ octahedra is decreased further by the fact that, owing to the lower electronegativity of nitrogen, the Si–N bond is considerably more covalent than the Si–O bond, whereby the nonmetallic character of silicon and therefore its sp^3 character are strengthened.

Since $[\text{SiN}_4]$ tetrahedra are larger than $[\text{SiO}_4]$ tetrahedra, $r(\text{N}^{3-}) > r(\text{O}^{2-})$, and the stronger covalent character of the Si–N bond means that the effective charge of the Si atoms in $[\text{SiN}_4]$ is lower than in $[\text{SiO}_4]$ tetrahedra, the repulsive forces between the Si atoms of linked $[\text{SiN}_4]$ tetrahedra in nitridosilicates are lower than the equivalent forces in oxosilicates. Despite the low number of known nitridosilicate structures, the above statement is supported by the finding that both corner-sharing ($L=1$) and edge-sharing ($L=2$) tetrahedra exist in $\text{BaSi}_7\text{N}_{10}$,^[6i] whereas edge-sharing of $[\text{SiO}_4]$ tetrahedra has never been satisfactorily established in the much more numerous oxosilicates (see footnote^[**] on p. 1733).

While oxosilicates only contain oxygen atoms which are either bound to none, one, or two silicon atoms ($\text{O}^{[0]}$, $\text{O}^{[1]}$, $\text{O}^{[2]}$), nitrogen atoms have been found in nitridosilicates that are bound to three or four silicon atoms ($\text{N}^{[3]}$, $\text{N}^{[4]}$), in addition to the species $\text{N}^{[1]}$ and $\text{N}^{[2]}$ (Scheme 1, Table 1). The histogram in Figure 2 shows the frequency distribution of known nitridosilicates relative to the occurrence of the different $\text{X}^{[\text{CN}(\text{X/Si})]}$ species.



Scheme 1. Linkages of the X anions with silicon in oxosilicates ($\text{X}=\text{O}$) and nitridosilicates ($\text{X}=\text{N}$); (–): not observed.

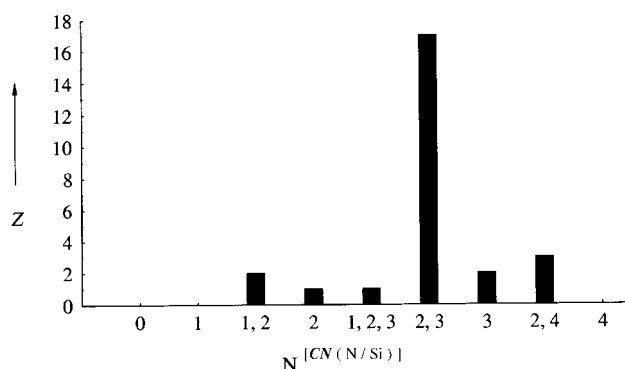


Figure 2. Number Z of nitridosilicate structures in which the different $\text{N}^{[\text{CN}(\text{N/Si})]}$ species have been found.

From the restrictions given for $\text{CN}(\text{Si/N})$, $\text{CN}(\text{N/Si})$ and L in Table 1, it follows that the degree of condensation of the $[\text{SiN}_4]$ tetrahedra in nitridosilicates can range from 0.25 for isolated single tetrahedra (not observed up to now, probably

because of its high formal charge of -8) and 0.75 (realized in the tetrahedral frameworks of α - and β - Si_3N_4).

Oxonitridosilicates: It would be expected that the oxonitridosilicates are structurally intermediate between the oxo- and the nitridosilicates. This is true for the few representatives of this group of compounds for which exact structure determinations are available,^[4,5] and for a range of further examples for which isotopy with known oxosilicates, such as Ca_2SiO_4 , åkermanite ($\text{Ca}_2\text{MgSi}_2\text{O}_7$), and pseudowollastonite ($\text{Ca}_3\text{Si}_3\text{O}_9$), could be established from powder X-ray diffraction data.^[17] They contain $[\text{SiX}_4]$ tetrahedra with O:N ratios ranging from 3:1 in $\text{LaEu}^{II}[\text{SiO}_3\text{N}]$ ^[17a] to 1:3 in $\text{Si}_2\text{N}_2\text{O}$.^[4b]

$\text{Ce}_{16}\text{Si}_{15}\text{O}_6\text{N}_{32}$

This extraordinary phase was recently synthesized by Köllisch and Schnick^[5] at about 1500°C and normal pressure and structurally investigated by single-crystal X-ray diffraction. It contains both tetrahedrally and octahedrally coordinated silicon atoms, and is not formally a pure oxonitridosilicate, but comes very close with a molar atomic ratio of $\text{Si}^{[4]}:\text{Si}^{[6]}=14:1$.

At first sight, the existence of $\text{Si}^{[6]}$ in this phase is surprising since it does not contain strongly electronegative cations ($\chi_{\text{Ce}}=1.08$) and was not synthesized under high pressure. Even more interesting is the fact that, although the tendency to form $[\text{SiX}_6]$ octahedra is lower for $\text{X}=\text{N}^{3-}$ than for $\text{X}=\text{O}^{2-}$, the structure determination reveals that the 14 $\text{Si}^{[4]}$ atoms per formula unit are probably statistically surrounded by oxygen and nitrogen atoms, whereas the $\text{Si}^{[6]}$ atom is, with high probability, only coordinated by nitrogen atoms. Therefore, there must be a particular reason that a few of the silicon atoms in $\text{Ce}_{16}\text{Si}_{15}\text{O}_6\text{N}_{32}$ are, against all expectations, octahedrally coordinated.

A possible reason, given by the authors, is that the structure of this cerium oxonitridosilicate bears certain similarities with the perovskite type $\text{A}^{[12]}\text{G}^{[6]}\text{X}_3$ (see footnote^[**] on p. 1733). Therefore, the structure can be described as a cubic $4 \times 4 \times 4$ superstructure of the ideal cubic perovskite type, in which four of the 64 $\text{G}^{[6]}$ positions per superstructure unit cell are occupied with $\text{Si}^{[6]}$, 56 with $\text{Si}^{[4]}$, and four are unoccupied, whereas the 64 $\text{A}^{[12]}$ positions are all occupied by Ce^{3+} ions. The valence is compensated for by 24 O^{2-} and 128 N^{3-} ions, so that 40 X positions of the perovskite superstructure cell are unoccupied. The cerium oxonitridosilicate phase is therefore a perovskite variant with an ordered distribution of vacancies, both in the sublattice of the octahedrally coordinated G cations and in the anion sublattice, and with a very extensive substitution of the $\text{G}^{[6]}$ cations by $\text{Si}^{[4]}$ cations. It can therefore be described by the formula $\text{Ce}_{16}(\text{Si}^{[6]}\text{Si}^{[4]}\square)(\text{O}_6\text{N}_{32}\square_{10})$, where the coordination number of the Ce^{3+} ions is considerably lower than 12 as a consequence of the vacancies ($\square$) in the anion sublattice. In addition, the change in coordination of $\text{G}^{[6]}$ to $\text{Si}^{[4]}$ requires that the positions of the O^{2-} and N^{3-} ions deviate considerably from the X positions in ideal perovskite.

Such a large difference between the structure of the cerium compound and that of the ideal perovskite type clearly provokes the question whether this structure relationship is crystal chemically and energetically relevant or whether it is

coincidence, imagination, or unrealistic. To answer this question, the various modifications of the ideal perovskite type are discussed below.

Stoichiometric Perovskites

The starting point is the ideal perovskite structure with a cubic unit cell ($a \approx 3.93 \text{ \AA}$), the space group $Pm\bar{3}m$, and one formula unit of $A^{[12]}G^{[6]}X_3$. The structure is usually represented as a three-dimensional framework of corner-shared, regular octahedra with centers occupied by the **G** cations, while the large **A** cations occupy the dodecahedral sites between eight octahedra.

This aristotype structure can, while maintaining the topology of the bonds, be deformed to hettotype structures^[*] with lower symmetries, in the sense of Megaw,^[18] by one of three mechanisms: a) distortion of the $[X_6]$ octahedra, b) shifting of the $G^{[6]}$ cations from the octahedra centers, and c) cooperative tilting of the $[X_6]$ octahedra around two-, three-, or fourfold rotation axes.

Two or all three of these mechanisms can occur simultaneously, and the resulting structures can be either ordered, or statically or dynamically disordered. The symmetry of such a distorted perovskite structure can be cubic, tetragonal, rhombohedral, or octahedral, down to triclinic subgroups of $Pm\bar{3}m$.^[19]

Such distortions can be caused by changes in temperature or pressure, or by the choice of the chemical species in the sites $A^{[12]}$, $G^{[6]}$, and **X**. Stoichiometric perovskites of the general formula $A^I G^{II} X_3^{-I}$, $A^{II} G^I X_3^{-I}$, $A^I G^V X_3^{-II}$, $A^{II} G^{IV} X_3^{-II}$, and $A^{III} G^{III} X_3^{-II}$ are known, in which the **A** and **G** cations and the **X** anions can vary over a wide range in relation to their size. $ThTaN_3$,^[20] a nitridoperovskite with the general formula $A^{IV} G^V X_3^{-III}$, and the oxonitridoperovskites $LaWO_{0.6}N_{2.4}$ and $BaTaO_2N$ ^[21] have also been synthesized. The ordered distribution of two different cations **A** and **A'** or/and **G** and **G'** on the **A** or **G** sites often leads to a multiplication of the volume of the unit cell of the ideal perovskite structure, for example to a tetragonal or cubic $2 \times 2 \times 2$ superstructure cell in $(CaFe)-Ti_2O_6$ or $(CaCu_3)Ge_4O_{12}$.^[22] In connection with the cerium oxonitridosilicate^[5] described by Köllisch and Schnick, it is worth mentioning that stoichiometric perovskites with the composition $CaSiO_3$ ^[23] and $MgSiO_3$ ^[24] have been synthesized under high pressure and structurally analyzed.

Nonstoichiometric Perovskites (Defect Perovskites)

If the **A** or **G** site, or even both, are each occupied by cations of different valence, the valence balance is achieved by the vacancies in the **A** and/or **X** sublattice, and more rarely in the **G** sublattice. Many AGO_3 phases are known in which the element in the **G** position, such as Nb, Ta, Ti, Fe, Mn, Cr, or W, can take on different oxidation states relatively easily. In some cases, it can be extremely difficult or even impossible to synthesize stoichiometric AGO_3 perovskite phases with such cations. In the ceramic and geosciences literature, composi-

tions are very often given as AGO_x or A_xGO_3 , since it is not always known how the valence balance has been achieved. With the cubic tungsten bronzes A_xWO_3 , for example, x can be as low as $x = 0$, so that WO_3 and the isotopic ReO_3 can be considered as defect perovskites with a maximum concentration of vacancies in the **A** sublattice. The **G** position can also be occupied by atoms of different chemical elements with differing valencies, such as in $Ca_2AlSiO_{2.5}$.^[25]

If the **G** positions are occupied by smaller cations, such as Si, which have a strong preference for tetrahedral coordination, then the perovskite type can even “cope” with the substitution of $[GO_6]$ octahedra with $[TO_4]$ tetrahedra. This is accompanied by the appearance of vacancies in the anion sublattice. In this way, the calcium sodium and europium sodium silicates $Na_2Ca_2(SiO_3)_3$, $Na_2Ca(SiO_3)_2$, $Na_2Eu(SiO_3)_2$, and $Na_4Ca(SiO_3)_3$, which have been described as cyclosilicates with rings of six and twelve $[SiO_4]$ tetrahedra, respectively,^[26] can be considered as defect perovskites $[(Na,Ca)_{6\Box}]_A [(Na,Ca)_2^{[6]}Si_6^{[4]}]_G [O_{18\Box}]_X$, $[(Na,Ca)_{7\Box}]_A [(Na,Ca)_2^{[6]}Si_6^{[4]}]_G [O_{18\Box}]_X$, and $[(Na,Eu)_{7\Box}]_A [(Na,Eu)_2^{[6]}Si_6^{[4]}]_G [O_{18\Box}]_X$ with a rhombohedral $2 \times 2 \times 2$ superstructure or $[(Na,Ca)_8]_A [(Na,Ca)_{1.92}^{[6]}Si_6^{[4]}]_{\Box 0.08}]_G [O_{18\Box}]_X$ with a cubic $4 \times 4 \times 4$ superstructure. The many minerals of the lovozerite group^[27] also belong to this homeotypic group of silicates. It is apparent that the phase described by Köllisch and Schnick,^[5] $[Ce_{16}]_A [Si_6^{[6]}Si_{14\Box}^{[4]}]_G [O_{6-N_{32\Box 10}}]_X$, also belongs to this group of defect perovskites.

The decreasing degree of structural similarity to the ideal perovskite type of the derivatives described here confirms that the description of the cerium compound as a defect perovskite^[5] is justified. The large variability of the perovskite type can also be seen as an expression of its strong stability. This stability does decrease with decreasing degree of structural similarity, but still provides a contribution to the stability of such complicated structures that should not be overlooked. This contribution could be the reason that $Ce_{16}Si_{15}O_6N_{32}$, against all expectations, contains $[SiN_6]$ octahedra, even if they are only few in number (see above).

What significance does this stabilizing influence have to such a variable structure type as perovskite? A comparison of different perovskite derivatives shows that the cations on the **A** positions do not deviate (or at the most very slightly) from the highly symmetrical special positions of the ideal perovskite structure. The deviations of the cation positions of the **G** substructure are also very slight, even when the coordination number is reduced from six to four. Only the anion positions of the **X** substructure deviate, at times extensively, from the ideal, highly symmetrical positions. Therefore, one interpretation could be that the stability of the structure is primarily determined by the highly symmetrical potential distribution of the cations. The anions take over the function of shielding the cations from each other, so that their contribution to the stabilization is also considerable, even when the arrangement deviates strongly from the special positions in ideal perovskite.

One indication that this very simplified interpretation is reasonable is given by the investigations by Vegas et al.^[28] They established, with the help of statistical analysis, that the topology of the cations in the spinels $G_2^{[6]}T^{[4]}O_4$, the delafossites $Q^{[2]}G^{[6]}O_2$, and the bixbyites $G_2^{[6]}O_3$ resembles the

[*] The aristotype of a structure type has the highest possible space group symmetry. Structure-type variants that are derived from the aristotype by reduction of its symmetry to a subgroup symmetry are designated hettotypes.

topology of the corresponding metal, and even that the distances between the cations are very similar to the distances in the relevant metal structures. Borides of the types AB_2 , AB_4 , and AB_6 show similar behavior.^[29]

Outlook

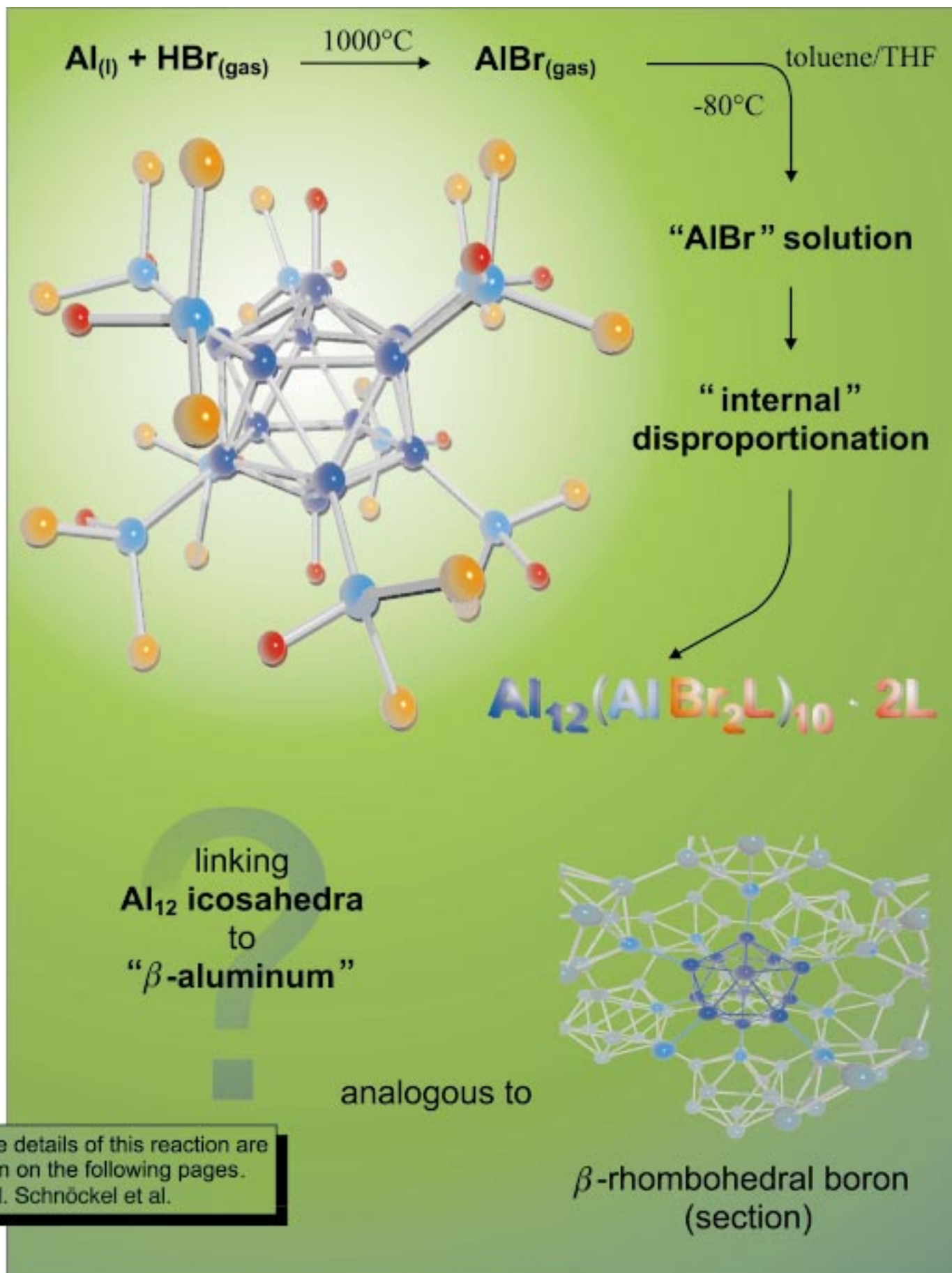
The cerium compound $\text{Ce}_{16}\text{Si}_{15}\text{O}_6\text{N}_{32}$ described by Köllisch and Schnick^[5] is no unimportant exotic from the rarities collection of chemical fundamental research. On the contrary, it encourages conclusions to be drawn of wide-ranging crystal chemical significance. Its high concentration of vacancies in the anion sublattice leads to the expectation of high ionic conductivity. The close relationship to other defect perovskites encourages the targeted synthesis of new materials with interesting electrical, magnetic, and optical properties.

It is unlikely that oxonitrido- or nitridosilicates will occur as minerals in the crust and in the upper mantle of the earth owing to the high oxygen fugacity. However, they could possibly exist in further depths of the earth, on planets and planetlike bodies with low oxygen but high nitrogen fugacity, or in interstellar space. The discovery of the mineral sinoite ($\text{Si}_2\text{N}_2\text{O}$) in meteorites and in cosmic dust^[30] could serve as the first example.

German version: *Angew. Chem.* **1999**, *111*, 1845–1850

Keywords: cerium • nonstoichiometric compounds • perovskite phases • silicates • solid-state structures

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Al₂₂Br₂₀ · 12 THF: The First Polyhedral Aluminum Subhalide—A Step on the Path to a New Modification of Aluminum?*

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For a long time polyhedral compounds were only known for the element boron in Group 13 (the triels (E)). Large polyhedral clusters such as B₂₀H₂₆^[1] are accessible by pyrolysis of smaller boron compounds. However, so far no neutral species with a *closo* framework^[2] has been found for the binary boranes. In contrast, for the boron halides several examples for this type of framework with its regular deltahedron exist, although according to the Wade's rules^[18] one would expect a *precloso* structure. For instance, B₄Cl₄, B₈Cl₈, and recently B₉X₉ (X = Cl, Br, I)^[3, 4] have been structurally determined. However, for B₁₂Cl₁₂ only the preparation by disproportionation of B₂Cl₄ is known,^[5] and the icosahedral B₁₂F₁₂^{2−}^[6] exists as a dianion. Thus, no neutral icosahedral species has been structurally determined so far. Since B₁₂ icosahedra are present in *all* boron modifications as structural elements, the neutral icosahedral boron clusters, in particular, should be important intermediates in the transition from the molecule to the solid state.

For the heavy triels Al, Ga, In, and Tl polyhedral compounds have only been known for a short time. In the last ten years the use of stabilizing ligands has enabled the synthesis of polyhedral E^I compounds, whose first structurally characterized examples were [AlCp*]₄^[7] (Cp* = C₅Me₅), [GaC(SiMe₃)₃]₄^[8], [InCp*]₆^[9] and [TlC(SiMe₃)₃]₄^[10]. In the meantime a large number of such polyhedral compounds has been characterized;^[11] however, hitherto there were no polyhedra with halogen substituents analogous to the boron halides. Although tetrameric halides of monovalent aluminum can be prepared by cocondensation of the high-temperature particle AlX (X = Br, I) with NEt₃ or PET₃ as the donor and toluene as the solvent (e.g. [AlBr · NEt₃]₄^[12]), as a result of the high-donor strength of triethylamine and triethylphosphane, respectively, a planar Al four-membered ring with isolated 2e–2c bonds is formed instead of an Al₄ tetrahedron. Consequently, the application of weaker donors for the cocondensation should primarily favor the formation of polyhedral electron-deficient compounds. However, under such conditions the disproportionation of the metastable

aluminum monohalide solution to give AlX₃ and elemental aluminum is also favored.

Herein, we report on the cocondensation of AlBr with toluene and THF as donor. This weaker donor with low steric requirements apparently allows the formation of Al multi-center bonds while still preventing the disproportionation to elemental aluminum to a great extent so that isolable intermediates are to be expected. Extremely sensitive pale yellow crystals of the composition Al₂₂Br₂₀ · 12 THF (**1**) were isolated from the solution heated to room temperature. They are mechanically labile and decompose on removal of the solvent or in anhydrous perfluoropolyether (at room temperature within a minute) to give a white amorphous solid. The result of the X-ray structural analysis^[13] (Figure 1) shows that

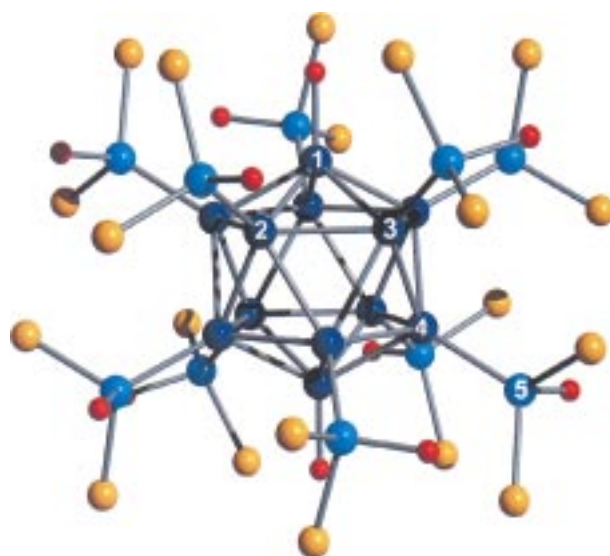


Figure 1. Molecular structure of **1** (C and H atoms are omitted for clarity). Dark blue: Al atoms of icosahedral core; light blue: Al atoms of AlBr₂ ligands; orange: Br atoms; red: O atoms of THF molecules. Selected distances [Å] (average ± maximal deviation determined from analogous bonds; results of the DFT calculations in parentheses): Al1–Al2 2.650 ± 0.023 (2.615), Al2–Al3 2.762 ± 0.005 (2.824), Al3–Al4 2.692 ± 0.016 (2.684), Al4–Al5 2.526 ± 0.019 (2.541), Al5–O1 1.892 ± 0.038 (1.937), Al5–Br1 2.299 ± 0.039; for comparison: selected Al–Al distances in other Al compounds (see text): [Al(Bu)₃]₂^{2−}: 2.670–2.696, [AlCp*]₄: 2.769, [AlBr₂ · OMePh]₂: 2.530, metal: 2.860.

this structure is better described by the formula Al₁₂[AlBr₂ · THF]₁₀ · 2 THF. The central structural element is a compressed Al₁₂ icosahedron. This is the second example of a molecular icosahedral unit for the heavy elements of Group 13; several years ago Uhl et al.^[14] prepared an [Al(Bu)₃]₁₂^{2−} ion. To our knowledge **1** is the only neutral molecular icosahedral cluster compound outside boron chemistry. The Al–Al distances lie in the expected range for subvalent compounds (see legend for Figure 1).

AlBr₂ units are bonded to ten of the twelve Al atoms of the icosahedron. As shown in Figure 2 the 20 bromine atoms are arranged above the triangular faces of the icosahedron in such a way that they adopt the geometry of a distorted pentagonal dodecahedron;^[15] a THF molecule (omitted for clarity in Figure 2) protrudes from the center of each of these faces. Therefore, two *para*-positioned Al atoms of the icosahedron

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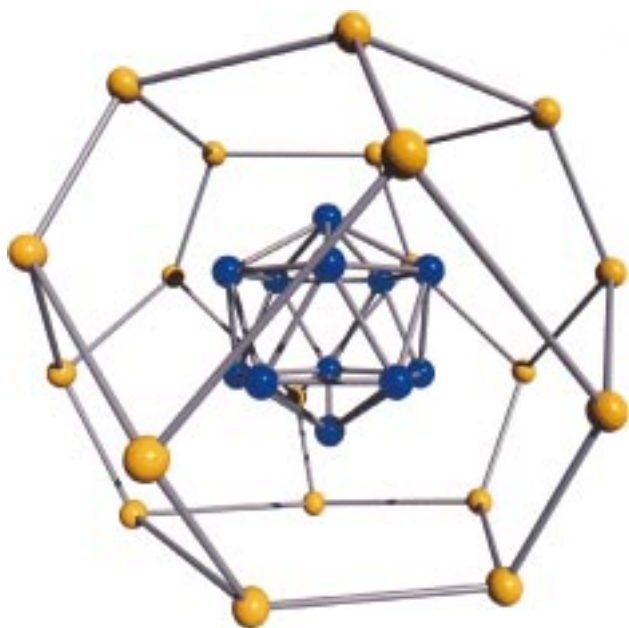


Figure 2. Pentagonal dodecahedron of the Br atoms around the Al icosahedron. The Br–Br distances lie in the range of the sum of the van der Waals radii of 3.80 Å. Color code see Figure 1.

are not bound to AlBr_2 units but are each only coordinated by a THF molecule. Thus, in analogy to the 1,12-carboranes an icosahedral framework is formed which is compressed in 1-12 direction. A compound topologically comparable with **1** was reported by Hawthorne:^[16] deca(dichloromethyl)-*para*-carborane,^[17] which, owing to the good steric shielding of the icosahedral framework by its substituents, was described as a „camouflaged carborane“. As shown in the space-filling model in Figure 3 the subvalent Al atoms in **1** are also completely surrounded by a closed shell of Br atoms and the methylene groups of the THF molecules.

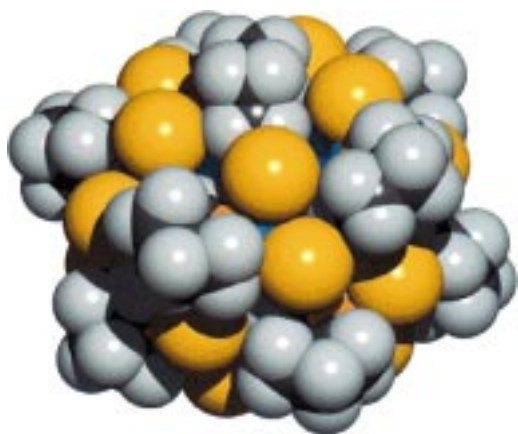


Figure 3. Space-filling model of **1** based on the van der Waals radii. The Al_{12} cluster is completely surrounded by dodecahedrally arranged Br atoms and icosahedrally arranged THF molecules. In the crystal the molecules adopt an almost hexagonal primitive arrangement. Disordered solvent (toluene) is found in the spaces. Color code see Figure 1.

The bonding in **1** can be interpreted according to Wade's rules.^[18] Owing to the coordinated THF molecules the two *para*-positioned Al atoms of the icosahedron can each provide the polyhedral framework with three electrons, whereas the

remaining ten icosahedron Al atoms can only each provide two electrons because of their bond to the AlBr_2 groups. This results in 26 framework electrons ($=2n+2$ with $n=12$) for the bonding in the Al_{12} polyhedron; that is in accordance with the experiment the electron balance indicates the formation of a molecule of the *closo*-type (icosahedron). Thus, the discussion on three-dimensional aromaticity, for example, for *closo*-polyhedra of boron, should probably be extended to aluminum.^[19, 20]

Under formal considerations on the oxidation states in the Al_{22} group the atoms of the icosahedral unit have the oxidation state 0, whereas the remaining ten atoms have the oxidation state +2, that is in total this results in the mean oxidation state of 0.91. Thus, this aluminum subhalide can be considered as the product of an *internal* disproportionation of 24 AlBr units with formal elimination of $[\text{AlBr}_2]_2$.^[21]

For a better understanding of **1** density functional theory (DFT) calculations were carried out on the analogous but simpler model system $\text{Al}_{22}\text{Cl}_{20} \cdot 12 \text{H}_2\text{O}$ (**1a**).^[22] The calculated Al–Al distances are shown in parentheses in the caption of Figure 1 and are in good agreement with the measured values. Furthermore, Figure 4 shows a comparison of the calculated

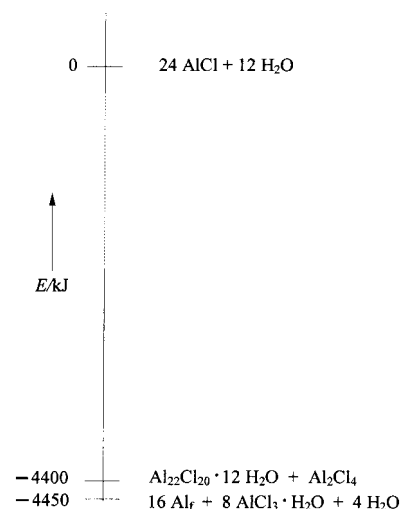


Figure 4. Energy diagram for the position of $\text{Al}_{22}\text{Cl}_{20} \cdot 12 \text{H}_2\text{O}$ (**1a**) relative to the starting material AlCl and the thermodynamic end products Al and AlCl_3 .

energetic position of this model system (**1a** + Al_2Cl_4) with the energetic positions of the corresponding starting materials ($24 \text{AlCl} + 12 \text{H}_2\text{O}$) as well as with the thermodynamically most stable final products ($16 \text{Al} + 8 \text{AlCl}_3 \cdot \text{H}_2\text{O} + 4 \text{H}_2\text{O}$): During the formation of **1a** almost the complete disproportionation energy is liberated.^[23] However, in the light of the polyhedral structure of **1**, it is questionable whether **1a** and **1** can be interpreted as intermediates of the disproportionation to AlX_3 and aluminum metal.

An important motivational factor for the research on clusters are findings about the change in material properties during the transition from the molecule to the solid state. The $\text{Al}_{77}\text{R}_{20}^{2-}$ cluster ($\text{R}=\text{N}(\text{SiMe}_3)_2$)^[24] is the largest metal cluster so far, whose structure has been determined by X-ray diffraction. It consists of a central Al atom, which is surrounded by a series of shells, first by 12 Al atoms, arranged

in the form of a distorted icosahedron, then by 44 Al atoms, and finally by a further 20 Al^{II}R units. Since the icosahedral Al₁₂ shell is distorted almost to the extent that it is a cuboctahedron, the twelve-coordinate central atom is already situated in an environment that is very reminiscent to that in cubic closest packed α -aluminum.

In contrast, compound **1** as the second largest aluminum cluster and as the largest neutral aluminum cluster shows a fundamental structural difference in the Al framework, because in this case the central Al atom is missing. Owing to the reactive Br substituents in the cluster shell further reactions resulting in the growth of the aluminum framework should be possible under comparably mild conditions. Finally, a further disproportionation and/or dehalogenation could lead to the formation of a solid-state structure. As long as no Al atom is present in the center of these icosahedral frameworks the linkage of the icosahedra would be more likely to result in a solid which is structurally more similar to boron and probably nonmetallic. Thus, the Al₂₂ unit already is a structurally analogous section from the structure of the β -rhombohedral modification of boron and is unique with regard to the ten M⁰–M^{II} bonds. Even in boron chemistry so far there has not been an intermediate such as a hypothetical B₁₂B₁₀R₂₀ molecule as the link between molecular B₁₂ icosahedra and the linked icosahedra in solid boron. It therefore appears plausible to interpret the formation of **1** as a first step in the direction of a new modification of aluminum.

Experimental Section

AlBr (40 mmol) was cocondensed with toluene (100 mL) and THF (15 mL) according to the method described in reference [11a]. A portion (20 mL) of the dark red solution (ca. 0.37 M) was concentrated to about half the volume in the cold and thus freed to a large extent from the donor THF. After several days at room temperature a slightly amorphous precipitate of aluminum occurred. From the further concentrated filtrate, **1** began to crystallize after several days in the form of pale yellow, hexagonal plates, which were mechanically and chemically highly labile. In contrast, in solution **1** is stable to above 110 °C. The total yield after several reprecipitations was about 10%.

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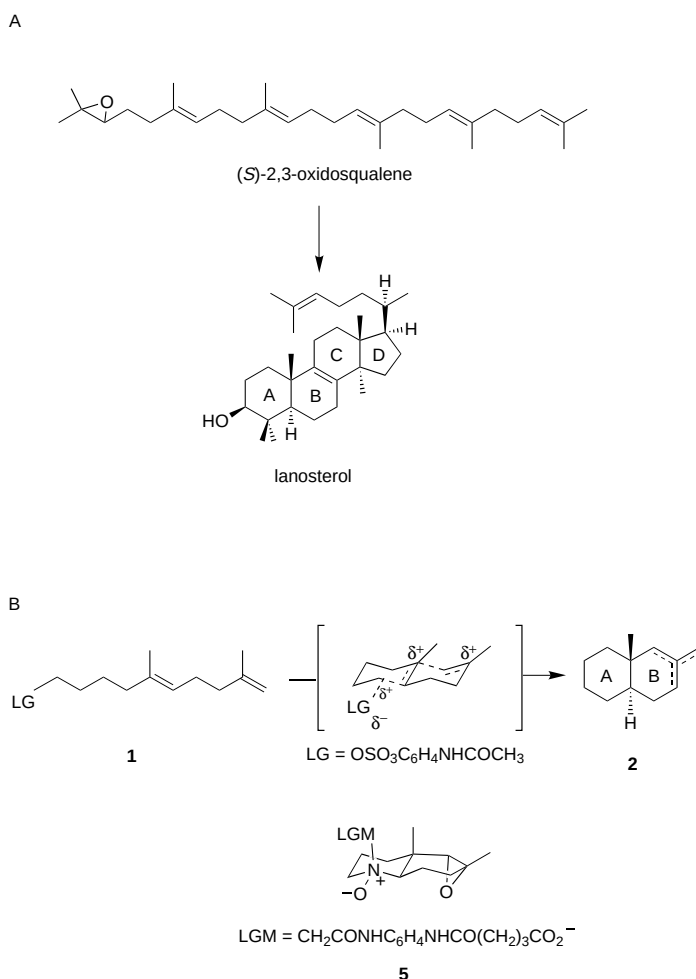
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Convergence of Catalytic Antibody and Terpene Cyclase Mechanisms: Polyene Cyclization Directed by Carbocation– π Interactions**

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Carbocation cyclization cascades catalyzed by terpenoid cyclases rank among the most important and the most complex carbon–carbon bond forming reactions in chemistry and biology.^[1, 2] For example, consider the pathway of

cholesterol biosynthesis in which the open-chain polyene squalene oxide is cyclized to yield the tetracyclic product lanosterol in a single chemical reaction catalyzed by lanosterol synthase (Scheme 1 A).^[2] More than one third of the carbon



Scheme 1. A) Cyclization catalyzed by lanosterol synthase. Lanosterol rings are labeled according to the standard steroid nomenclature. B) Polyene cyclization catalyzed by antibody HA5-19A4 yields a *trans*-decalin skeleton analogous to the lanosterol A and B rings. LGM = leaving group mimic plus carrier protein linker. For further information see the text.

atoms in the triterpene substrate undergo changes in bonding and/or hybridization during the cyclization cascade to yield a product containing seven precisely formed stereocenters. Remarkably, only 1 out of a possible 128 product stereoisomers is generated, so one key role of the cyclase is to mediate the structural and stereochemical precision of the cyclization reaction. Such precision is the hallmark of most terpenoid cyclases, which are responsible for the biosynthesis of myriad natural products in all forms of life.

Recent X-ray crystal structure determinations of terpenoid cyclases suggest an independent evolution of two classes of cyclases, yet members of each class exhibit convergent structural features important for catalysis.^[3] The active site cavities of each are nested deep within α -helical superstructures, where numerous hydrophobic residues help to sequester the linear polyene substrate from solvent and form

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a template to enforce the unique substrate conformation and stereochemistry required for cyclization. Furthermore, each active-site cavity contains aromatic residues that are poised to stabilize any carbocation cyclization intermediates (and their associated transition states) through cation– π interactions.^[4] Additional polar residues in each active site likely participate in electrostatic stabilization and govern the regiochemistry of the deprotonation and/or protonation steps required in the cyclization mechanism. Structural relationships within each class of terpenoid cyclases indicate divergence from the primordial class I and II cyclase ancestors early in the evolution of terpenoid biosynthetic pathways.^[3] The facile evolution of the ancestral terpenoid cyclase maximizes the potential product diversity that arises from a minimal polyene substrate pool.

Catalytic antibody technology^[5] provides an alternative approach that can be exploited to maximize the diversity of cyclization products starting from a polyene substrate. Importantly, catalytic antibody cyclases are not limited to a handful of naturally occurring polyene substrates, so their potential product diversity is even greater than that of naturally occurring terpenoid cyclases. Several antibodies have been generated to date that catalyze cationic cyclization reactions,^[6] and one in particular catalyzes a terpenoid-like cyclization reaction:^[7] antibody HA5-19A4 catalyzes the tandem cationic cyclization of polyene substrate **1** to form the bridge-methylated *trans*-decalin skeleton **2** (Scheme 1). This bicyclic product is analogous to that formed by the steroid A and B rings of lanosterol in the cyclization of squalene oxide (Scheme 1). The rate of the antibody-catalyzed reaction $k_{\text{cat}} = 0.021 \text{ min}^{-1}$ ^[7] means that the catalytic turnover approaches that measured for many terpenoid cyclases; the absolute rate enhancement over the uncatalyzed reaction rate is immeasurably large, as is typical for a terpenoid cyclase. Antibody HA5-19A4 was raised against hapten **5**, which partially mimics the productive chair–chair conformation required for polyene cyclization to the *trans*-decalin (Scheme 1). As part of a “bait-and-switch” strategy^[6–8] the hapten design also incorporated the *N*-oxide moiety, which was intended to elicit functional groups in the antibody combining site that would facilitate departure of the leaving group.

We have determined the X-ray crystal structure of the Fab fragment of the catalytic antibody HA5-19A4 complexed with hapten **5**. The structure of the complex shows that the bicyclic hapten is buried deep within the antibody combining site, and intermolecular interactions provide critical structural inferences on the mechanism of polyene cyclization (Figure 1). The antibody combining site is hydrophobic in nature with numerous aromatic residues in close proximity to the hapten and its linker. Together with the aliphatic residues in the antibody combining site the

aromatic residues provide for a highly complementary fit with the hapten. In accord with theories underlying hapten design^[7] the catalytic antibody serves as a precise template for catalysis: the active site has a specific contour that binds the polyene substrate in a productive chair–chair conformation, poised to proceed along the reaction coordinate of carbon–carbon bond formation once formation of the carbocation is triggered (Figure 2). This lowers the conformational entropic barrier to catalysis^[9] and accelerates the polyene cyclization reaction.

Hydrogen-bond interactions in the Fab–**5** complex provide the first confirmation of the “bait-and-switch” strategy for hapten design. This result highlights a subtle, yet important, point: not only can the structure of the hapten incorporate features anticipated in a catalytic transition state, but it can also include additional functional groups that will elicit complementary functional groups in the antibody combining

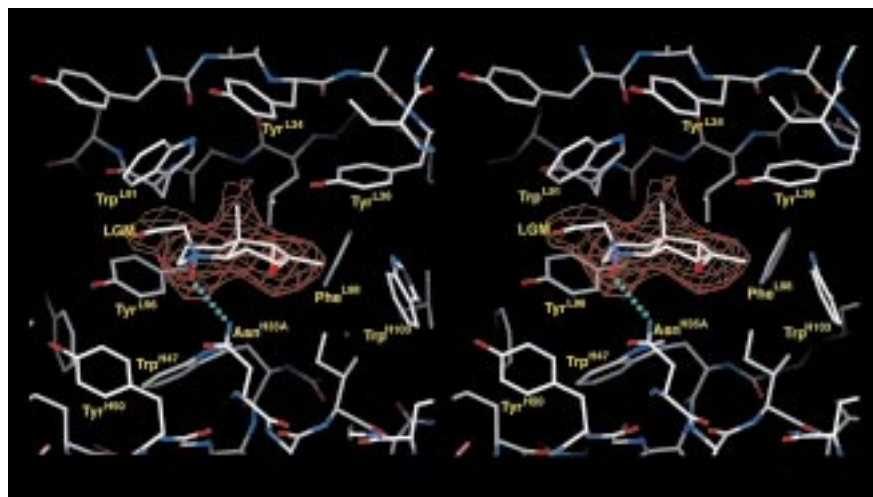


Figure 1. Top: C_{α} backbone of the variable region of the Fab, showing the location of the bound hapten.^[21] Bottom: Stereoview of the omit electron density map of the hapten (contoured at 3.4σ); numerous aromatic residues surround the hapten to form a highly complementary contour. For clarity only a portion of the leaving group mimic (LGM) is shown. A hydrogen bond between the oxygen atom of the *N*-oxide moiety and Asn^{H35A} (cyan dotted line) reflects the likely role of this residue in triggering the departure of the leaving group in catalysis.

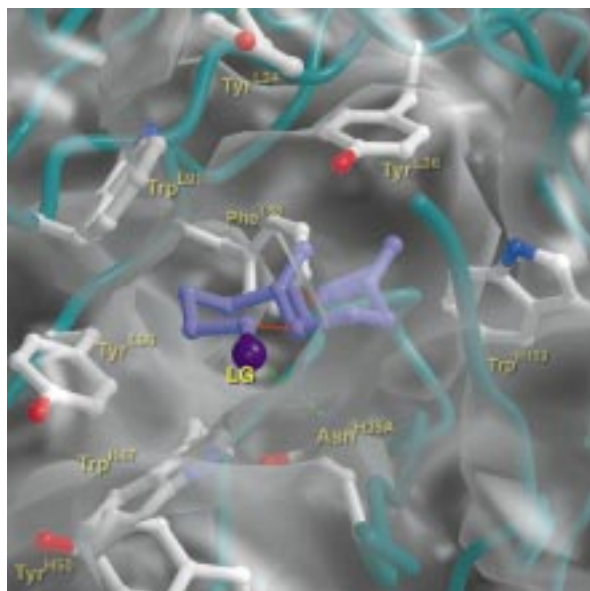


Figure 2. Model of polyene substrate **1** binding with the productive chair–chair conformation (lavender), based on the structure of the Fab-**5** complex; the reaction coordinates of the carbon–carbon bond formation are indicated by red dotted lines.^[21] Selected aromatic residues which contribute to the active site template for substrate binding are indicated. Additionally, Asn^{H35A}, which is believed to help trigger departure of the leaving group (LG) by hydrogen bonding with the sulfonate group of the substrate, is indicated (green dashed line; for clarity the leaving group is designated as a simple purple sphere).

site to participate in a specific chemical step of catalysis. The side chain of Asn^{H35A} donates a hydrogen bond to the negatively charged oxygen atom of the *N*-oxide moiety, and the positively charged nitrogen atom of the *N*-oxide moiety (which is isosteric with the substrate C1 atom) interacts with the π -electron clouds of Trp^{L91} and Tyr^{L96}, and to a lesser extent Tyr^{H50} (Figure 3A). Therefore, the initial formation of the carbocation is triggered by at least one hydrogen-bond interaction with the sulfonate leaving group and the electrostatic stabilization of the developing positive charge on C1 by aromatic π -electron clouds. A concerted attack of the C5–C6 π bond avoids any significant formation of an unfavorable primary carbonium ion at C1 and forms the 6-membered A ring of the *trans*-decalin, yielding an intermediate with a favorable tertiary carbonium ion at C5 (Scheme 2).^[7] Notably, the π -electron clouds of Tyr^{L36}, Trp^{L91}, and to a lesser extent Tyr^{L34}, are oriented for optimal electrostatic stabilization of the C5 carbocation (Figure 3A). This reaction sequence mimics the first step of the lanosterol synthase mechanism, where formation of the A ring of lanosterol occurs in concert with the opening of the epoxide ring of squalene oxide.^[10]

The next step in the catalytic antibody cyclase mechanism is attack of the C5 tertiary carbonium ion intermediate by the C9–C10 π bond to yield the B ring of the *trans*-decalin. The identification of minor side products **3a–c** and **4a,b**, which reflect an incomplete closure of the B ring (Scheme 2),^[7] suggests that this step most likely does not occur in concert with formation of the A ring. This is in accord with the cyclization pathway of tetracyclic triterpenes.^[2] The closure of the B ring yields an intermediate with a tertiary carbonium ion at C9, and the structure of the Fab-**5** complex reveals that

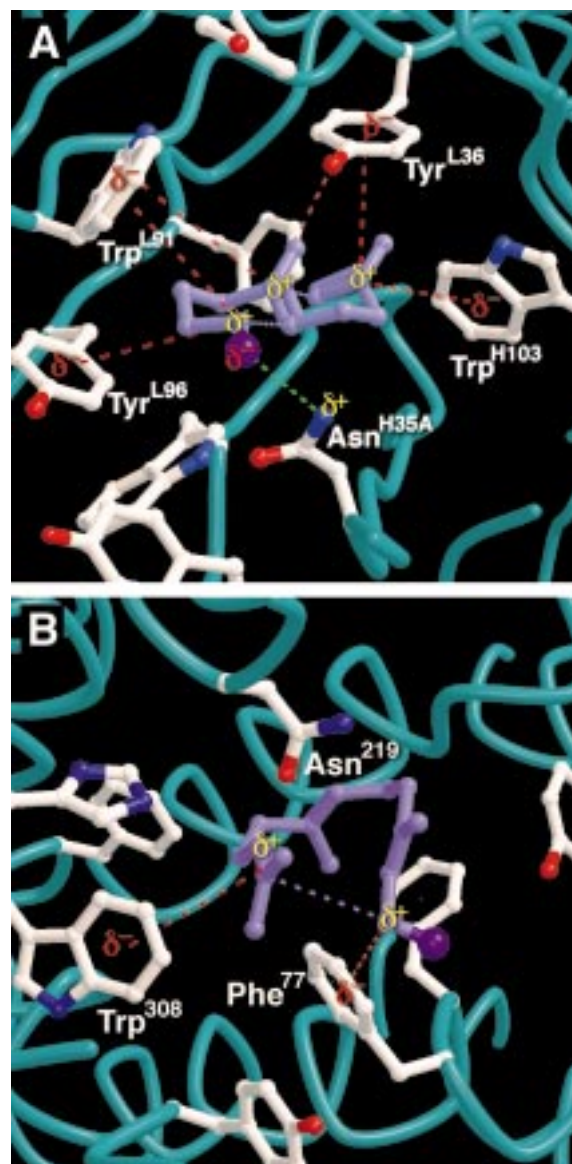
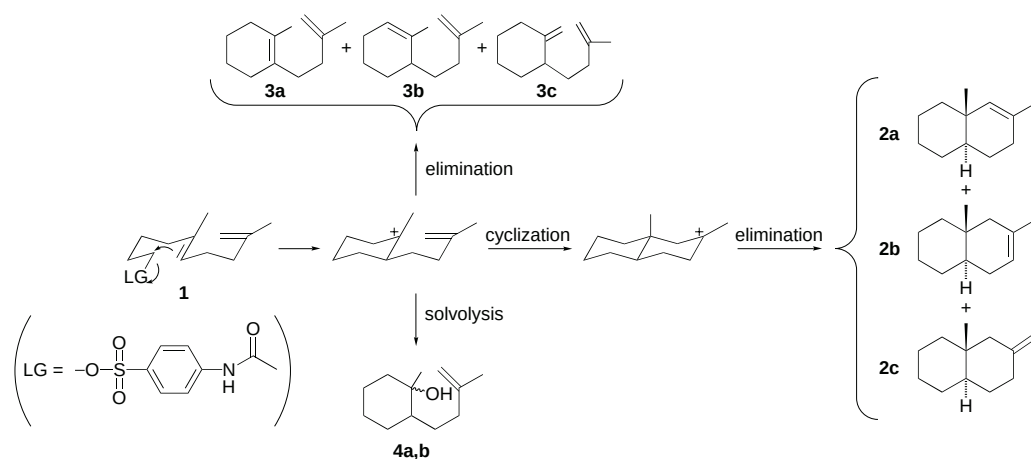


Figure 3. Comparison of A) catalytic antibody HA5-19A4 polyene cyclase and B) pentalenene synthase, which adopt β and α folds, respectively.^[21] The reaction coordinates of carbon–carbon bond formation are indicated by lavender dashed lines, and the leaving groups are symbolized by purple spheres, in the models of each enzyme–substrate complex. The departure of the sulfonate leaving group of polyene **1** appears to be triggered in part by a hydrogen bond interaction with Asn^{H35A} in the catalytic antibody (A); metal coordination triggers the departure of the diphosphate leaving group of farnesyl diphosphate in the pentalenene synthase reaction (B). Aromatic residues in each cyclase active site stabilize sites of developing positive charge by electrostatic interactions with aromatic π -electron clouds (red dashed lines). Additional aromatic residues in each active site help define the active site template that enforces the productive binding conformation of the flexible polyene substrate.

the π -electron clouds of Tyr^{L36} and Trp^{H103} are appropriately positioned to stabilize the C9 carbocation (Figure 3A). The fact that the tandem cationic cyclization yields only the *trans*-decalin skeleton **2** indicates that the antibody functions effectively as a chaperone that governs the substrate and intermediate conformations throughout the cyclization cascade to yield a product with exclusive and pre-designed stereochemistry. It is striking that such a broad constellation



Scheme 2. The mechanism of polyene **1** cyclization catalyzed by antibody HA5-19A4 yields *trans*-decalin regioisomers **2a–c** as the major products. For further information see the text.

of aromatic residues in the active site are appropriately oriented for electrostatic stabilization of developing positive charges at the C1, C5, and C9 atoms of the polyene substrate (Figure 3 A). In addition to the aromatic residues that interact directly with these substrate atoms, other aromatic residues line the antibody combining site and possibly contribute some weak, long-range stabilization to the carbocation intermediates. The stabilization of carbocation intermediates by point charges is a strategy known to accelerate polyene cyclization reactions.^[11] The stabilization achieved by multiple partial charges should likewise accelerate polyene cyclization reactions, which has been postulated for naturally occurring terpenoid cyclases.^[3] Thus, the “unnaturally evolved” antibody cyclase and the naturally evolved terpenoid cyclases have converged to identical strategies for managing and manipulating carbocations in complex cyclization cascades.

A carbocation cyclization cascade is terminated by the addition of a nucleophile (for example, solvent) or elimination of a proton. As part of the “bait-and-switch” hapten design strategy, an oxirane moiety was incorporated into hapten **5** to elicit antibody residues that would facilitate one of two possible termination routes:^[12] a) the epoxide oxygen atom could elicit a polar residue to interact with a water molecule that would terminate the C9 carbocation to form an alcoholic product, or b) the 3-membered epoxide ring fixes the B ring of the hapten in a half-chair conformation that could favor proton elimination from the C9 carbocation to form olefinic products with the corresponding half-chair conformations of the B ring. The latter termination route is favored, since olefinic products are predominantly observed.^[7] However, the analysis of the olefinic products enabled a mixture of the three possible elimination products to be identified (the closely related regioisomeric *trans*-decalins **2a–c**) present in ratios that reflected their expected thermodynamic stabilities (Scheme 2).^[7] The Fab structure reveals that there is no catalytic base to direct the regiochemistry of this final elimination step to yield one exclusive olefin.

The lack of an appropriate basic residue in Fab HA5-19A4 to terminate the carbocation cyclization cascade is reminiscent of the triterpene cyclase squalene–hopene cyclase

obtained from *Alicyclobacillus acidocaldarius*, which generates the pentacyclic products hopene (90 %, which arises from proton elimination) and hopan-22-ol (10 %, which arises from addition of a solvent nucleophile).^[13] As with the catalytic antibody cyclase the lack of an appropriate catalytic base in squalene–hopene cyclase results in a mixture of termination products. However, it should be noted that other terpenoid cyclases such as pentalenene synthase and epi-aristolochene synthase do contain active site residues that appear to mediate the regiochemistry of proton transfer steps,^[3] so it is likely that such residues can be engineered into any cyclase active site—either by nature or by design.

In closing, it is instructive to compare the general features of convergent evolution in the active sites of the catalytic antibody cyclase HA5-19A4 and pentalenene synthase,^[3] a bacterial terpenoid cyclase (Figure 3). Each cyclase exhibits a remarkably different protein fold: the antibody adopts the β fold of an immunoglobulin, and the terpenoid cyclase adopts an α fold. However, each cyclase has a deep, hydrophobic active-site cavity flanked by multiple loops that sequester highly reactive carbocation intermediates from solvent. Additionally, each cyclase contains numerous aromatic residues that, in addition to forming a template to bind the productive conformation of a flexible polyene substrate, stabilize carbocation intermediates by electrostatic interactions with their π -electron clouds. This comprises a highly effective chemical strategy for catalysis:^[4] electrostatic stabilization can be achieved without risk of quenching the carbocation and annihilating the protein catalyst if stabilization were achieved with a more polar protein atom. Remarkably, the naturally occurring terpenoid cyclase required millions of years to evolve to this mechanistic strategy, whereas the catalytic antibody required only weeks—the acceleration of convergent evolution highlights the fundamental appeal of catalytic antibody technology. Further exploration and optimization of catalytic antibody HA5-19A4 promises to yield further insight on carbocation cyclization reactions with diverse polyene substrates to yield novel product arrays far beyond the limits of those attainable with naturally occurring terpenoid cyclases.

Experimental Section

Fab HA5-19A4 was prepared by digestion of IgG HA5-19A4 with papain according to a standard protocol (Pierce No. 44885). The IgG was obtained from a standard hybridoma protocol described in the supporting information of Hasserodt and colleagues.^[6] For sequence determination the total RNA from 1×10^7 hybridoma cells was prepared with Tri Reagent according to the manufacturer's protocol (Molecular Research Center, Inc., Cincinnati, OH). Reverse transcription and PCR amplification of the light-chain and Fd-fragment sequences were performed as described.^[14] The primer 5'-GAYGTNCARCTCGAGGAGTCAGGACCT-3' was used to amplify the 5' end of the Fd-fragment sequence. Light-chain and Fd-fragment sequences were digested with *SacI/XbaI* and *XhoI/SpeI*, respectively, and ligated into the phagemid vector pComb3H.^[15] After electrotransformation into XL1-Blue *E. coli* cells, clones were randomly selected for protein expression induced with isopropyl- β -D-thiogalactopyranoside.^[16] Fab specific to HA5-BSA antigen was detected by enzyme-linked immunosorbent assay (ELISA) by using goat anti-mouse F(ab')₂ (Pierce) conjugated to alkaline phosphatase as a secondary antibody. Plasmid DNA from ELISA-positive clones was sequenced to determine the light-chain and Fd-fragment sequences.

Crystals of the HA5-19A4 Fab–5 complex were obtained by equilibrating 5 μ L of protein solution (11.4 mg mL⁻¹ Fab, 10 mM CdCl₂, 10 mM 5, 50 mM Tris (pH 7.0)) with 5 μ L of precipitant buffer (30% polyethylene glycol (*M_w* = 6000), 100 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulphonic acid (HEPES; pH 7.9)) in a hanging drop suspended over a 1 mL reservoir of precipitant buffer at room temperature. Small rectangular plates appeared within three days. These crystals diffracted X-rays with a resolution of 2.7 Å (93.5% complete, *R_{merge}* = 0.088). Crystal dimensions 0.6 mm $\times$ 0.1 mm $\times$ 0.05 mm, space group C2, *a* = 101.3, *b* = 70.3, *c* = 69.6 Å, β = 114.2°, one molecule in the asymmetric unit. The structure was solved by molecular replacement by using the atomic coordinates^[17] of Fab D2.3 to construct a polyalanine probe for rotation and translation function calculations with AmoRe.^[18] Refinement and rebuilding of the model were performed with X-PLOR^[19] and O;^[20] refinement converged smoothly to a final crystallographic *R* factor of 0.165 (*R_{free}* = 0.257). Two Cd²⁺ binding sites were identified: one between Glu^{L187} and Glu^{L213}, and a second between Asp^{H173} and Glu^{L81} of symmetry-related molecules in the crystal lattice. The final model has excellent stereochemistry with rms deviations from ideal bond lengths and angles of 0.011 Å and 1.7°, respectively. The Ramachandran plot shows that 318, 49, and 3 residues adopt the most favored, additionally allowed, and generously allowed backbone conformations, respectively; only 4 residues adopt disallowed backbone conformations, and these are found in poorly characterized loop regions. Atomic coordinates of the Fab–5 complex have been deposited in the Brookhaven Protein Data Bank (<http://www2.ebi.ac.uk/pdb>) with accession code 1CF8.

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A Polycationic Metallodendrimer with 24 [Fe(η^5 -C₅Me₅)(η^6 -N-Alkylaniline)]⁺ Termini That Recognizes Chloride and Bromide Anions

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Dendrimers^[1] are the first well-defined, monodisperse synthetic macromolecules that should be able to achieve various supramolecular functions.^[2–7] Several applications as, for example, antennas,^[3] boxes,^[4] and catalysts^[5] have already

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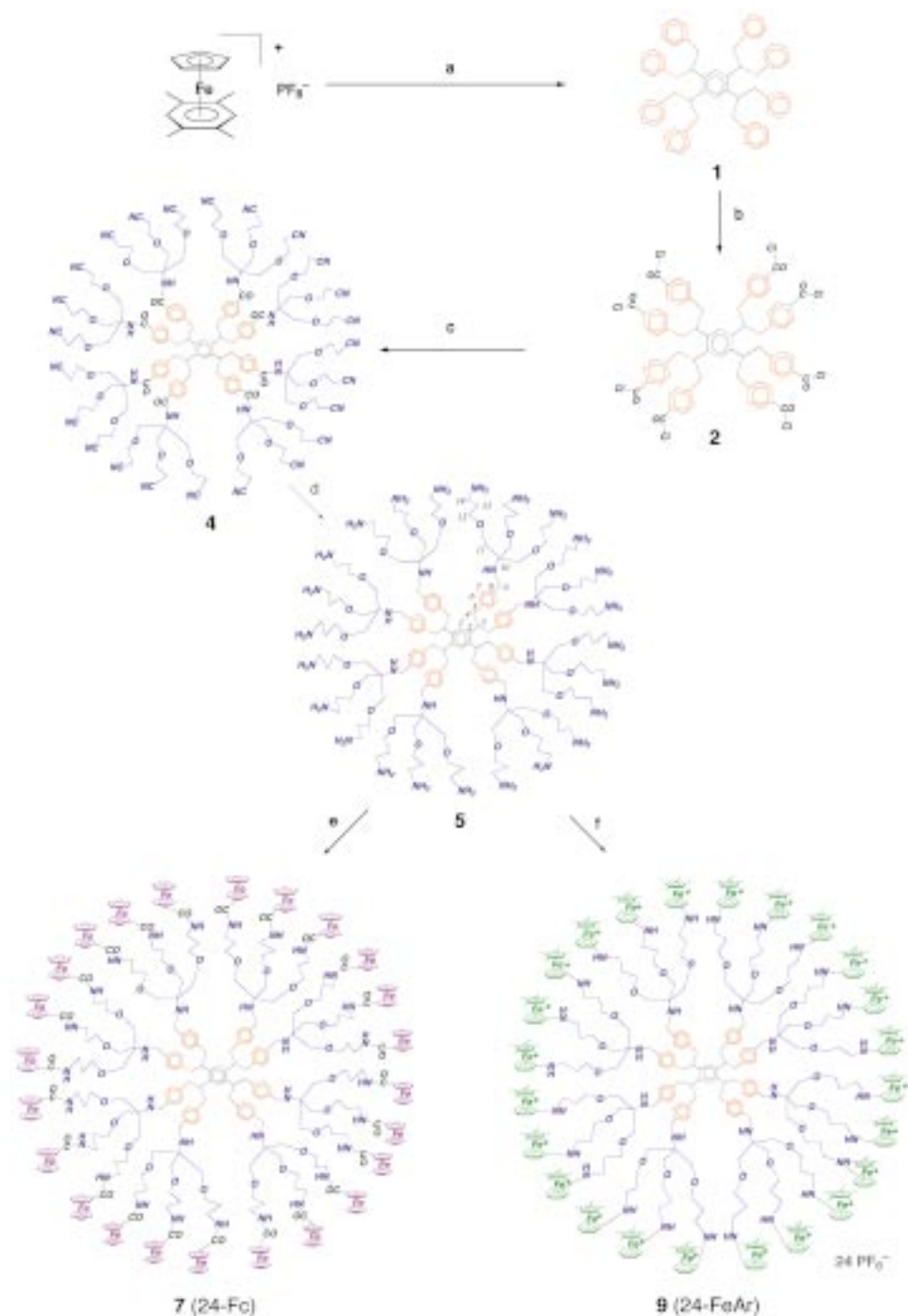
Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

been highlighted. Molecular recognition, including that of anions, is a function directly related to supramolecular chemistry,^[2] and has been studied using *endo* receptors.^[6–19] For instance, Lehn has recently reported hosts which are specific for chloride,^[19] and anion recognition is becoming more and more important for biological and environmental purposes.^[10] The design and investigation of new types of receptors is thus timely, especially in view of the multiple properties of dendrimers.^[1] Reports on anion sensing by Tomalia could find useful extensions in drug transport and gene therapy.^[20, 21] Indeed, dendrimers resemble viruses in view of the fractality of their surfaces and can consequently be considered as *exo* receptors.

The recognition of oxo anions by 9- and 18-amidoferrrocene dendrimers was recently determined with electrochemistry,^[22] and other polyferrocene dendrimers were shown to be promising biosensors.^[24, 25] The recognition abilities of such polyamidoferrrocene dendrimers could not be observed by NMR spectroscopy, except for H_2PO_4^- , because the neutral diamagnetic 18-electron form does not usually interact efficiently with anions. Recently, Beer et al. have shown with NMR spectroscopy that some neutral ferrocenoyl receptors can recognize anions.^[10c] Electrochemistry was the method of choice for studying oxo anions because the anodically generated cationic ferricinium form strongly interacts electrostatically with the anions; this interaction operates in synergy with $\text{X}^- \cdots \text{HN}$ bonding. Halides could not be recognized with these polyferrocene dendrimers, however, as they interact only weakly with the ferrocene moieties, even the cationic amidoferricinium forms.^[22]

We report here the synthesis of a new polycationic metallodendrimer, and present ^1H NMR results for its use as an *exo* receptor for the selective recognition of chloride and bromide anions. We also compare the remarkable recognition ability of this metallodendrimer, which is polycationic in its 18-electron form, to that of a polyamidoferrrocene dendrimer of comparable topology, whose metal moieties are neutral in their 18-electron forms.

To improve the selectivity in the recognition of various anions, we have chosen new, large cationic metal–sandwich termini with bulky C_5Me_5 ligands and a new type of core. The construction of the dendritic core is based on a regioselective chlorocarbonylation in the *para* position of the octabenzyl core **1** (68% yield of **2**), which is easily available by CpFe^+ -induced octabenzylation of 1,2,4,5-tetramethylbenzene (durene; Scheme 1).^[26] Reaction of fresh samples of Newkome's tripodal amine $\text{NH}_2\text{C}(\text{CH}_2\text{OCH}_2\text{CH}_2\text{CN})_3$ (**3**)^[27] with **2** at 20 °C in CH_2Cl_2 with NEt_3 gave the 24-nitrile dendrimer **4**



Scheme 1. a) PhCH_2Br , KOH , DME , 2 d, RT; b) $(\text{COCl})_2$, AlCl_3 , CH_2Cl_2 , 15 °C, 1 h; c) $\text{NH}_2\text{C}(\text{CH}_2\text{OCH}_2\text{CH}_2\text{CN})_3$, NEt_3 , CH_2Cl_2 , 3 d, RT; d) $\text{BH}_3 \cdot \text{Me}_2\text{S}$, THF , RT, 1 d; e) $[\text{FeCp}^*(\eta^6\text{-C}_6\text{H}_5\text{F})][\text{PF}_6]$ (**6**), CH_2Cl_2 , 4 d; f) $[\text{FeCp}^*(\eta^6\text{-C}_6\text{H}_5\text{F})][\text{PF}_6]$ (**8**), CH_2Cl_2 , RT, 15 d.

(61 % yield after chromatographic purification on neutral alumina with dichloromethane as eluent). This dendrimer was characterized by its molecular peak (m/z 3328.57 [$M+Na^+$]) in the MALDI-TOF mass spectrum (MALDI-TOF = matrix-assisted laser desorption/ionization time-of-flight).

Reduction of the 24 nitrile and 8 carbonyl groups of **4** with $BH_3 \cdot SME_2$ (THF, 20 °C) followed by methanolysis gave the 24-amino dendrimer **5** (90 % yield) as a white, hygroscopic, water-soluble powder. Nucleophilic substitution of chloride by amine was known for $[Fe(\eta^5-C_5H_5)(\eta^6-C_6H_5Cl)]^+$,^[28] but initial attempts with this substrate showed that the sandwich termini were relatively fragile. The use of Cp^* ($Cp^* = \eta^5-C_5Me_5$) as ligand considerably increases the stability of the sandwich complexes. However, the analogous nucleophilic substitution reactions were unknown for the Cp^* compounds, which were clearly going to be less reactive because of the decreased positive charge on the arene ligand. Reaction of the 24-amine dendrimer with a twofold excess of $[FeCp^*(\eta^6-C_6H_5F)][PF_6]$ (**8**)^[29] in CH_2Cl_2 in the presence of NEt_3 gave the robust 24- $[FeCp^*(\eta^6-arene)]^+$ dendrimer **9** as an orange powder in 30 % yield after chromatography on alumina with methanol as eluent. To compare the recognition ability of **9** with those of a mononuclear compound and of a tripod having structures resembling that of the dendrimer, we also synthesized the mononuclear compound **10** and the trinuclear species **11** (Figure 1; these compounds also gave satisfactory analytical and spectroscopic data).

Titration of **9**^[30, 31] and cationic complexes **10** and **11** with nBu_4N^+ salts of Cl^- , Br^- , $H_2PO_4^-$, and HSO_4^- was monitored by the variation of the ^1H NMR chemical shift δ_{NH} of the exocyclic amine proton in $[D_6]DMSO$. The results are in contrast to those obtained with amidoferrocene dendrimers such as **7** (synthesized by the reaction of **5** with ferrocenylcarbonyl chloride and NEt_3 in CH_2Cl_2) and others^[22] (Figure 1 and Table 1).

The changes in δ_{NH} and the apparent association constant K_{app} upon addition of Cl^- to **9** are larger than those obtained upon addition of the other anions; a sharp transition is observed for eight equivalents of Cl^- per dendrimer, which corresponds to one Cl^- ion per tripodal dendrimer branch (Figure 1a). There is a distinction between the relatively sharp transition with Cl^- and the smooth transition with Br^- . Moreover, the magnitude of the variation of δ_{NH} (Figure 1a) and K_{app} for Cl^- (Table 1) is much larger with dendrimer **9** than with the tripodal compound **11**; thus, the dendritic effect is strong. In fact, in the case of **10** and **11**, no clear equivalence point can be obtained. The dendritic effect can be measured by the low-field shift in the NMR spectrum and the increase in K_{app} , when a given anion is added to the solution of the dendrimer, obtained in switching from **11** to **9** (tripodal **11** has the same structure as the tripods of dendrimer **9**). Molecular models show that in **9** the tripod branches are forced by the bulky groups at the periphery to approach one another more closely than in **11**. The channels defined by the large organo-iron groups become narrow because of the steric compression at the dendrimer periphery, so that Cl^- presumably fits well in the tripodal semicavities of the dendritic periphery of **9**.

Upon addition of Br^- to **9**, the change of the ^1H NMR shift smoothly reaches a plateau after approximately one equivalent of Br^- per dendrimer branch. A large dendritic effect is

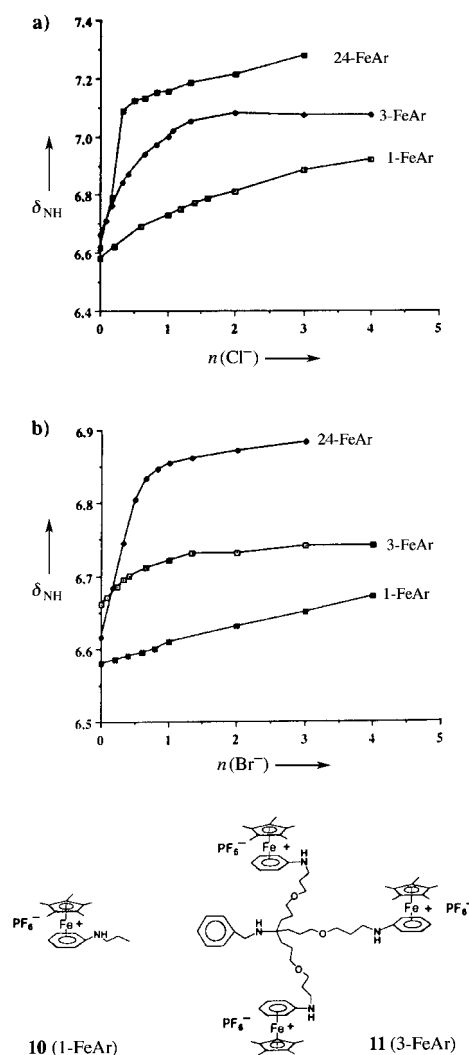


Figure 1. Variation of δ_{NH} for the exocyclic amine proton measured by ^1H NMR spectroscopy upon addition of nBu_4NCl (a) and nBu_4Br (b), given in number of equivalents n per branch, to **10** (1-FeAr), **11** (3-FeAr), and **9** (24-FeAr).

Table 1. Apparent association constants K_{app} [$dm^3 mol^{-1}$], determined from the variation of the δ_{NH} signal in $[D_6]DMSO$ at 20 °C with the EQ-NMR program^[31] (the uncertainty is 10 %). Small values of the order of 10 obtained with Cl^- and Br^- for the monometallic complex **10** have no physical meaning, but signify that no equivalence point is reached for one equivalent of anion per FeAr branch (this emphasizes the dendritic effect). Also note the negative dendritic effect for HSO_4^- upon comparison of the values obtained for **9** and **11**.

	10 (1-FeAr)	11 (3-FeAr)	9 (24-FeAr)
Cl^-	10	118	1221
Br^-	2	129	431
HSO_4^-	14	461	6

also found, the ^1H NMR shifts and apparent association constants K_{app} increasing as the number of branches is raised (1-FeAr $\rightarrow$ 3-FeAr $\rightarrow$ 24-FeAr, Figure 1b and Table 1). As for Cl^- , in the titration of complexes **10** and **11** with Br^- there is no sharp equivalence point, which is in contrast to what was found for **9**. The affinity of **9** for Br^- is weaker than for Cl^- because the $NH \cdots X^-$ hydrogen bond is also weaker (a well-known trend^[2]) due to a reduced electrostatic attraction.^[7] In

the case of Br^- , the recognition is simply accounted for by a 1:1 hydrogen-bonding interaction between the anion and the dendrimer branch. On the other hand, it is probable that for Cl^- each anion interacts through hydrogen bonding with the three dendrimer arms of each tripodal dendrimer branch.

The addition of $[\text{nBu}_4\text{N}][\text{H}_2\text{PO}_4]$ to **9** provoked the precipitation of the corresponding salt of the dendrimer, which was not the case for the other anions. With HSO_4^- , δ_{NH} changed continuously even beyond one equivalent per branch without reaching a break point. This indicated that there is no specificity of the dendrimer for this anion. On the other hand, the tripodal compound **11** is selective; equivalence is reached at one equivalent of HSO_4^- per branch, and the apparent association constant ($K_{\text{app}} = 461$) is two orders of magnitude larger than for **10** ($K_{\text{app}} = 14$) and **9** ($K_{\text{app}} = 6$). This means that the semicavities defined by the eight tripodal branches at the dendrimer surface cannot open to encapsulate HSO_4^- anions, unlike the situation with the more flexible **11**. This lack of selective recognition contrasts with the favorable dendritic effect found for oxo anions with ferrocene dendrimers such as **7** and other neutral and less bulky dendrimers.^[22]

The new polycationic metallo dendrimer **9**, designed with large cationic termini, shows large dendritic effects for recognition of Cl^- and Br^- according to ^1H NMR spectroscopy. These halides could not be recognized by neutral polyferrocene dendrimers such as **7** (no change in the NMR shift δ_{NH} upon addition of the anion), which has a topology comparable to that of **9**, and other structures that have already been reported.^[22–25] Likewise, electrochemistry was a workable sensing technique in the case of amidoferrocene dendrimers, but only little effects are found for the polycationic dendrimer **9**. These striking differences of behavior between neutral and cationic dendrimers with the same topology demonstrate the essential role of the positive charge for efficient interaction with anions and for the recognition ability observed by ^1H NMR spectroscopy. For instance, a polycationic dendrimer such as **9** must have a much larger hydrodynamic radius than the compact, neutral 24-amido-ferrocene dendrimer **7**. Besides the ferrocene groups, we believe that another important factor is the number of hydrogen bonds. Whereas the amido group is a chelating zwitterion that is potentially able to form two hydrogen bonds with chelating anions such as H_2PO_4^- , this feature is not available with **9** because only one anchoring nitrogen atom can enter into hydrogen bonding with the single-atom halogen anion.

In summary, the recognition of chloride and bromide, as determined by the NMR shifts of δ_{NH} for **10**, is due to the synergy between the single $\text{X}^- \cdots \text{HN}$ hydrogen bond per branch, the electrostatic attraction, and the shape selectivity brought about by the peripheral cavities formed by the dendrimer branches and tripods.

Experimental Section

For the numbering of the atoms, see the formula of **5** (Scheme 1). Descriptions of the syntheses as well as spectroscopic data and elemental analyses for compounds **2–11** can be found in the supporting information.

9: A mixture of **5** (0.12 g, 0.0365 mmol), NEt_3 (1.22 mL, 8.76 mmol), and **8** (0.76 g, 1.75 mmol) in CH_2Cl_2 (10 mL) was stirred for 15 d at ambient

temperature. After removal of the solvent under vacuum, CH_2Cl_2 (50 mL) was added. The organic phase was washed with a 1M solution of KPF_6 (3×20 mL) and then water (2×20 mL), dried over Na_2SO_4 , and filtered. The solvent was removed under vacuum. After chromatography on alumina (activity II–III) with methanol as eluent, **9** was obtained as a yellow-brown powdery solid (0.143 g, 30% yield). Elemental analysis calcd for $\text{C}_{562}\text{H}_{774}\text{O}_{24}\text{N}_{32}\text{Fe}_{24}\text{P}_{144}$: H 5.92, C 51.21, N 3.4; found: H 6.12, C 51.80, N 3.89; cyclic voltammetry (scan rate 0.3 V s^{-1} , Pt, DMF, 20°C , 0.1 M Bu_4NPF_6): a single reversible cathodic 24-electron wave at $E_{1/2} = -1.88 \text{ V}$ vs. SCE with ferrocene as internal reference.

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A New Route to Organic Intercalates Consisting of Vanadium Pentoxide and Pyridines: $(4\text{-H}_2\text{N-C}_5\text{H}_5\text{NH})\text{V}_2\text{O}_5^{*}$

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Layered intercalation compounds have attracted increased research attention due to their applications in such diverse areas as rechargeable batteries, heterogeneous catalysis, and ion exchange.^[1] Interest is particularly high in the insertion of organic molecules into layered hosts for the purpose of synthesizing organic–inorganic composite materials with

well-defined stoichiometries and organized structures.^[2–7] Such materials may possess hybrid properties that have a synergism of the properties shown by the host and the guest.^[3, 8] Pyridine and its derivatives are the class of organic Lewis bases that has been most investigated for intercalation in a variety of layered hosts including transition metal dichalcogenides,^[4] metal phosphorus trisulfides,^[9] metal oxides,^[10, 11] and metal phosphates.^[12, 13]

The conventional synthetic scheme for intercalating such molecules into layered hosts entails a direct reaction of the guest with the host lattice.^[1c] Such heterogeneous reactions usually afford products with insufficient crystallinity. As a result, definitive structural information with regard to the arrangement of the organic molecules in the interlamellar space is difficult to obtain. Contradicting structural models have sometimes been proposed for the same host–guest system based on X-ray powder diffraction results.^[14] Recently, hydrothermal techniques have been demonstrated to facilitate the synthesis and single-crystal growth of organic-based intercalation compounds.^[15] For example, α - and β -(enH_2)_{0.5}- V_2O_5 (en = ethylenediamine)^[16] as well as $(\text{DABCOH}_2)\text{V}_6\text{O}_{14}$ (DABCO = 1,4-diazabicyclo[2.2.2]octane)^[17] have been synthesized by the hydrothermal technique and structurally characterized by X-ray single-crystal analysis.

We have been interested in synthesizing novel microporous–mesoporous composite materials using layered compounds containing intercalated small molecules (e.g. pyridines, alkylamines, and alkalimetal ions) as starting materials in liquid crystal templating reactions. Here we describe the hydrothermal synthesis and X-ray structure of $(4\text{-H}_2\text{N-C}_5\text{H}_5\text{NH})\text{V}_2\text{O}_5$ (**1**), the first pyridine intercalation compound to be characterized by single-crystal X-ray analysis. This study has not only provided insight into the molecular orientation of the pyridine ring with respect to the host layer, but has also shed light on the formation mechanisms of the pyridine intercalate under hydrothermal conditions.

The structure of **1** was determined by X-ray single-crystal analysis.^[18] Compound **1** crystallizes in the monoclinic system with an asymmetric unit containing two V, five O, five C, two N, and seven H atoms in general positions. Both V atoms have square-pyramidal coordination with double-bonded O atoms occupying the axial positions. The structure can best be described as a two-dimensional intercalation compound of the organic $(4\text{-H}_2\text{N-C}_5\text{H}_5\text{NH})^+$ cations into the anionic $[\text{V}_2\text{O}_5]^-$ layers (Figure 1). Each $[\text{V}_2\text{O}_5]^-$ layer is formed parallel to the [001] plane consisting of distorted VO_5 square pyramids linked by edge- and corner-sharing O atoms. Figure 2 gives a projection of a $[\text{V}_2\text{O}_5]^-$ layer in **1**, and the bonding modes of the V and O atoms in the layer. The square pyramids share two edges of their base with neighboring square pyramids to form double ribbons along the [100] direction in such a way that the axial vertices alternatively point up and down. The ribbons are then connected with one another along the same direction through corner sharing. Because all the ribbons within a layer adopt the same orientation, the vertices of neighboring ribbons point in opposite directions across the corner-shared O atoms. Overall, the orientation of the square pyramids alternates up and down along the [001] direction. The O atoms are engaged in terminal (O2 and O4), doubly

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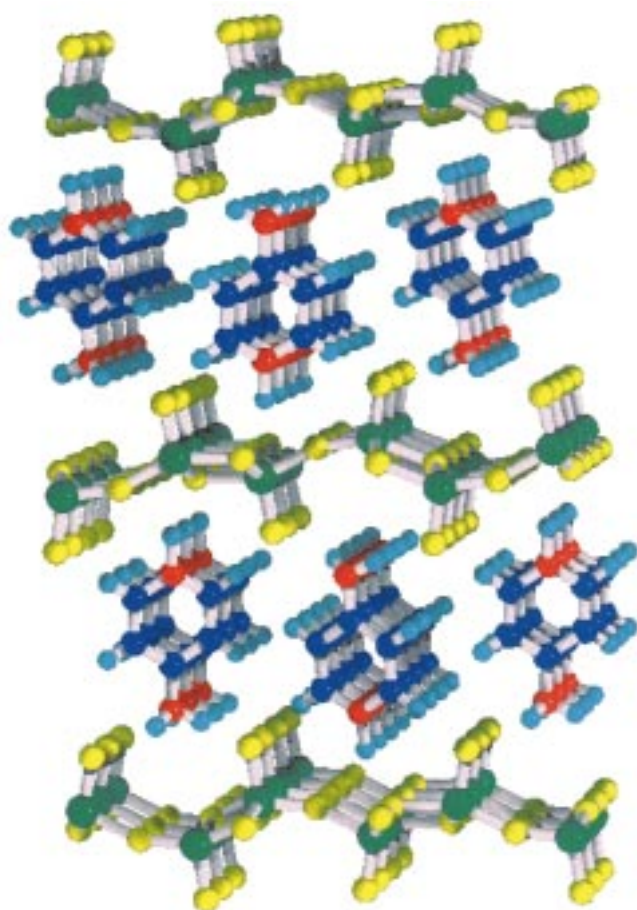


Figure 1. The structure of **1**, as viewed down the *a* axis. V: green, O: yellow, C: blue, N: red, H: turquoise.

bridging (O3), and triply bridging coordination (O1 and O5). The layers are stacked along the [001] direction with the 4-aminopyridine molecules occupying the interlamella space with their C_2 axis perpendicular to the layers (Figure 1). There are significant π - π stacking interactions between the aromatic organic molecules in each column along the *a* axis; the rings are parallel to each other and are separated by about 3.57 Å.

The FT-IR spectrum of **1** clearly shows that the pyridine N atom is protonated (characteristic $\nu(\text{N-H})$ bands at 3322 (s) and 3172 cm^{-1} (s) and stretching vibrations of the pyridinium ring at 1667 (s and br), 1600 (s), and 1543 cm^{-1} (s)), while the NH_2 group remains unprotonated (characteristic $\nu(\text{N-H})$ band at 3497 cm^{-1}). The latter is a strong and extremely broad band, indicating that this amino substituent is involved in significant hydrogen bonding. These findings are consistent with the corresponding $\text{p}K_a$ values of 9.11 and 1.18. In fact, the two N atoms in the $4\text{-H}_2\text{N-C}_5\text{H}_3\text{NH}^+$ ion form hydrogen bonds with the triply-bridging and terminal O atoms in the vanadium oxide layers above and below, respectively (N1-O1 2.97(1), N2-O2 2.90(1), N2-O4 2.89(1) Å). On the other hand, electron spectroscopy chemical analysis (ESCA) gave two overlapping peaks for $\text{V}2\text{p}_{3/2}$ at 517.5 and 516.0 eV with approximately equal intensities, suggesting a mixed valence state of $\text{V}^{5+}/\text{V}^{4+}$ in a 1:1 ratio in **1**. Although V1 and V2 are both in a square-pyramidal environment, the V-O bond

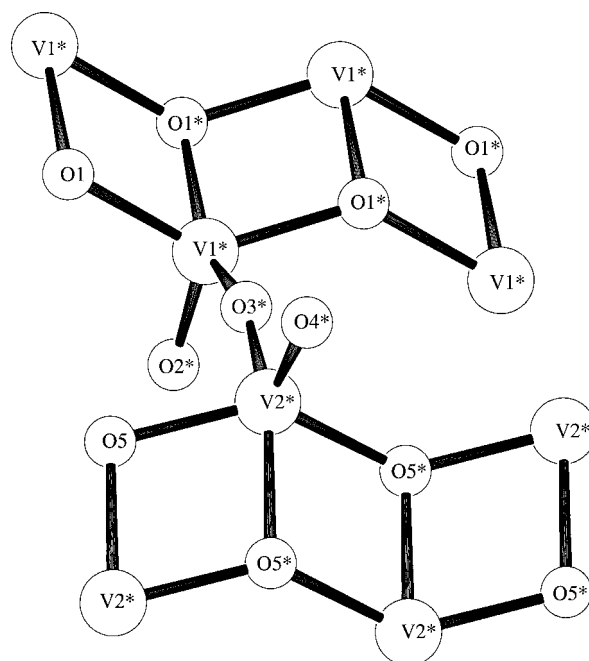
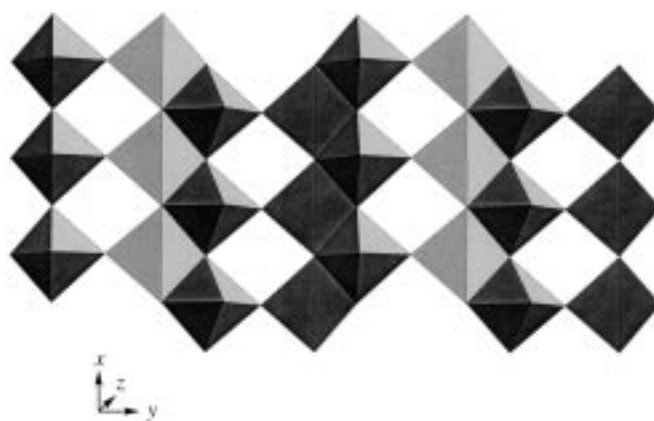


Figure 2. The projection of a $[\text{V}_2\text{O}_5]^-$ layer in **1** along the *c* axis and the bonding modes of the V and O atoms in **1**. Selected bond lengths [Å]: V1-O1 1.935(7), V1-O1 1.948(7), V1-O1 2.000(6), V1-O2 1.605(7), V1-O3 1.902(7), V2-O3 1.720(7), V2-O4 1.615(7), V2-O5 1.919(7), V2-O5 1.899(7), V2-O5 2.003(7).

lengths around V2 are shorter than their counterparts around V1, and the differences are statistically significant (see the legend to Figure 2). We therefore assign the oxidation state of V1 as +4, and of V2 as +5. This assignment of oxidation states was supported by the valence sum calculations,^[19] which gave a value of 4.21 for V1 and 4.99 for V2.

The magnetic susceptibility measurements performed with a SQUID showed that **1** is paramagnetic in the entire temperature region between 300 to 2 K (Figure 3a). The molar susceptibility rises sharply when the temperature is decreased from 3 to 2 K (Figure 3b). The field dependency measurements confirmed the ferromagnetic behavior at 1.8 K (Figure 3c). The fitting of the high-temperature (above 130 K) data with the Curie-Weiss law yielded $\theta = 197$ and

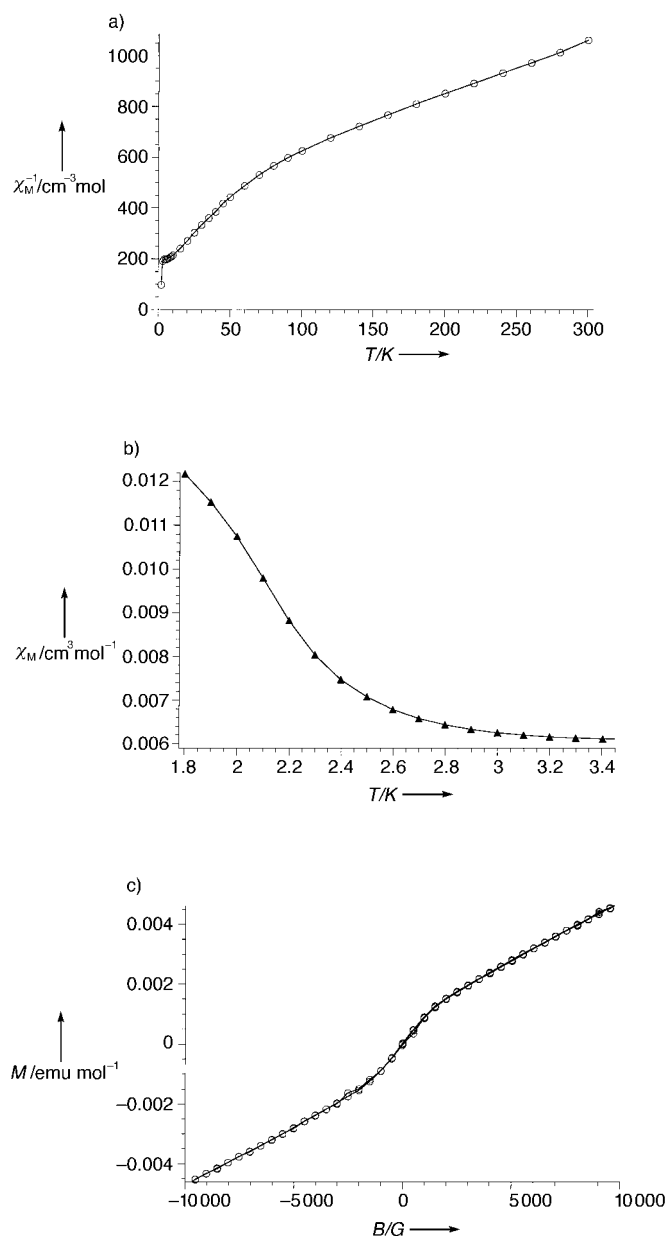


Figure 3. a) Inverse molar magnetic susceptibility of **1** as a function of temperature at 200 G. b) The temperature dependency of the molar magnetic susceptibility near 2 K at 500 G. c) The field dependency of the magnetization at 1.8 K.

$C = 0.51$. The latter is close to 0.374, suggesting an $S = 1/2$ spin system. The magnetic behavior of **1** is distinct from those of the other layered mixed-valence V^{4+}/V^{5+} oxide materials reported thus far in the literature. For example, at low x values the xerogel bronzes $A_xV_2O_5 \cdot nH_2O$ ($A = K, Cs$; $0.05 \leq x \leq 0.6$) are Curie–Weiss-type paramagnets showing temperature-independent paramagnetism (TIP) because the V^{4+} centers are isolated.^[20] As the concentration of the V^{4+} spins increases, the materials show strong antiferromagnetic coupling between the neighboring d^1 centers. Recently, Zubieta et al. described the synthesis and magnetic properties of the new layered vanadium oxide compounds $(en)_2M[V_6O_{14}]$ ($M = Zn, Cu$; **2**) and $[(en)_2Cu]_2[V_{10}O_{25}]$ (**3**), which feature interlamellar coordination with metal–amine complexes.^[21] The

layers in **2** and **3** are built up from $V^{4+}O_5$ square pyramids and $V^{5+}O_4$ tetrahedra and show paramagnetism with anomalously low magnetic moments, probably due to the spin pairing within the states that are delocalized in the entire layers.

Careful comparison of the structural details of the $[V_2O_5]^-$ layers in **1** with those of the starting material V_2O_5 reveals a substantial reorganization of the VO_5 pyramids in the formation of **1**.^[22] A naturally occurring mineral form of vanadium pentoxide, known as shcherbinaite, has an orthorhombic layered structure that also consists of edge-shared VO_5 double ribbons connected by corner sharing.^[23] However, because the neighboring ribbons adopt opposite orientations, the vertices of neighboring ribbons point in the same direction across corner-shared O atoms. Figure 4 gives a schematic

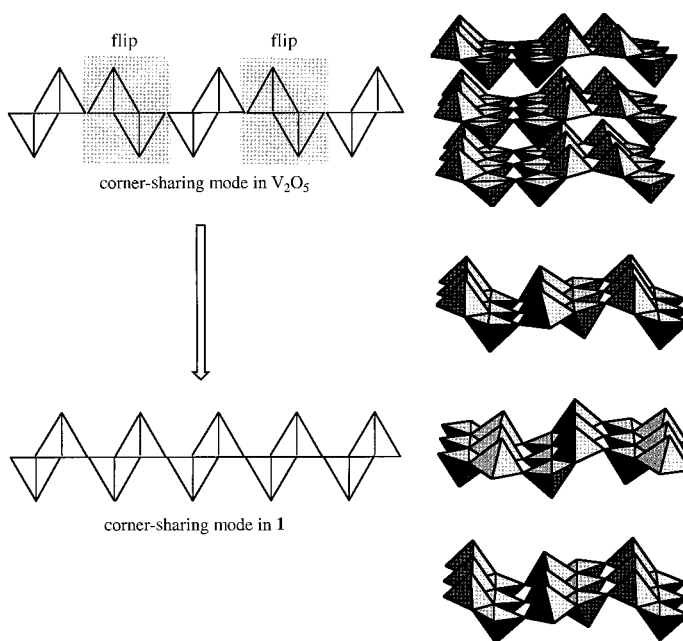
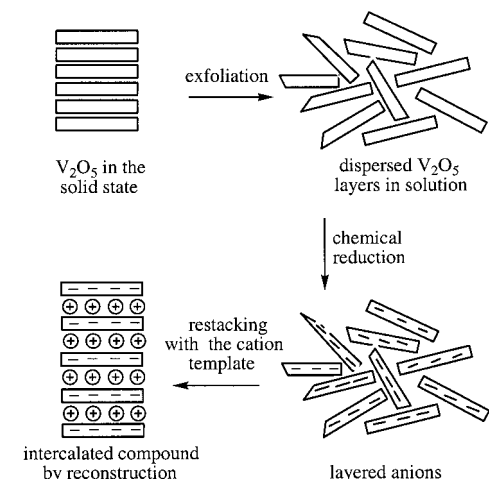


Figure 4. Schematic representation of the layer reorganization upon formation of **1** from V_2O_5 .

representation of the layer reorganization from V_2O_5 to **1**. Thus, the formation of **1** cannot be considered a topotactic reaction. This term is usually reserved for reactions in which the structural integrity of the host lattice is retained upon insertion of a guest species.

Instead, **1** is likely to be formed through the layer exfoliation–reduction–restacking process illustrated in Scheme 1. Vanadium pentoxide dissolves slightly in water to form a pale yellow solution containing dispersed V_2O_5 layers.^[24] Because of methanol present in the hydrothermal synthesis, the exfoliated layers are readily reduced to give lamella anions,^[25] which are restacked with the organic cations to form the new intercalation compound.^[7b, 8c] We suggest that the structural reorganization in the $[V_2O_5]^-$ layers is caused by the cation templating effect; that is, in addition to the above-mentioned hydrogen bonds, the narrower grooves in **1** also allow for C–H $\cdots$ O hydrogen bonding between the aromatic ring and the terminal O atoms (C4–O2 3.02(2), C2–O4 3.07(1) Å; see also Figure 1).



Scheme 1. Possible layer exfoliation–reduction–restacking process in the formation of **1**.

Experimental Section

A sample of V_2O_5 (100 mg, 0.55 mmol), 4-aminopyridine (104 mg, 1.10 mmol), and $H_2O/MeOH$ (50/50 v/v, 0.5 mL) was sealed in a thick-walled Pyrex tube. The tube was heated at $110^\circ C$ for eight days, and pure black, thin needles of **1** were obtained in about 92% yield based on V_2O_5 . The product was purified by washing with water and acetone.

The phase identity and homogeneity of **1** was confirmed by comparing the experimental X-ray powder diffraction patterns of the bulk material with those calculated from the single-crystal X-ray data.

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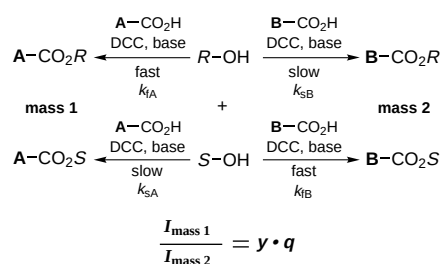
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Measurement of Enantiomeric Excess by Kinetic Resolution and Mass Spectrometry**

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The powerful nature of combinatorial synthetic methods for the preparation of diverse libraries of small molecules has often made the analysis of such compounds the rate-limiting step in their application.^[1] An intriguing target is the discovery of new enantioselective catalytic processes by the testing of large numbers of candidate catalysts.^[2] Optimal use of combinatorial synthesis in this endeavor will require the high-throughput measurement of enantiomeric excesses on a small scale, a task for which current techniques are evolving.^[1, 3] We describe here a method for the determination of enantiomeric excesses of alcohols and amines on the nanomole scale by diastereoselective derivatization and automated quantitative electrospray ionization mass spectrometry (ESI-MS).^[4, 5] The capabilities of mass spectrometry for the analysis of component mixtures^[4, 6] allows the chemical procedures and reagents used here to be simple and generally applicable.

The technique employs an equimolar mixture of pseudo-enantiomeric "mass-tagged" chiral acylating agents that differ in a substituent remote to the chiral center, such that the mass of the molecule is correlated to its absolute configuration. In general, the reactions of the enantiomers of any pair of chiral reagents will proceed with nonequal rate constants ($k_f > k_s$; f = fast, s = slow),^[7] as shown in Scheme 1 for the reaction of



Scheme 1. Generalized reaction of chiral alcohols with mass-tagged chiral acids in the presence of DCC and base. I = mass spectrum peak intensity, q = ionization correction factor, y = corrected intensity ratio.

alcohols with chiral mass-tagged acids **A**-CO₂H and **B**-CO₂H in the presence of 1,3-dicyclohexylcarbodiimide (DCC). The relative amounts of the product esters, measured in this case by mass spectrometry, can be used to determine the enantiomeric composition of the starting substrate using Equation (1)

$$\% ee = \left[\frac{(y-1)(s+1)}{(y+1)(s-1)} \right] \cdot 100 \quad \text{where} \quad \begin{cases} s = \frac{k_f}{k_s} \\ y = \text{corrected intensity ratio} \end{cases} \quad (1)$$

and two calibration measurements.^[8] The method represents an elaboration of Horeau's procedure for the determination of the absolute configuration of secondary alcohols.^[9] Although developed independently, it may also be regarded as an application of "parallel kinetic resolution" described recently by Vedejs and Chen.^[10]

While several elegant nonenzymatic systems have been developed for kinetic resolution in acylation reactions,^[10, 11] we employed the mass-tagged *N*-acylprolines **1a** and **1b**, along with DCC, as simple chiral acylating agents.^[12] As discussed below, these structures were chosen not primarily for their ability to discriminate between nucleophile enantiomers, but rather because they are readily prepared and afford products which should be detected with high sensitivity in the electrospray technique. Although enantiomeric discrimination in the esterification of phenethyl alcohol (**2**) by these and related *N*-acylproline structures was only modest ($s = k_f/k_s = 2.0$ – 2.6 at room temperature), it proved to be sufficient to determine enantiomeric excesses using ESI-MS to measure small differences in the amounts of the mass-tagged products. Alcohols were analyzed by reaction with a 20-fold excess of an equimolar mixture of acids **1a** and **1b** in the presence of DCC and a catalytic amount of 4-dimethylaminopyridine (DMAP),^[13] whereas amines were similarly derivatized with **1a**, **1b**, DCC, and 1-hydroxybenzotriazole. For these preliminary studies, 1 μ mol of each alcohol and 10 nmol of each amine were used, but we have subsequently found that equal success may be achieved with 1–10 nmol (or less) of both types of substrates. After acylation, each mixture was simply evaporated, redissolved, diluted, and analyzed by ESI-MS in 5- μ L injection aliquots, each of which contained no more than 50 pmol of the target esters or amides. Repeat injections were found to give reproducible intensity ratio to standard deviations of approximately 1–3%.^[14] We used two different instruments, one showing dominant $[M+H]^+$ ions and the other dominant $[M+Na]^+$ ions, both with good results.

Calibration must be performed on a racemic sample and a sample of known enantiomeric excess for each substrate structure. The observed mass ratio for the racemate defines a correction factor q which accounts for differences in electrospray ionization efficiencies between compounds differing by a methyl group. For the nonracemic calibration samples we chose enantiomerically pure (>98% *ee*) compounds available from commercial sources. The observed value of y (Scheme 1) and the known enantiomeric excess for the calibration sample are applied to a rearranged version of Equation (1)^[15] to give s . The enantiomeric excess of other samples can then be measured from their observed MS intensity ratios, and the values of q and s obtained from calibration.

Figure 1 shows the results for nine secondary alcohols (**2**–**10**) and five primary and secondary amines (**11**–**15**) of varying structure, including aliphatic and aromatic substituents, each as samples of 20, 50, 70, and 90% *ee*. Compound **15** is epibatidine, a potent nonopioid analgesic that has received

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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.

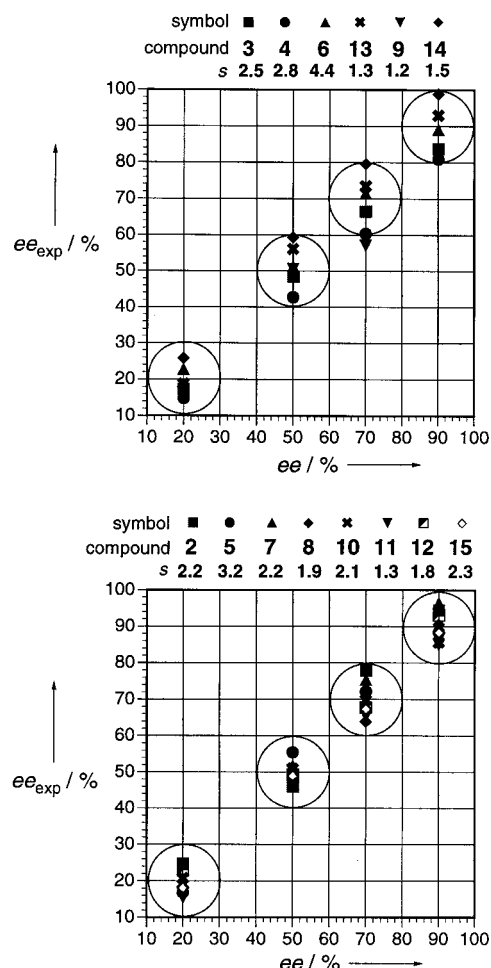
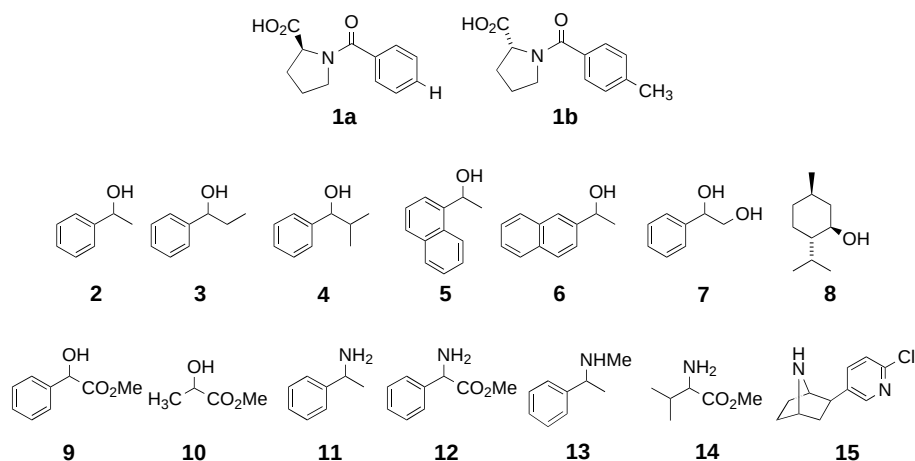


Figure 1. Plots of the actual versus measured enantiomeric excesses and s values for samples of **2–15** enriched in the *S* enantiomer (top) or the *R* enantiomer (bottom).



much recent attention.^[16] Each point shown in Figure 1 is the averaged result of two independent acylation reactions per sample, each reaction being analyzed by three averaged ESI-MS injections. In every case but one (**9** at 70% ee), the measured value falls within 10% ee of the true value. In general, the greater the enantiomeric discrimination in the

acylation process (s or $1/s$), the better is the ee measurement. Remarkably, the mass spectrometer is sufficiently reliable to provide effective measurement of enantiomeric excesses for substrate–mass tag combinations showing k_t/k_s ratios as low as 1.2. Thus, the kinetic resolution process used to read the enantiomeric content of the desired species must proceed with an energy difference between competing diastereomeric transition states of only about $0.1 \text{ kcal mol}^{-1}$. Such a small energetic requirement means that a single pair of mass tags may be used to analyze many different types of structures, as observed here.^[17]

The method for determining enantiomeric excesses described here has several distinctive features:

1) Because the necessary level of kinetic resolution is so low, it is likely that readily available chiral acids can be found for the analysis of chiral compounds of widely varying structures.

2) The technique can be reversed (mass-tagged chiral nucleophiles used to measure the enantiomeric composition of acylating agents) or used with many other bond-forming reactions as “reporter” processes, as long as they afford a small degree of kinetic resolution.

3) Instead of making use of known reactivity (the relative rate s) to measure unknown composition (ee), this approach may find utility for rapidly establishing structure–activity relationships by surveying reactivity (s) for compounds of known composition (structures of interest in racemic and enantiomerically pure form). Thus, the s values shown in Figure 1 reveal that better enantiomeric recognition occurs for the alcohols containing large aromatic groups (**5** and **6**), and highlights a potentially interesting difference between **4** and **9**, which are approximately the same size but undergo kinetic resolution with different efficiencies.

4) The method does not rely on chromatographic separation, and thus may be especially useful for cases in which chromatography is either ineffective or inconvenient.^[18]

5) Since the mass-tagged chiral reagents are used in large excess and the desired masses can be selected from a complicated mass spectrum, this method is tolerant of reactive achiral impurities such as water and requires little or no purification.

6) The method is rapid,^[19] amenable to automation, and usable for small amounts of substrate (10 nmol or less).^[20] It is presently intended for the screening of asymmetric catalysts, for

which purpose the determination of enantiomeric excess to $\pm 10\%$ is sufficient.

The development of new mass-tagged analytical reagents to improve the scope, sensitivity, and accuracy of the method is underway, as is its application to the screening of candidate catalytic asymmetric processes in a microtiter plate format.

Experimental Section

Representative procedure for alcohols: A solution of alcohol (1 μmol in 50 μL of toluene) was placed in a small vial or microtiter plate. A mixture of **1a** (10 μmol) and **1b** (10 μmol) in 100 μL of CH_2Cl_2 was added followed by a mixture of DMAP (0.1 μmol) and DCC (10 μmol) in 50 μL of CH_2Cl_2 . The vessel was sealed for 24 h to ensure complete reaction, although the method does not depend on reaching any particular percent conversion beyond what is necessary for detection of the desired esters. The solvent was then allowed to evaporate.

Representative procedure for amines: A solution of amine (10 nmol in 50 μL of toluene (THF in the case of **15**)) was placed in a 96-well microtiter plate. A mixture of **1a** (100 nmol) and **1b** (100 nmol) in 20 μL of CH_2Cl_2 , 1-hydroxybenzotriazole (10 nmol) in 20 μL of THF, and DCC (100 nmol) in 20 μL of CH_2Cl_2 were sequentially added. The plate was sealed for 24 h, and the solvent was then allowed to evaporate. Two sets of identical reactions were set up for all samples, using the same stock solutions.

The reaction product obtained from each of the above procedures was taken up in 500 μL of methanol, and the precipitate was allowed to settle. For the alcohols, an aliquot of 5 μL of the supernatant was diluted to 1.0 mL before transfer to an HPLC autosampler vial. For the amines, an aliquot of 50 μL of the supernatant was diluted to 100 μL for analysis. The autosampler was used to deliver 5- μL samples to the electrospray mass spectrometer (Hewlett-Packard 1100 MSD). The flow rate of the mobile phase (methanol) was 0.5 mL min^{-1} , and samples were injected directly into the spectrometer at intervals of 2 min. A curtain gas of ultrapure nitrogen was pumped into the interface to aid desolvation of the charged droplets and to prevent particulate matter from entering the analyzer. The drying gas flow was set at a rate of 12 L min^{-1} . The drying gas temperature was set to 350 °C. The nebulizer pressure was set to 1810 Torr. A capillary voltage of 3500 V was applied to the interface sprayer to charge the sample droplets. The fragmentor was set at a relatively low potential (80 V) to minimize ion fragmentation. The data was acquired at a step size of 0.15 m/z , and was set to scan the relevant mass range. With this instrument and under these conditions, $[M+\text{Na}]^+$ ions were dominant. Samples **5–7** were analyzed in earlier runs on a Perkin-Elmer SCIEX API100 single quadrupole LC-MS instrument (nebulizer gas 1.6 L min^{-1} , electrospray potential 4200 V, ultrapure nitrogen curtain gas at 1.6 L min^{-1} , 80 V orifice potential); under these conditions spectra were dominated by $[M+\text{H}]^+$ peaks. Each sample was analyzed with three averaged injections. A wash of 20 minutes at a flow rate of 2 mL min^{-1} was performed every 36 injections. Complete experimental details, including the preparation and characterization of mass-tagged reagents, are available in the supporting information.

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- [13] Under DCC-mediated esterification conditions, we observe a small degree of epimerization of the *N*-acylprolines, but this does not interfere with the present application.
- [14] The use of more concentrated samples, containing approximately 25 nmol of desired esters or amides along with the other components of the acylation reaction mixtures, showed poorer sensitivity and gave less accurate results (standard deviations for repeat injections of approximately 5 %).
- [15] $s = [\% ee(y + 1) + 100(y - 1)] / [\% ee(y + 1) - 100(y - 1)]$ for 100 % *ee*, $s = y$.
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- [18] Note, for example, that not all of the compounds analyzed here could have been accommodated by a single chiral HPLC or GC column. Of course, the techniques are related in that the chemical interactions responsible for molecular discrimination in chromatography are likely to be similar to those giving rise to diastereoselectivity in the derivatization process that precedes mass spectrometry readout.
- [19] Each mass spectrometry injection presently requires approximately two minutes. The analysis can be made more efficient by combining compounds of differing molecular weights in each injection.
- [20] Our present sensitivity limit for quantitative ESI-MS of small molecules is approximately 500 fmol per μL .^[6]

A Method for High-Throughput Screening of Enantioselective Catalysts**

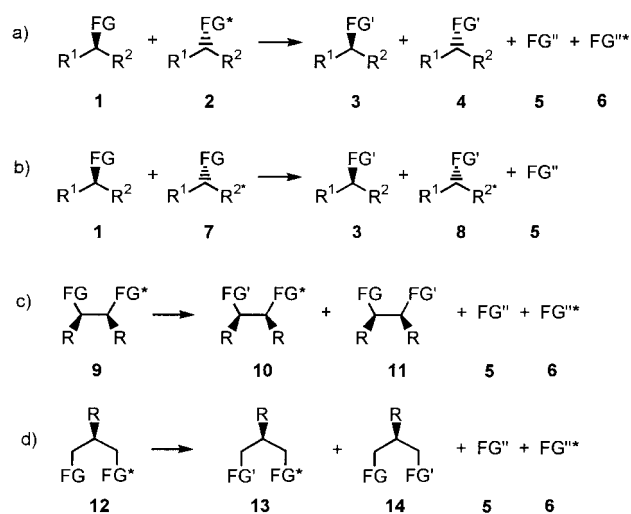
Manfred T. Reetz,* Michael H. Becker,
Heinz-Werner Klein, and Detlef Stöckigt

Whereas the principles of combinatorial chemistry are well established in pharmaceutical research,^[1] extension to the area of catalysis is not as advanced.^[2] One reason is that few general methods for high-throughput screening of heterogeneous and homogeneous catalysts have been devised. This applies all the more to enantioselective catalysts.^[3] We have previously developed a screening system for the catalytic enantioselective hydrolysis of chiral *p*-nitrophenol esters in which the course of the reactions of the *R*- and *S*-configured substrates is monitored in a parallel manner by UV/Vis spectroscopy.^[4] With the use of microtiter plates crude screening of about 800 different enantioselective catalysts is

possible per day, in this case mutant lipases created by directed evolution.^[4, 5] Accordingly, following expression of a library of mutant genes in *E. coli*/*P. aeruginosa*, the bacterial colonies on agar plates were collected and cultivated individually in the wells of microtiter plates, each supernatant containing a mutant lipase suitable for screening. By nature this particular screening system is restricted to chiral acids and cannot be used in the evaluation of asymmetric catalytic reactions involving chiral alcohols, diols, amines, amino alcohols, alkyl halides, or epoxides. Recently, IR thermography was introduced as a means to detect metal- or enzyme-catalyzed enantioselective reactions, but quantification still needs to be accomplished.^[6]

We now describe a method based on electrospray ionization mass spectrometry (ESI-MS)^[7] which enables the determination of enantioselectivity in about 1000 catalytic or stoichiometric asymmetric reactions per day. Two basically different stereochemical processes can be monitored by this approach, namely, kinetic resolution of racemates and asymmetric transformation of substrates which are prochiral due to the presence of enantiotopic groups.

The underlying principle is based on the use of isotopically labeled substrates in the form of *pseudo*-enantiomers or *pseudo*-prochiral compounds (Scheme 1).^[8] The course of the asymmetric transformation—that is, the relative amounts of reactants and/or products—is detected by ESI-MS.^[9, 10]



Scheme 1. a) Asymmetric transformation of a mixture of *pseudo*-enantiomers involving cleavage of the functional groups FG and labeled functional groups FG*. b) Asymmetric transformation of a mixture of *pseudo*-enantiomers involving either cleavage or bond formation at the functional group FG; isotopic labeling at R² is indicated by the asterisk. c) Asymmetric transformation of a *pseudo*-meso substrate involving cleavage of the functional groups FG and labeled functional groups FG*. d) Asymmetric transformation of a *pseudo*-prochiral substrate involving cleavage of the functional group FG and labeled functional group FG*.

In the case of kinetic resolution, compounds **1** and **2**, differing in absolute configuration and in labeling at the functional group FG*, are prepared in enantiomerically pure form and then mixed in a 1:1 manner, simulating a racemate (Scheme 1a). Following asymmetric functional group transformation (in an ideal kinetic resolution up to 50 % conversion), true enantiomers **3** and **4** are formed, in addition to

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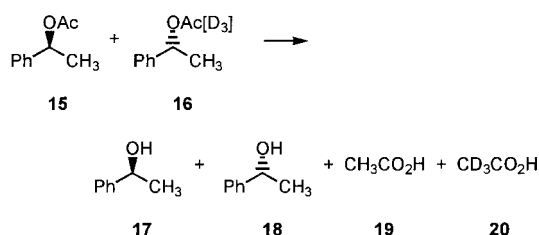
[**] We thank H. Husmann for performing GC analyses and H. Hinrichs for performing LC analyses as well as Novo Nordisk (Denmark) for enzyme samples.

nonlabeled and labeled achiral products **5** and **6**. The ratios of the total intensities of **1/2** and **5/6** in the mass spectra (m/z intensities of the quasi-molecular ions) allow for the determination of enantioselectivity. In some cases it may be advantageous to use an internal standard in order to determine the conversion.^[11]

As a variation of this theme, kinetic resolution of the *pseudo*-enantiomers **1** and **7**, in which labeling occurs at residue R², affords a new pair of *pseudo*-enantiomers **3** and **8** (Scheme 1b). Based on the m/z intensities of the quasi-molecular ions of **1/7** and **3/8**, the conversion, the enantioselectivity, and the selectivity factor (*s* or *E* value) can be obtained. An internal standard is not necessary.

In the case of prochiral substrates having enantiotopic groups, for example *meso* compounds (Scheme 1c), the synthesis of a single *pseudo-meso* compound suffices, for example **9**, since the stereodifferentiating reaction of interest delivers a mixture of two *pseudo*-enantiomers **10** and **11** that are detectable by MS. The same applies to other *pseudo*-prochiral substrates of the type **12** (Scheme 1d).

Although our method is independent of the type of catalyst or reagent used, we restrict ourselves in this paper to lipase-catalyzed reactions. The lipase-catalyzed hydrolytic kinetic resolution of racemic 1-phenylethyl acetate, corresponding to Scheme 1a, served as the first example. In exploratory experiments *pseudo*-enantiomers **15** and **16** were prepared in enantiomerically pure form and mixed in various ratios [Eq. (1)]. The resulting mixtures were analyzed by gas



chromatography in order to ascertain the exact *pseudo-ee* values. Thereafter the samples were analyzed employing ESI-MS.^[12] A typical ESI-mass spectrum is shown in Figure 1. Comparison of the two sets of data shows an agreement of $\pm 5\%$ (Table 1). Similar observations were made in control experiments involving other substrates, thereby demonstrating the accuracy and precision of such *ee* determinations.

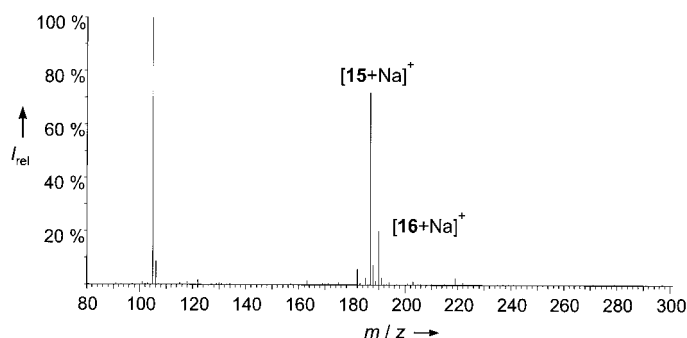
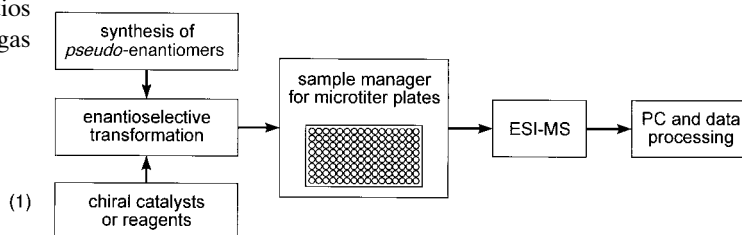


Figure 1. ESI mass spectrum of **15** and **16** (sample 6, Table 1).

Table 1. Enantiomeric excesses of samples prepared by mixing **15** and **16** as determined by GC and ESI-MS.

Sample	<i>ee</i> [%] (GC)	<i>ee</i> [%] (ESI-MS)
1	100	100
2	91	90
3	81	79
4	74	73
5	60	57
6	56	54
7	48	48
8	28	27
9	10	10
10	23	20
11	40	38
12	45	43
13	55	53
14	65	60
15	75	74
16	95	93
17	100	100

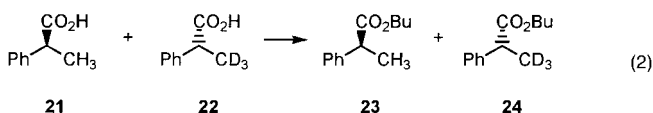
Following these encouraging results, the task remained to devise an experimental setup capable of high-throughput screening of enantioselective reactions. This was achieved by combining an automated liquid sampler for microtiter plates with an ESI-MS system, both commercially available (Scheme 2). With such a configuration about 1000 precise



Scheme 2. Schematic description of the experimental setup for the determination of *ee* values.

determinations of the *ee* value and the conversion of the reaction in Equation (1) can be performed in a single day.^[13] In other cases the number varies between 700 and 1400, depending upon the particular substrate. It should be emphasized that neither chromatographic separation of enantiomers or *pseudo*-enantiomers nor of the products is involved. Thus, the method constitutes a fast and accurate way to determine the enantioselectivity and conversion of lipase-catalyzed (or otherwise induced) hydrolysis reactions leading to chiral alcohols.

Our approach can be used in the kinetic resolution of all types of chiral compounds, for example in the lipase-catalyzed stereoselective esterification of 2-phenylpropionic acid using a 1:1 mixture of **21** and **22** [Eq. (2)],^[13] which correspond



to Scheme 1b. As before, about 1000 *ee* determinations can be performed per day. To exclude possible secondary kinetic isotope effects, the reaction of the *pseudo*-enantiomers **21** and

22 was compared to that of the true racemate of **21**. The data in Figure 2 demonstrate agreement within the limits of experimental error.

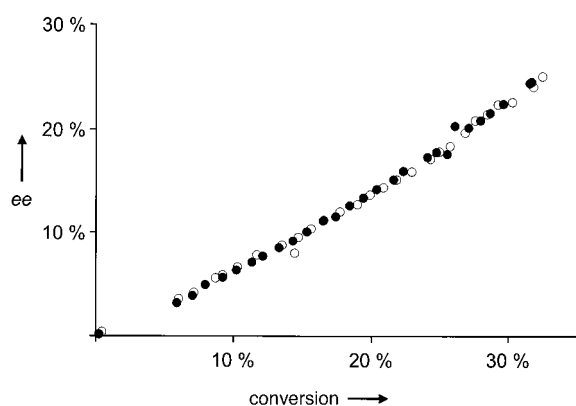
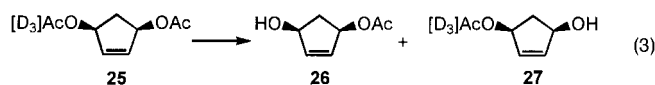


Figure 2. Comparison of the *ee* values obtained for the lipase-catalyzed (Novo SP 435 lipase, Novo Nordisk) esterification of the racemate of **21** (○) with that of a 1:1 mixture of **21** and **22** (●).

Finally, the lipase-catalyzed enantioselective hydrolysis of *meso*-1,4-diacetoxy-2-cyclopentene was investigated,^[13] which is an example of Scheme 1c. Accordingly, the *pseudo-meso* compound **25** was first prepared by acylation of (1*S*,4*R*)-4-acetoxycyclopent-2-ene-1-ol (**26**) using CD₃C(O)Cl (>99 atom % D). Hydrolysis of **25** afforded the expected mixture of *pseudo*-enantiomeric products **26** and **27** [Eq. (3)], which



were analyzed by MS as described above for the other substrates. In Figure 3 ESI mass spectra of two representative samples are depicted, one involving a mutant lipase (*P. aeruginosa*)^[4] having no enzyme activity (no conversion; Figure 3a) and the other involving an active enzyme (porcine liver esterase, Sigma) leading to an *ee* value of 73 % (Figure 3b). In control experiments several samples were also analyzed by gas chromatography using a chiral stationary phase. Again, the correspondence between the traditional chromatographic (slow) and the present method (fast) turned out to be excellent.

In summary, we have developed a highly efficient method to assay the enantioselectivity of asymmetric reactions of chiral compounds or of prochiral substrates bearing enantiotopic groups.^[14] Although isotopically labeled *pseudo*-enantiomers or *pseudo*-prochiral compounds need to be prepared, once this task has been performed a large number of enantioselective biocatalysts or chemical catalysts can be screened for the particular reaction in question within a short time. Since the position of isotopic labeling is chosen so as to preclude primary isotope effects which might influence the results, reliable data are obtained. The present method is not restricted to ESI-MS; preliminary studies show that other ionization techniques such as matrix-assisted laser desorption/ionization (MALDI) are also efficient. Other screening methods are required for systems involving prochiral sub-

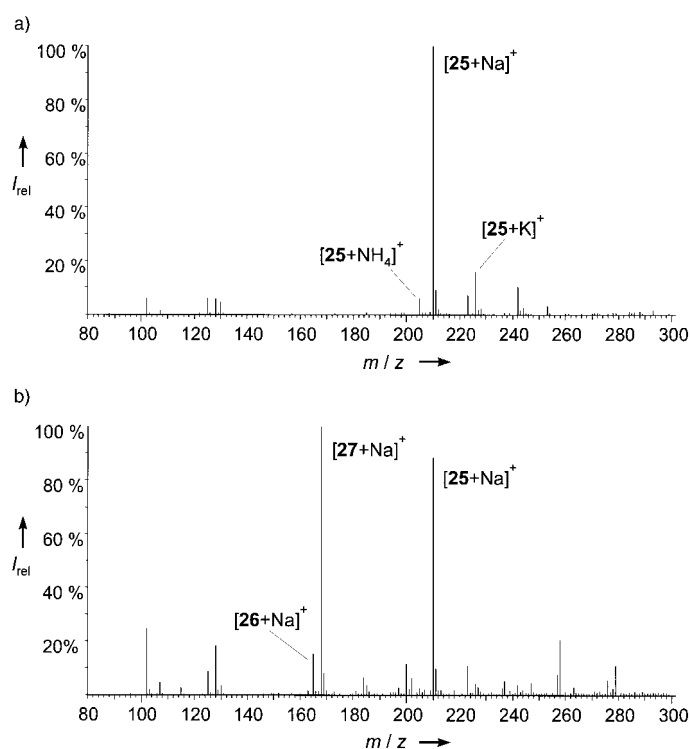


Figure 3. a) ESI mass spectrum of a sample obtained from the use of a nonactive mutant lipase in the attempted hydrolysis of **25**. b) ESI mass spectrum of a sample obtained from the use of an active esterase in the hydrolysis of **25** (ESI-MS: *pseudo-ee* = 73 %; GC: *ee* = 73 %).

strates having enantiotopic faces, as in the enantioselective reduction of ketones or olefins.

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- [10] In principle it should be possible to extend Horeau's method^[9a] to the determination of *ee* values in high-throughput screening. However, this requires an additional step (derivatization) and an excess of a 1:1 mixture of the *pseudo*-enantiomeric reagent as well as the assumption that conversion is complete without any enrichment of one of the diastereomers.
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- [12] Aliquots (10 μ L) of 1 mM 1:1 mixtures of **15** and **16** in CH₃OH were injected into a Rheodyne port of an ESI-MS system (ESI-MS conditions: MS: Hewlett Packard 5989B MS engine quadrupole mass spectrometer equipped with a Hewlett Packard 59987A API electrospray source II with hexapole ion guide (Analytica of Branford) and ChemStation data system; data acquisition: positive-ion mode scan spectra; *m/z* 90–300; step size *m/z* 0.1, unit resolution, gaussian mass filter *m/z* 0.3; gaussian time filter 0.05 min; API source conditions: potential difference between spray needle and first electrode –5250 V, pressure of N₂ nebulization gas: 4140 Torr, flow of N₂ drying gas ca. 9 L min^{–1} (150 °C), solvent flow 0.06 mL min^{–1}, CH₃OH/H₂O 8/2). ESI mass spectra were collected, and the ratios of **15** and **16** were determined on the basis of absolute intensities of the peaks of the corresponding sodium adducts ([**15**+Na]⁺ and [**16**+Na]⁺, Figure 1). The ratios of the peak intensities (and thus the *ee* values of the synthetic mixtures of **15** and **16**) were obtained automatically using a macro to submit the data from the *m/z* intensity table of each measurement to an Excel spread sheet.
- [13] All enzyme-catalyzed hydrolytic reactions and esterifications were performed in deep-well microtiter plates (total volume 1.2 mL). For ESI-MS analysis a defined volume of the resulting product mixtures was extracted with diethyl ether. The extracts were automatically transferred in microtiter plates and diluted with methanol to a final concentration of 0.5–2.0 mM. The microtiter plates were placed in an automated sample manager (Scheme 2) equipped with a Rheodyne port for the injections.
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- [[[(η^7 -C₂B₁₀H₁₂)(η^6 -C₂B₁₀H₁₂)U][K₂(thf)₅]]₂]:
A Metallocarborane Containing the Novel
 η^7 -C₂B₁₀H₁₂^{4–} Ligand****
- Zuowei Xie,* Chaoguo Yan, Qingchuan Yang, and
Thomas C. W. Mak
- It has been well-documented that C₂B₁₀H₁₀R₂ (R = H, alkyl, aryl) can be reduced by alkali metals to form the *nido*-C₂B₁₀H₁₀R₂^{2–} dianion, which can be bound in a η^6 manner to transition metals to afford a series of 13-vertex *closo*-metallacarboranes.^[1] Treatment of [(η^6 -C₂B₁₀H₁₂)Co(η^5 -C₅H₅)] with Na/naphthalene followed by reaction with C₅H₅Na and CoCl₂ gave the 14-vertex *closo*-metallacarborane [(η^6 : η^6 -C₂B₁₀H₁₂)-{Co(η^5 -C₅H₅)₂}].^[2] The proposed geometry of the cage is the bicapped hexagonal antiprism; X-ray confirmation of this species has not been reported. We are interested in this tetraanion ligand and its bonding mode to transition metals, and describe herein the isolation and structural characterization of the first metallacarborane bearing a η^7 -C₂B₁₀H₁₂^{4–} ligand.
- Interaction between *o*-C₂B₁₀H₁₂ and excess K metal in THF at room temperature followed by treatment with a suspension of UCl₄ in THF gave, after workup, **1** as deep red crystals in 58 % yield [Eq. (a)]. Compound **1** is extremely air- and
- $$4\text{ }o\text{-C}_2\text{B}_{10}\text{H}_{12} + 12\text{ K} + 2\text{ UCl}_4 \xrightarrow{\text{THF}} \text{[[[(}\eta^7\text{-C}_2\text{B}_{10}\text{H}_{12})(\eta^6\text{-C}_2\text{B}_{10}\text{H}_{12})\text{U][K}_2(\text{thf})_5\text{]]}_2\text{]} + 8\text{ KCl} \quad (\text{a})$$
- 1**
- moisture-sensitive, but remains stable for months at room temperature under an inert atmosphere. Contact with traces of air immediately results in conversion of the intensely colored **1** into a yellow powder. Compound **1** is soluble in polar organic solvents such as THF and pyridine, sparingly soluble in toluene, and insoluble in hexane.
- An X-ray diffraction study^[3] reveals that **1** is a centrosymmetric dimer with a bent sandwich structural motif. As shown in Figure 1, each U atom is η^6 -bound to *nido*-C₂B₁₀H₁₂^{2–}, η^7 -bound to *arachno*-C₂B₁₀H₁₂^{4–}, and coordinated to two B–H bonds from the C₂B₅ bonding face of the neighboring *arachno*-C₂B₁₀H₁₂^{4–} ligand. This results in a highly distorted tetrahedral geometry at U with a cent(S)–U–cent(L) angle of 136.3° (cent(S) and cent(L) are the centroids of the C₂B₄ and C₂B₅ bonding faces, respectively). Compound **1** represents not only the first metallacarborane containing a novel η^7 -C₂B₁₀H₁₂^{4–} ligand, but also the first organoactinide compound bearing a η^6 -C₂B₁₀H₁₂^{2–} ligand.
- The average distance between U and a cage atom of the C₂B₄ bonding face in **1** (2.867(7) Å) is longer than that between U and a cage atom of the C₂B₅ bonding face in

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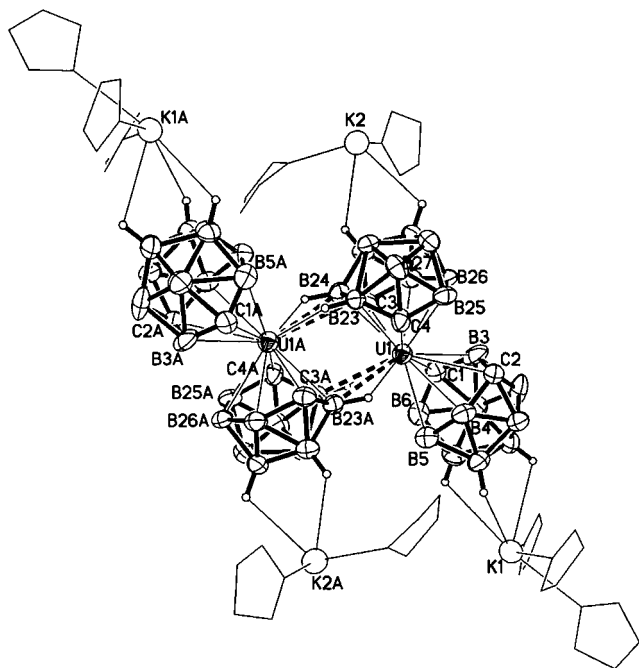


Figure 1. Molecular structure of **1**. Selected distances [Å]: U1–C1 2.744(6), U1–C2 2.958(5), U1–B3 2.969(7), U1–B4 2.702(7), U1–B5 2.840(7), U1–B6 2.991(7), U1...B23A 2.980(6), U1...B24A 2.989(6).

[Li(thf)₄]₂[(η^5 -C₂B₉H₁₁)₂UCl₂] (2.73(2) Å)^[4] and [Li(thf)₄]₂[(η^5 -C₂B₉H₁₁)₂UBr₂] (2.72(2) Å).^[5] This measured distance can be compared with the values of 2.841(3) Å in [(Me₂Si(η^6 -C₂B₁₀H₁₁)(η^5 -C₅H₄))Sm^{III}(thf)₂],^[6] 2.976 Å in [(η^6 -C₂B₁₀H₁₂)Eu^{II}(MeCN)₃],^[7] and 3.03 Å in [(η^6 -C₂B₁₀H₁₂)₂-Eu^{II}(thf)₂]²⁻.^[7] The Shannon radius for U^{IV} is 0.079 and 0.25 Å smaller than that of Sm^{III} and Eu^{II}, respectively.^[8] The distances from the U atom to the five B atoms of the C₂B₅ bonding face from the η^7 -C₂B₁₀H₁₂⁴⁻ ligand range from 2.772(6) to 2.791(6) Å with an average value of 2.780(6) Å, which is close to those in [(η^5 -C₂B₉H₁₁)₂UX₂]²⁻ (X = Cl,^[4] Br^[5]) but shorter than the average distance between U and a cage atom of the C₂B₄ bonding face of the η^6 -C₂B₁₀H₁₂²⁻ ligand (2.867(7) Å). The U...B distances in the two U–H–B units are 2.980(6) Å and 2.989(6) Å, which can be compared with the Sm^{II}...B distances of 3.025(8) to 3.058(8) Å found in [(C₂B₉H₉(CH₂C₆H₅)₂)Sm(dme)₂]₂ (dme = 1,2-dimethoxyethane),^[9] and the Th^{IV}...B distances of 3.086(3) and 3.101(3) Å observed in [(C₅Me₅)₂ThMe]₂[(C₂B₉H₁₁)₂Fe].^[10] Further comparisons are difficult due to the lack of other related compounds.

The most interesting features of this structure are the boat shape of the C₂B₅ bonding face, the coordination environment of each cage atom of the novel η^7 -C₂B₁₀H₁₂⁴⁻ ligand, and the unexpectedly short U–C3 and U–C4 bond distances of 2.414(5) and 2.443(5) Å (Figure 2). These values are very close to those of U–C σ bonds normally observed in organouranium compounds, for instance, 2.43(2) Å in [(C₅H₅)₃U(*n*Bu)],^[11] 2.56(1) Å in [(C₅H₅)₃U(*n*Bu)]⁻,^[12] 2.48(3) Å in [(C₅H₅)₃U[CH₃C(CH₃)₂]],^[13] 2.54(2) Å in [(C₅H₅)₃U(*p*-CH₂C₆H₄CH₃)],^[11] and 2.436(4) Å in [(Me₃SiC₅H₄)₃U(CH=CH₂)].^[14] The five B atoms of the C₂B₅ bonding face are almost coplanar, and the two C atoms

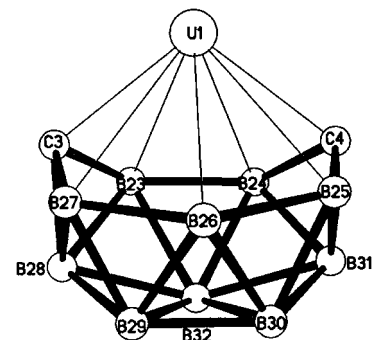


Figure 2. Interaction between the U atom and the C₂B₁₀H₁₂⁴⁻ ligand in **1**. Selected distances [Å]: U1–C3 2.414(5), U1–C4 2.443(5), U1–B23 2.772(6), U1–B24 2.785(6), U1–B25 2.777(6), U1–B26 2.776(6), U1–B27 2.791(6).

are about 0.6 Å above this plane. The coordination number for the C atoms is one less than those of B atoms in this bonding face.^[15] Based on the above-mentioned findings, the U–C3 and U–C4 bonds are best described as σ bonds.

The mechanism of the formation of **1** might be proposed as follows: In the presence of excess K metal, [C₂B₁₀H₁₂]₂K₂^[1, 7] reacts with UCl₄ in THF to give the presumable intermediate [(C₂B₁₀H₁₂)₂U(thf)₂]. Further reduction of the *nido*-C₂B₁₀H₁₂²⁻ ligands by K metal to *arachno*-C₂B₁₀H₁₂⁴⁻ affords **1**. Direct reduction of C₂B₁₀H₁₂ to C₂B₁₀H₁₂⁴⁻ by excess K metal seems very unlikely.^[2, 16]

Experimental Section

1: A mixture of *o*-C₂B₁₀H₁₂ (0.145 g, 1.0 mmol) and K (0.98 g, 25.0 mmol) in THF (40 mL) at room temperature was stirred overnight. To this pale yellow suspension was added UCl₄ (0.19 g, 0.50 mmol), and the mixture was stirred at room temperature for 4 d to afford a dark red suspension. After removal of excess K metal and precipitates, the dark red solution was evaporated to give a very dark red solid. Recrystallization from toluene/THF afforded **1** as very dark red crystals (0.278 g, 58%). ¹H NMR (300 MHz, [D₅]pyridine, 25 °C, TMS): δ = 1.55 (brs, thf), 3.59 (brs, thf), 27.78 (brs, cage CH), 52.62 (brs, cage CH); ¹¹B NMR (160 MHz, [D₅]pyridine, 25 °C, BF₃·OEt₂): δ = –3.5 (4B), –9.0 (2B), –9.9 (4B), –14.0 (4B), –14.6 (2B), –21.5 (2B), –36.9 (2B); IR (KBr): $\tilde{\nu}$ = 3075 (w), 2969 (m), 2870 (m), 2490 (vs), 2278 (m), 1049 (s), 902 cm^{–1} (m); elemental analysis calcd for C₄₀H₁₁₂B₄₀K₄O₈U₂ (**1** + 2 thf): C 26.89, H 6.32; found: C 26.48, H 6.21.

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- Crystal data for **1**: C₄₈H₁₂₈B₄₀K₄O₁₀U₂, *M*_r = 1930.4, monoclinic, space group *P*2₁/*c*, *a* = 17.892(3), *b* = 11.506(2), *c* = 21.628(4) Å, β = 91.32(1)°, *V* = 4451(1) Å³, *T* = 296 K, *Z* = 2, ρ_{calcd} = 1.440 g cm^{–3}, $2\theta_{\text{max}}$ = 50°, $\mu(\text{MoK}\alpha)$ = 0.71073 Å, absorption corrections applied by using ABCOR,^[17] relative transmission factors in the range 0.785–1.274. A total of 7563 reflections were collected and led to 5355 unique reflections, 4817 of which with *I* > 2 σ (*I*) were considered as observed,

$R_1 = 0.0778$, $wR_2 (F^2) = 0.1857$. This structure was solved by direct methods and refined by full-matrix least squares on F^2 by using the Siemens SHELXTL/PC package of crystallographic software.^[18] All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were geometrically fixed using the riding model. 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-112284. 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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ESI Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS): A Rapid High-Resolution Analytical Method for Combinatorial Compound Libraries**

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Test systems for the search for active materials are largely automated and designed for the reliable recognition of in vitro

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activity with minimal sample consumption (high-throughput screening). Compound collections for mass screening are nowadays prepared by the methods of combinatorial chemistry.^[1, 2] The parallel synthesis of hundreds and thousands of compounds per day is state of the art (high-throughput synthesis). The requirement for rapid characterization of the compounds (high-throughput analysis) arises from this development. An increase in the efficiency of "off-bead" analysis by ESI/MALDI-TOF/quadrupole-MS^[3, 4] and reverse-phase HPLC^[5] as well as "on-bead" analysis by NMR^[6] and FT-IR spectroscopy^[7] has been rapidly achieved. Previously less utilized methods such as FT-IR microscopy even allow simultaneous "on-bead" analysis of polymer-bound compound collections on hundreds of resin beads.^[8]

FT-ICR mass spectrometry^[9] sets new standards with respect to effectiveness, informative value, and sample consumption. In Figure 1, measurements obtained by ESI-quadrupole

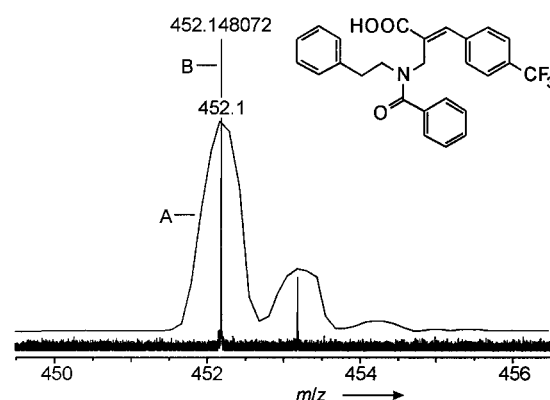


Figure 1. Superimposed measurements of a compound obtained by solid-phase synthesis with ESI-quadrupole (A) and ESI-FT-ICR mass spectrometry (B). Starting points for elemental analysis: $[M-H]^-$ measured: 452.1480720 Da, charge -1, min./max. double bond equivalents (DBE) 0.5/30, tolerance ± 0.0005 Da. Elemental analysis for $M_r = 452.1480720$: C 26, O 3, N 1, S 0, H 21, F 3; $[M-H]^-$ calculated: 452.1478911, 15.5 DBE, deviation 0.4 ppm. The calculation of possible molecular formulas on the basis of the very precise ESI-FT-ICR measurement thus gave solely the correct composition.

rupole (A) and ESI-FT-ICR mass spectrometry (B) on a compound prepared by solid-phase organic synthesis^[10] are superimposed. The ESI-FT-ICR measurement gives a mass accuracy which is greater by a factor of about 550 (A: $\delta = 221$ ppm; B: $\delta = 0.4$ ppm). Accordingly, the difference between calculated and measured mass of the FT-ICR determination corresponds to 0.00018 Da in this example. The resolution is about a factor of 15 better than for the comparable measurement with a quadrupole analyzer (A: 4500; B: 70000). The FT-ICR measurement was taken in the broad-band mode with a scanned mass range of 1400 Da. This measurement mode is not designed for the highest resolutions, but nevertheless a resolution of 70000 was obtained. The corresponding measurement in the high-resolution mode at a scanned mass range of 100 Da gave a resolution of 350000 (data not shown).

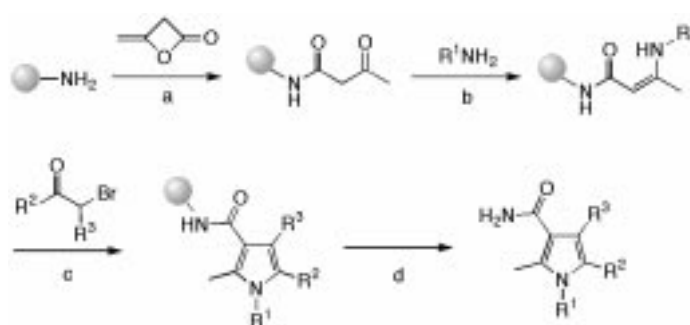
The amount of sample required for an FT-ICR measurement is 10 times less than for the quadrupole measurement (A: 40 pmol; B: 4 pmol). The tolerance of the FT-ICR measurement resulting from the mean accuracy of the

measurements is very small (0.0005 Da), for which reason the number of postulated molecular formulae is small. Owing to the high resolution, the isotope peaks are especially easy to recognize. When the exclusion rules are taken into consideration (nitrogen rule, double bond rule, valences), the calculation for this example gives solely the elemental composition which corresponds to the actual molecular formula. The FT-ICR measurement thus enables rapid elementary analysis^[11] of individual compounds in the pharmacologically interesting range of up to 500 Da.

This rapid molecular formula determination may also be applied to complex mixtures such as those which arise from combinatorial chemistry. To demonstrate the performance of this method in this respect, a split–mix compound collection of 140 different pyrrole amides consisting of 10 subcollections of 14 compounds each was synthesized on Rink amide AM resin (Scheme 1).^[12]

The results of the subcollection with 2-bromoacetophenone as building block ($R^2 = C_6H_5$, $R^3 = H$) are illustrated as an example. The total amount of sample of 20 pmol gives a mean sample consumption of 1.4 pmol per compound for the routine measurement. All 14 expected products could be detected by mass spectrometry (Figure 2).

The resolution averaged over all 14 compounds ($m/\Delta m_{50\%}$) is 38000. The mean mass accuracy Δm is 0.00032 Da (1.05 ppm) at a standard deviation of 0.00026 Da. The possible molecular formulas were calculated for all 14 masses of the measurement. The exact masses for the elements C, H, N, O, and S were considered by the program, and results with a nitrogen content of over 50 weight percent were rejected (Table 1). The very high mass accuracy allows only one or two, at the most three, possible elementary compositions (hits) for each compound. The individual compounds are thus so precisely characterized that, with prior knowledge of the reaction conditions, the structural formulae may also be determined.



Scheme 1. Solid-phase synthesis of pyrrole amides by the split–mix method. a) Acetoacetylation with diketene; b) separation of the resin into 14 aliquots, enaminone formation with 14 different primary amines; c) combination of the 14 aliquots, separation into 10 aliquots, reaction with 10 different α -bromoketones; d) cleavage from the polymer support.

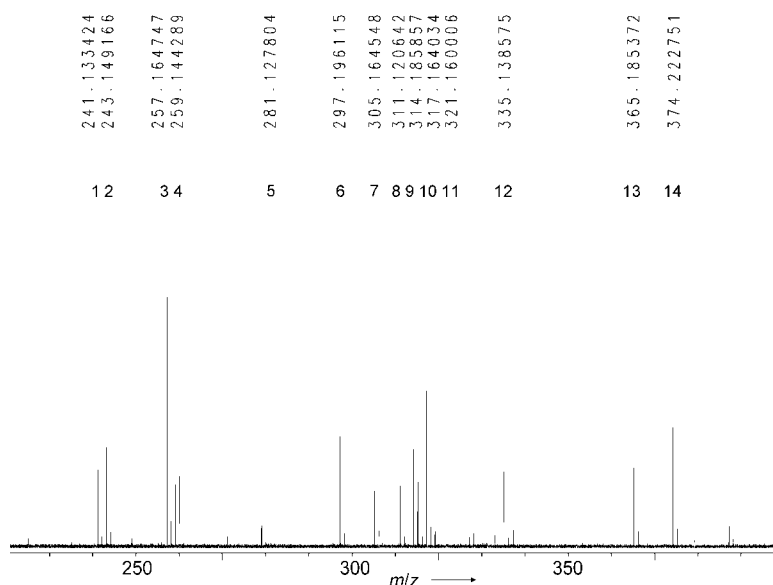


Figure 2. Automated routine ESI-FT-ICR measurement of the pyrrole amide collection (section from m/z 220–400). The duration of the measurement was about 30 s; the measurement range of 1400 m/z was measured 16 times during this time. Processing of the data collected (selection of smoothing parameters and number of data points, peak labeling) was automated.

Table 1. Results of the ESI-FT-ICR measurement illustrated in Figure 3. The high mass accuracy (mean value $\delta = 1.05$) allows only one or two, at the most three, possible molecular compositions (hits) per compound.

Compd	Amine (R ¹)	Molecular formula	Mass calcd [M+H] ⁺	Mass found [M+H] ⁺	Δ mass	δ	Hits	
1	cyclopropylamine	C ₁₅ H ₁₆ N ₂ O	241.133530	241.133424	0.000106	0.44	1	
2	isopropylamine	C ₁₅ H ₁₈ N ₂ O	243.149179	243.149166	0.000013	0.05	1	
3	1-butylamine	C ₁₆ H ₂₀ N ₂ O	257.164829	257.164747	0.000082	0.31	1	
4	3-amino-1-propanol	C ₁₅ H ₁₈ N ₂ O ₂	259.144093	259.144289	0.000196	0.75	2	
5	furfurylamine	C ₁₇ H ₁₆ N ₂ O ₂	281.128444	281.127804	0.000640	2.27	2	
6	aminomethylcyclohexane	C ₁₉ H ₂₄ N ₂ O	297.196127	297.196115	0.000012	0.04	1	
7	2-phenylethylamine	C ₂₀ H ₂₀ N ₂ O	305.164829	305.164548	0.000281	0.92	2	
8	2-(2-aminoethyl)thiophene	C ₁₈ H ₁₈ N ₂ OS	311.121294	311.120642	0.000652	2.09	3	
9	N-(2-aminoethyl)morpholine	C ₁₈ H ₂₃ N ₃ O ₂	314.186290	314.185857	0.000433	1.37	1	
10	1-aminoindane	C ₂₁ H ₂₀ N ₂ O	317.164829	317.164034	0.000795	2.50	2	
11	tyramine	C ₂₀ H ₂₀ N ₂ O ₂	321.159743	321.160006	0.000263	0.81	2	
12	piperonylamine	C ₂₀ H ₁₈ N ₂ O ₃	335.139008	335.138575	0.000433	1.29	2	
13	3,4-dimethoxyphenylethylamine	C ₂₂ H ₂₄ N ₂ O ₃	365.185955	365.185372	0.000583	1.59	2	
14	4-amino-N-benzylpiperidine	C ₂₄ H ₂₇ N ₃ O	374.222674	374.222751	0.000077	0.20	2	
					mean value:	0.000326	1.05	1.57
					standard deviation:	0.000264	0.83	

The very high accuracy and resolution of the measurements alone represent a considerable increase in the efficiency of mass spectrometric methods. The precise separation by mass of the direct measurement of a compound mixture frequently avoids prior separation by HPLC. As the result of direct determination, time-consuming and expensive tagging systems for compound mixtures of up to 100 components become unnecessary. The time saved even for low sample availability is considerable.

To meet the demands of high-throughput analysis, we automated the sample input. For this purpose an HPLC system was coupled directly to the FT-ICR mass spectrometer. The integrated automatic sample injector inputs a new sample for measurement into the FT-ICR mass spectrometer by flow injection every two minutes. Together with the synchronized start of the FT-ICR measurement, this allows a sample throughput of 300 hundred samples per night. At the end of the measurement the raw data collected are processed automatically according to the wishes of the user (number of data points, choice of smoothing parameters, peak labeling, etc.). After the automatic sample injector is reset the mass spectrometer is ready for use.

Mass spectrometric characterization of isomers requires the coupling of HPLC and mass spectrometer. The results of HPLC/FT-ICR-MS analysis of the aforementioned subcollection is illustrated in Figure 3 as a contour diagram.

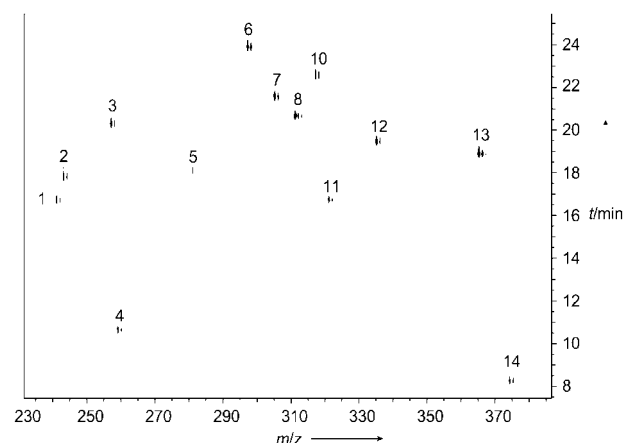


Figure 3. Contour diagram of the HPLC/FT-ICR-MS coupling of the pyrrole amide compound collection with $R^2 = C_6H_5$, $R^3 = H$. Isomers with the inherent separation problems could also be detected with this method. The isotope resolution of the molecule ion peaks is readily seen.

The measurement described utilizes fully all technical possibilities for improving the performance of mass spectrometric methods for use in high-throughput analysis. These are currently the best possible resolution and mass accuracy over a broad mass range, short measurement times, lowest sample consumptions, and automated sampling and processing. This method is thus an important aid in synthesis optimization and control of individual compounds and compound collections in combinatorial chemistry. The system is also ideally suited for use in protein analysis, for the elucidation of natural products by MS^n experiments, or for metabolic studies.

Experimental Section

The measurements were carried out with an Apex II-ESI-FT-ICR mass spectrometer (4.7 T) from Bruker Daltonik. The number of data points per measurement was 256 K in a mass range of 100–1500 Da. The mass range was scanned once a second, and 16 scans were taken. The ESI-FT-ICR-MS and automatic sampler were coupled through a fused silica capillary (75 μ m internal diameter, 365 μ m external diameter). The samples were injected into the continuous solvent stream (flow rate 30 μ L min⁻¹, 60% acetonitrile, 40% water, each with 0.1% formic acid) by the automatic sample injector of the HPLC system (Hewlett Packard, HP-1100 series). Measurements with a known compound collection provided the data for three-point calibration of the mass spectrometer. The subcollection for the HPLC/FT-ICR-MS coupling was separated on an RP-C 18 column (GROM-Sil ODS7; 1 $\times$ 6 mm; gradient of 10–80% acetonitrile/water plus 0.1% formic acid in 25 min at a flow rate of 50 μ L min⁻¹).

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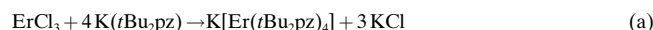
Supramolecular Architecture and a New Mode of Pyrazolate Coordination (η^3) in the First Homoleptic Lanthanide Pyrazolate Complexes**

Glen B. Deacon,* Ewan E. Delbridge, and Craig M. Forsyth

The recent preparations of the first homoleptic pyrazolate complexes $[\text{Ti}(\eta^2\text{-Me}_2\text{pz})_4]$ (R_2pz = 3,5-disubstituted pyrazolate)^[1a,b] and $[\text{Ta}(\eta^2\text{-Me}_2\text{pz})_3(\eta^1\text{-Me}_2\text{pz})_2]$ ^[1c] for relatively small ions (ionic radii of Ti^{4+} and Ta^{5+} : 0.74 Å)^[2] are in contrast to the invariable isolation of tris(η^2 -pyrazolate) derivatives of the larger (ca. 1.0 Å)^[2] lanthanide ions as $[\text{Ln}(\eta^2\text{-R}_2\text{pz})_3(\text{L})_n]$ complexes (L = thf, dimethoxyethane (dme), or Ph_3PO),^[3] rather than as homoleptic $[\text{Ln}(\text{R}_2\text{pz})_3]$ species. We now report the first homoleptic pyrazolatolanthanide(III) complexes **1** and the charge-separated **2**. Additional novel features are the K^+ / $[\text{Er}(\eta^2\text{-}t\text{Bu}_2\text{pz})_4]^-$ supramolecular framework and a new pyrazolate coordination mode (η^3 to K^+) in **1**, as well as the nature of the K^+ /toluene interaction in **2**. Pyrazolate ions commonly bind as $\mu\text{-}\eta^1\text{:}\eta^1$, η^1 , or η^2 ligands,^[4] and $\mu_3\text{-}\eta^1\text{:}\eta^2\text{:}\eta^1$ (to K^+)^[5] and $\mu\text{-}\eta^2\text{:}\eta^2$ (to Yb^{2+})^[6] coordination modes were recently observed.



Complex **1** was prepared by reaction of anhydrous erbium chloride with potassium 3,5-di-*tert*-butylpyrazolate in 1,2,4,5-tetramethylbenzene at 200 °C under vacuum [Eq. (a)] and



obtained in good yield by crystallization from toluene, while **2** was isolated by crystallization of **1** from dme/PhMe containing [18]crown-6. Not only are these the first homoleptic pyrazolatolanthanide complexes, but they are also the first mononuclear anionic lanthanide pyrazolates. The cages $[\text{Ln}_3(\text{Me}_2\text{pz})_6(\mu_3\text{-O})\text{Na}_2\text{L}_2]$ (L = thf or Me_2pzH) can be viewed as polynuclear “ate” species.^[7]

Analytical, IR, and Vis/near-IR data for **1** and **2** were consistent with the proposed compositions. Conductivity measurements in the low-polarity solvent CH_2Cl_2 suggest greater ion separation in **2**, although its conductivity is lower than that typical for a 1:1 electrolyte in this solvent.^[8] X-ray crystallography^[9] on **1** revealed a linear supramolecular array of $[\text{Er}(\eta^2\text{-}t\text{Bu}_2\text{pz})_4]^-$ anions and potassium cations. The latter are sandwiched between two of the pyrazolate ligands (η^3 bonded), and the chains are linked by intermolecular potassium–carbon interactions (Figure 1). Discrete $[\text{Er}(\eta^2\text{-}t\text{Bu}_2\text{pz})_4]^-$ anions and $[\text{K}([\text{18crown-6})(\text{dme})(\text{PhMe})]]^+$ cations are present in **2** (Figure 2).

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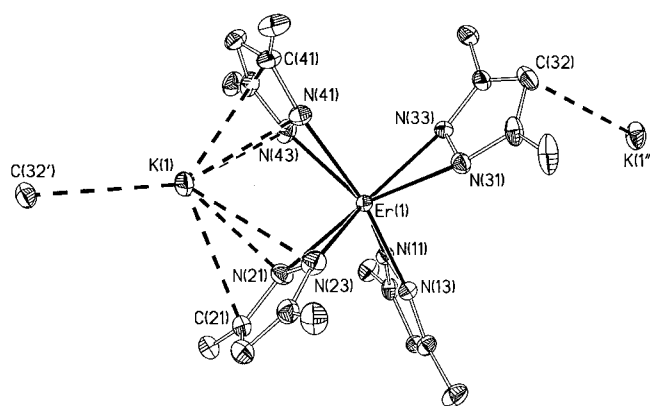


Figure 1. ORTEP plot of **1** (30 % probability thermal ellipsoids; hydrogen atoms and methyl groups omitted for clarity). Selected bond lengths [Å] and angles [°]: Er–N(11) 2.342(6), Er–N(13) 2.292(6), Er–N(21) 2.346(6), Er–N(23) 2.416(6), Er–N(31) 2.350(6), Er–N(33) 2.324(6), Er–N(41) 2.361(6), Er–N(43) 2.385(6), K–N(21) 2.811(7), K–N(23) 3.077(7), K–C(21) 3.187(7), K–N(41) 2.842(6), K–N(43) 3.130(6), K–C(41) 3.093(8), K–C(32') 3.162(7), K...C(31') 3.665(7), K...C(33') 3.577(7); cen(1)–Er–cen(2) 98.1, cen(1)–Er–cen(3) 102.2, cen(1)–Er–cen(4) 137.2, cen(2)–Er–cen(3) 136.7, cen(2)–Er–cen(4) 91.5, cen(3)–Er–cen(4) 98.7 (cen(x) = center of the N(x1)–N(x3) bond).

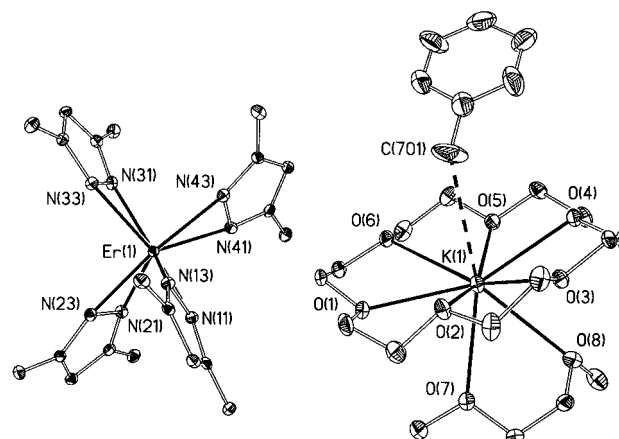


Figure 2. ORTEP plot of **2** (30 % probability thermal ellipsoids; hydrogen atoms and the methyl groups of the *t*Bu substituents omitted for clarity). Selected bond lengths [Å] and angles [°]: Er–N(11) 2.332(3), Er–N(13) 2.368(3), Er–N(21) 2.359(3), Er–N(23) 2.348(3), Er–N(31) 2.332(3), Er–N(33) 2.378(3), Er–N(41) 2.396(3), Er–N(43) 2.336(6), K–O(1) 2.885(3), K–O(2) 2.868(3), K–O(3) 2.863(3), K–O(4) 2.841(3), K–O(5) 2.782(3), K–O(6) 2.900(3), K–O(7) 2.823(3), K–O(8) 3.075(3), K–C(701) 3.423(4); cen(1)–Er–cen(2) 94.7, cen(1)–Er–cen(3) 135.8, cen(1)–Er–cen(4) 99.6, cen(2)–Er–cen(3) 100.9, cen(2)–Er–cen(4) 136.7, cen(3)–Er–cen(4) 96.7 (cen(x) = center of the N(x1)–N(x3) bond).

The erbium centers of **1** and **2** are coordinated to eight nitrogen atoms of four $\eta^2\text{-}t\text{Bu}_2\text{pz}$ ligands, and the average Er–N distances (**1**: 2.35; **2**: 2.36 Å) are comparable with those of eight-coordinate $[\text{Er}(\eta^2\text{-}t\text{Bu}_2\text{pz})_3(\text{thf})_2]$ ^[3a] and $[\text{Er}(\eta^2\text{-Ph}_2\text{pz})_3(\text{Ph}_3\text{PO})_2]$ ^[3c] (av 2.34 Å). The geometries around the erbium atom, as defined by the centers of the N(x1)–N(x3) bonds (cen(x), $x = 1\text{--}4$) are highly distorted from tetrahedral [cen–Er–cen 91.5–136.7° (**1**); 94.7–136.7° (**2**)]. Most likely, these deviations minimize the steric repulsions between the bulky *t*Bu groups, since in the less crowded $[\text{Ti}(\eta^2\text{-}t\text{Bu}_2\text{pz})_2\text{Cl}_2]$ ^[1b] the range of pseudotetrahedral angles (100.5–113.3°) is markedly narrower. The similarity of the

geometries of the $[\text{Er}(\eta^2\text{-}t\text{Bu}_2\text{pz})_4]^-$ anions in **1** and **2** is consistent with the supramolecular nature of the $t\text{Bu}_2\text{pz}\cdots\text{K}\cdots t\text{Bu}_2\text{pz}$ interactions in **1**.

Two of the K–N distances of **1** [K(1)–N(21) and K(1)–N(41)] are comparable with the $\mu\text{-}\eta^1\text{:}\eta^1$ and η^2 K–N distances of $[\text{K}(t\text{Bu}_2\text{pz})(\text{thf})]_6$.^[5] Additionally, the adjacent nitrogen and carbon atoms of both pyrazolate ligands [e.g., N(23) and C(21) for N(21)] are only about 0.3 Å further from the potassium atom. These longer distances are similar to the K–N and K–C distances (3.122(5)–3.255(5) Å) of a π -bound $\text{K}(\eta^5\text{-pyrrolyl})$ moiety^[10] and hence are regarded as significant interactions that give overall η^3 coordination. The central K–N(21) and K–N(41) bonds of the η^3 -pyrazolate ligands are virtually normal (94.2, 92.5°) to the ring planes, and this implies π -donor bonding to potassium. Notably, the potassium atom lies within the smallest of the intracentroid angles (cen(2)–Er–cen(4) 91.5°), and this also implies bonding. Since the K $\cdots$ C distances of the remaining two C atoms of the pyrazolate rings of each ligand are about 0.4 Å longer, it is unlikely that they are significant interactions, especially in view of the position of K⁺ (see above), although they are within the range found for potassium(η^6 -arene) bonding (K–C max. 3.72(1) Å; av 3.37 Å).^[11, 12] The sevenfold coordination of the potassium atom in **1** is completed by a potassium–carbon (C32') contact (3.162(7) Å) to a neighboring $[\text{Er}(\eta^2\text{-}t\text{Bu}_2\text{pz})_4]^-$ anion (see ref. [13] for a similar Ag–C(Me₂pz) interaction). The next nearest carbon atoms (see legend to Figure 1) are significantly more distant (by 0.4 Å or more) and are considered nonbonding.

The potassium cation in **2** is located in the cavity of an [18]crown-6 molecule, and a chelating dme ligand is bound on one side of the K([18]crown-6) moiety. A toluene molecule, orientated with the methyl group toward the potassium ion, occupies the vacant face opposite to dme. Formation of this supramolecular K([18]crown-6)/toluene “lock and key” interaction (K–C(Me) 3.423(7) Å) is remarkable given the excess of the potential donor dme in the crystallization solution.

Experimental Section

1: Potassium 3,5-di-*tert*-butylpyrazolate (1.20 g, 5.5 mmol) was prepared from KH and *t*Bu₂pzH in PhMe and heated with ErCl₃ (0.38 g, 1.4 mmol) in 1,2,4,5-tetramethylbenzene (1.00 g) at 200°C/10^{−3} Torr in an evacuated sealed Carius tube for 24 h. Extraction of the pink residue with light petroleum (to remove C₆H₅Me₄) followed by PhMe and subsequent concentration yielded large pink crystals of **1** (2.58 g, 77%). IR (Nujol): $\tilde{\nu}$ = 1568 m, 1310 w, 1285 w, 1250 m, 1226 m, 1205 m, 1129 w, 1016 m, 993 s, 798 vs, 782 s, 728 vs, 628 w cm^{−1}; Vis/near IR (PhMe): λ_{max} (ϵ) = 379 (60), 490 (9), 522 (20), 595 (5), 654 (5), 980 nm (8); Λ_{m} (CH₂Cl₂) = 0.22 Scm² mol^{−1} (2.60 × 10^{−3} M); elemental analysis calcd for C₄₄H₇₆N₈ErK: C 57.23, H 8.30, N 12.13, Er 18.11; found: C 56.30, H 8.93, N 11.86, Er 18.35.

2: Compound **1** (0.10 g, 0.11 mmol) and [18]crown-6 (0.50 g, 1.9 mmol) were dissolved in PhMe (20 mL) and dme (5 mL). Cooling of the solution afforded a few pink acicular crystals of **2**. IR (Nujol): $\tilde{\nu}$ = 1606 w, 1515 m, 1486 m, 1411 m, 1319 s, 1303 m, 1285 m, 1251 m, 1225 m, 1206 m, 1191 m, 1114 brs, 1019 m, 997 s, 986 m, 960 s, 851 s, 840 s, 781 s, 776 s, 727 vs, 694 vs, 628 w cm^{−1}; Λ_{m} (CH₂Cl₂) = 9.7 Scm² mol^{−1} (1.31 × 10^{−3} M); elemental analysis calcd for C₆₇H₁₁₈N₈O₈ErK: C 58.74, H 8.68, N 8.18; found: C 58.08, H 7.88, N 8.35.

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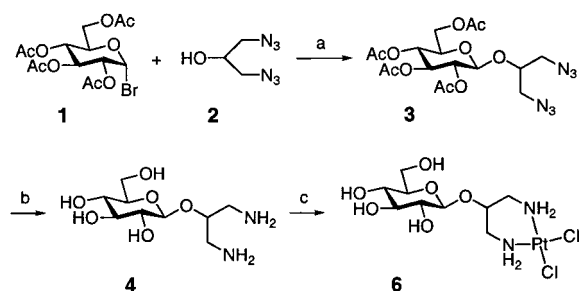
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- [9] Crystal data for **1**: pink crystal, 0.25 × 0.15 × 0.15 mm, monoclinic, space group *P2₁/c*, *a* = 20.7775(10), *b* = 12.3035(6), *c* = 18.8274(6) Å, β = 90.527(1)°, *V* = 4812.8(17) Å³, ρ_{calcd} = 1.275 g cm^{−3}, $2\theta_{\text{max}}$ = 56.5°, *N* = 11 516 (*R*_{int} = 0.072), *N*_o = 7754, *R* = 0.063, *R*_w = 0.132 (all data: *R* = 0.112, *R*_w = 0.147). Crystal data for **2**: pink crystal, 0.25 × 0.13 × 0.13 mm, monoclinic, space group *P2₁/c*, *a* = 13.6803(2), *b* = 21.6001(2), *c* = 25.4376(4) Å, β = 99.445(1)°, *V* = 7414.8(3) Å³, ρ_{calcd} = 1.227 g cm^{−3}, $2\theta_{\text{max}}$ = 60.1°, *N* = 20 450 (*R*_{int} = 0.055), *N*_o = 14 904, *R* = 0.051, *R*_w = 0.108 (all data: *R* = 0.088, *R*_w = 0.129). A total of *N* unique reflections (*N*_o with (*I*) > 2σ(*I*) observed) were collected (Enraf-Nonius CCD, monochromatic MoK α radiation, λ = 0.71073 Å, *T* ≈ 123 K) and used in least-squares refinement (anisotropic *U* for non-hydrogen atoms, H atoms constrained (*x*, *y*, *z*, *U*_{iso})) after structure solution and expansion by Patterson and Fourier techniques. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-112942 (**1**) and -112941 (**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).
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A Carbohydrate-Linked Cisplatin Analogue Having Antitumor Activity**

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Since the antitumor activity of cisplatin was first discovered by Rosenberg et al.,^[1] much effort has been devoted to investigating the interaction of platinum compounds with cellular targets (especially DNA)^[2] and developing more potent cisplatin analogues with improved pharmacological properties.^[3] Of particular interest is the synthesis of platinum(II) complexes with biologically important ligands^[4] because of their reduced toxicity. Although carbohydrates play a key role in various biological processes,^[5] their usage in platinum-based cancer chemotherapy has remained virtually unexplored; this is especially true of complexes in which an intact carbohydrate moiety is connected to platinum through an appropriate linker.^[6] Here we report on the synthesis, characterization, and cytotoxicity of a novel carbohydrate-linked cisplatin analogue, *cis*-dichloro[(2- β -D-glucopyranosyl)propane-1,3-diamine]platinum (**6**, see Scheme 1).

Glycosylation of 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide (**1**) with 1,3-diazidopropan-2-ol (**2**)^[7] was promoted by HgO/HgBr₂ in CH₂Cl₂ to give glycoside **3** (Scheme 1).



Scheme 1. Synthesis of complex **6**. Reagents and conditions: a) **1** (1.0 equiv), **2** (1.5 equiv), HgBr₂ (0.2 equiv), HgO (1.0 equiv), CH₂Cl₂, RT, 2 d in the dark, 63% of **3**; b) cat. NaOMe, MeOH, RT, 6 h, then Pd/C (12 wt %), H₂ (1810 Torr), RT, 8 h, 96% of **4**; c) K₂[PtCl₄] (**5**, 1.0 equiv), H₂O, RT, 2 d, 75% of **6**.

Deacetylation of **3** and subsequent hydrogenation afforded the diamino ligand **4** with an unprotected glucose unit. Compound **4** was treated with a stoichiometric amount of K₂[PtCl₄] (**5**) in water to give **6** within two days. After the coordination reaction was complete, however, the carbohydrate-linked cisplatin analogue remained dissolved in the solution together with the resulting KCl salt. Complex **6** was isolated from the mixture by use of a gel filtration column with Bio-gel P2 resin

(yield 75%). Colorless single crystals were obtained from solutions of purified **6** in D₂O at room temperature.

Complex **6** was characterized by ¹H and ¹³C NMR spectroscopy,^[8] which confirms complex formation in water. New peaks occur at δ = 4.23, 2.86, and 2.68 in the ¹H NMR spectrum, and at δ = 46.2, 45.1 in the ¹³C NMR spectrum for **6** as compared with the noncoordinated ligand **4**.

The X-ray crystal structure analysis of **6**^[9] (Figure 1) reveals that there are two independent molecules in the crystal. In

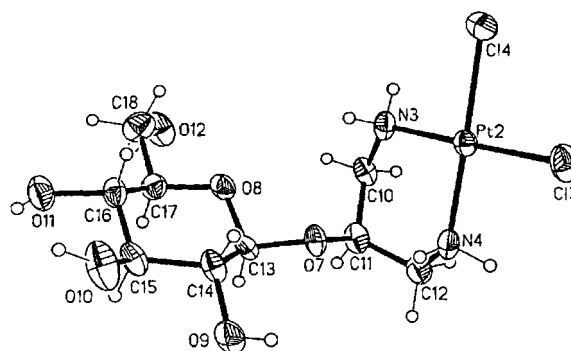


Figure 1. Molecular structure of one of the two molecules of **6** in the crystal. Selected bond lengths [pm] and angles [°]: Pt2–N3 202.3(7), Pt2–N4 202.6(8), Pt2–Cl3 230.2(2), Pt2–Cl4 232.0(2), N3–C10 148.2(12), N4–C12 148.1(12), C10–C11 152.2(13), C11–C12 149.5(14); N3–Pt2–N4 93.3(3), N3–Pt2–Cl3 175.4(2), N4–Pt2–Cl3 87.4(2), N3–Pt2–Cl4 86.3(2), N4–Pt2–Cl4 177.9(2), Cl3–Pt2–Cl4 92.72(9), C10–N3–Pt2 120.9(6), C12–N4–Pt2 118.8(6), N3–C10–C11 111.9(7), C12–C11–C10 113.7(8), N4–C12–C11 111.8(8).

both molecules, the intact and unprotected β -D-glucopyranose moiety is connected to platinum through the 2-propane-1,3-diamine linker group, which acts as a neutral bidentate ligand and coordinates through the two amino groups. The coordination about platinum is roughly square planar as expected, and the six-membered chelate ring is in a chair conformation as is the glucopyranose ring. The mean Pt–Cl and Pt–N distances (231 and 202 pm, respectively) are consistent with those found in PtCl₂N₂ systems.^[10] However, the Cl–Pt–Cl and N–Pt–N angles (92.7 and 93.3°, respectively) present a major difference from those forming five-membered chelate rings,^[11] in which the “bite” of the diamine ligand reduces the N–Pt–N angle to less than 90° with an accompanying increase of the Cl–Pt–Cl angle to more than 90°. The two independent molecules adopt very similar structures except for the conformation of the CH₂OH moiety from the glucose ring, which very likely results from molecular packing controlled by intermolecular hydrogen-bonding interactions. From the crystal structure two types of hydrogen-bonding interactions can be identified: direct hydrogen bonding between two ligand hydroxyl groups, and water-bridged hydrogen bonding from a ligand hydroxyl group to a water molecule and then to another ligand hydroxyl group.

The *in vitro* antitumor activity of complex **6** (Table 1) shows that it is as active as cisplatin against human ovarian cancer cell A2780S and human melanoma cancer cell MeWo, but less active against human ovarian cancer cell A2780cP. Thus with the attachment of an intact and unprotected carbohydrate moiety, **6** exhibits cytotoxicity comparable to that of the parent compound. While more detailed pharmacokinetic study regarding *in vivo* behavior is underway, the retained activity of **6**, together with its improved solubility in water,^[13]

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Table 1. In vitro antitumor activity^[a] of complex **6**.

Cell line	Tumor of origin	MED ^[b] [μ M]	
		6	Cisplatin
A2780S ^[c]	ovarian	2.5	3.3
A2780cP ^[c]	ovarian	112	36
MeWo ^[d]	melanoma	11.2	8

[a] Cytotoxicity was assessed by clonogenic survival assay as described.^[12] [b] Median effect dose. [c] A2780S and its cisplatin-resistant variant, A2780cP, were obtained from Dr. Marshal Sklar (University of Miami) and maintained in Paul G. Braunschweiger's laboratory. [d] Obtained from Dr. Jorgen Fogh and maintained in Paul G. Braunschweiger's laboratory.

should open new avenues to explore this important area of cancer chemotherapy.

Moreover, complex **6** is, to the best of our knowledge, the first example of a carbohydrate-containing transition metal complex in which the carbohydrate moiety is not only unprotected, but also unbound to the metal center.^[6, 14] Such complexes should be obtainable as single crystals, which can then be used to determine the solid-state structure of intact and unprotected carbohydrates.

Experimental Section

6: A mixture of **1** (2.2 g, 5.36 mmol), **2** (1.14 g, 8.0 mmol), and 4-Å molecular sieves (3.5 g) was stirred under Ar for 1 h at RT in CH₂Cl₂ (50 mL), and HgBr₂ (0.39 g, 1.07 mmol) and HgO (1.16 g, 5.36 mmol) were added. After being stirred in the dark for 2 d, the mixture was filtered through a layer of Celite. The filtrate was washed with aqueous NaHCO₃ solution, dried, and concentrated. Chromatography of the residue in hexanes/ethyl acetate (1/1) on silica gel afforded **3** (1.59 g, 63%). Compound **3** (0.78 g, 2.56 mmol) and a catalytic amount of NaOMe were stirred in MeOH (30 mL) for 6 h at RT. The mixture was neutralized by addition of Dowex H⁺ ion-exchange resin and filtered, and Pd/C (60 mg) was added. After being stirred under H₂ (35 psi) for 8 h, the mixture was filtered through a layer of Celite, and the filtrate concentrated. The residue was redissolved in H₂O and lyophilized to give **4** (0.4 g, 96%). Compound **4** (0.18 g, 0.71 mmol) and **5** (0.3 g, 0.71 mmol) were stirred in H₂O (10 mL) for 2 d at RT, and concentrated. Chromatography of the residue on a gel filtration column with Bio-gel P2 resin afforded **6** (0.28 g, 75%).

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- [7] Compound **2** was prepared from 1,3-dibromo-2-propanol by reaction with NaN₃ in DMF.
- [8] **6:** ¹H NMR (500 MHz, D₂O): δ = 4.39 (d, J (H1,H2) = 8.0 Hz, 1H; H1), 4.23 (m, 1H; CH(CH₂NH₂)₂), 3.70 (dd, J (H6a,H6b) = 12.5, J (H5,H6a) = 2.0 Hz, 1H; H6a), 3.55 (dd, J (H6a,H6b) = 12.5, J (H5,H6b) = 5.0 Hz, 1H; H6b), 3.34–3.23 (m, 3H; H3, H4, H5), 3.17 (dd, J (H2,H3) = 9.5, J (H1,H2) = 8.0 Hz, 1H; H2), 2.89–2.84, 2.72–2.65 (2m, 2 $\times$ 2H; CH(CH₂NH₂)₂); ¹³C NMR (125 MHz, D₂O): δ = 102.2 (C1), 76.5, 76.2 (C3, C5), 74.1 (CH(CH₂NH₂)₂), 73.7 (C2), 70.1 (C4), 61.2 (C6), 46.2, 45.1 (CH(CH₂NH₂)₂).
- [9] Crystal structure data (Bruker P4/CCD diffractometer) for **6** · 1.5H₂O: C₉H₂₃Cl₂N₂O_{7.5}Pt, M_r = 545.28, crystal dimensions 0.32 $\times$ 0.22 $\times$ 0.06 mm³, T = 295(2) K, monoclinic, space group $P2_1$, a = 690.390(10), b = 3174.58(6), c = 809.74(2) pm, β = 115.0650(10)°, Z = 4, V = 1.60758(6) nm³, ρ_{calcd} = 2.253 g cm⁻³, MoK α radiation (λ_0 = 0.71073 Å), μ = 9.096 mm⁻¹, 2θ = 2.56–56.68°; of 9952 reflections collected, 6498 were independent ($R(\text{int})$ = 0.031); refinement method: full-matrix least squares on F^2 , 397 refined parameters, empirical absorption correction (SADABS software, T_{min} and T_{max} undefined), GOF = 1.008 (based on F^2), $R1$ = 0.0383, $\omega R2$ = 0.0915 ($\sigma > 2\sigma(I)$), absolute structure parameter 0.007(8), residual electron density –2.559/3.782 e Å⁻³. The structure was solved and refined with the programs SHELXS-93 and SHELXTL. The hydrogen atoms were placed in their geometric positions (riding model), except that no hydrogen atoms were placed in the solvent molecules. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-113805. 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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- [13] Solubility of **6** in water: > 20 mg mL⁻¹ at 25 °C.
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Mixed Crossed Aldol Condensation between Conjugated Esters and Aldehydes Using Aluminum Tris(2,6-diphenylphenoxide)

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Crossed aldol condensation between two different carbonyl compounds is one of the earliest and synthetically most significant reactions for carbon–carbon bond formation.^[1]

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Our recent discovery^[2] that aluminum tris(2,6-diphenylphenoxide)^[3, 4] (ATPH, Figure 1) and lithium diisopropylamide (LDA) trigger mixed crossed aldol condensation of conjugated aldehydes or ketones with aldehydes encouraged us to

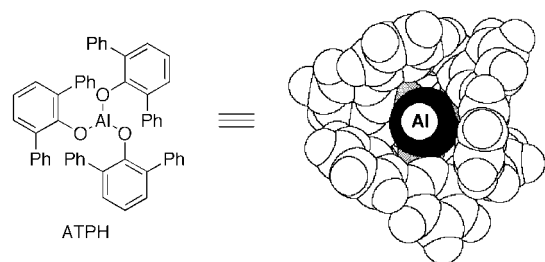
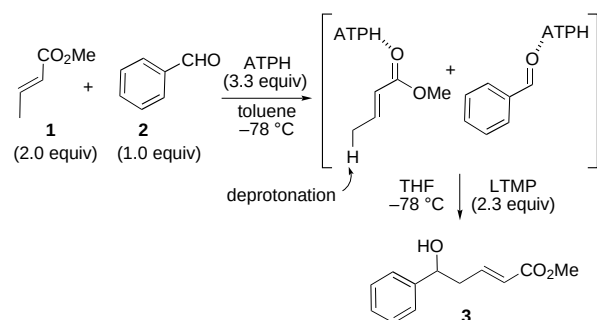


Figure 1. The molecular structure (left) and space-filling model of ATPH (right).

improve this reaction to a more synthetically useful level. Reported herein is the mixed aldol condensation between conjugated esters and aldehydes^[5] in the presence of ATPH; lithium 2,2,6,6-tetramethylpiperidide (LTMP) instead of LDA has proven to be an effective reagent.

Precomplexation of methyl crotonate (**1**, 2.0 equiv) and benzaldehyde (**2**, 1.0 equiv) with ATPH (3.3 equiv) was followed by treatment with a solution of LTMP (2.3 equiv) in THF at -78°C under an argon atmosphere (Scheme 1). After stirring of the mixture at this temperature for 30 min, quenching with aq. NH_4Cl , and purification by column chromatography on silica gel, aldol adduct **3** was obtained in



Scheme 1. Crossed-aldol condensation of methyl crotonate (**1**) with benzaldehyde (**2**) using ATPH and LTMP.

97% yield with retention of the *E* configuration at the olefin moiety (see entry 1 of Table 1). None of the *Z* isomer was detected by ^1H NMR spectroscopy or GC-MS analysis.

Similar to the case of the conjugated aldehyde–aldehyde aldol system,^[2] deprotonation and subsequent alkylation occurred exclusively at the allylic terminus of the unsaturated ester, and not at the α -carbon atom. The use of a smaller amount of the ester, ATPH, and LTMP (1.0:2.2:1.2) proved disappointing (yield of **3** 57%). Under otherwise identical conditions, the reaction with other lithium amides such as LDA, lithium hexamethyldisilazide (LHMDS), and lithium dicyclohexylamide proceeded less efficiently than with LTMP, producing **3** with consistently lower yields of 33, 23, and 42%, respectively. It should be emphasized that the ester and aldehyde must be precomplexed with ATPH: when benzaldehyde (**2**) was exposed to the ATPH complex (-78°C , 5 min) prior to treatment with LTMP, the desirable aldoliza-

tion was obtained with similar ineffectiveness (recovery of **2** 90%, yield of **3** 10%).

We thus chose to focus on the use of LTMP, and various examples of ester–aldehyde combinations are listed in Tables 1 and 2. Benzaldehyde (**2**) and pivalaldehyde (**22**) were particularly well suited to this process. In contrast, valeraldehyde (**24**) might suffer from competitive deprotonation at the α -carbon atom. This difficulty was easily circumvented when cyclohexanecarboxaldehyde (**23**) branched at the α -carbon atom was used to provide **28** (90%) and **29** (87%) with equal effectiveness. Aldolization at the γ -positions of lactones proceeded with similar success, despite low diastereoselectivities (Table 1, entries 4 and 5). Highly conjugated esters including dienolate **8**, trienolate **9**, tetraenolate **10**, and even pentaenolate **11** participated in this transformation; however, the reaction proceeded less readily with **11** to give **20** in moderate yield (Table 1, entries 9–12).^[6]

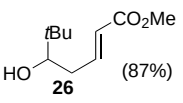
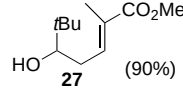
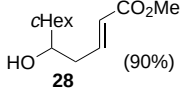
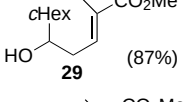
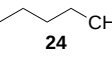
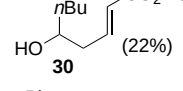
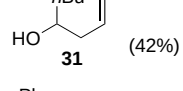
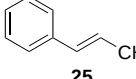
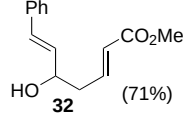
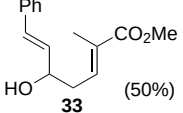
One area where this system could be applied with particular benefit is the rapid elaboration of elongated conjugated esters, which have been demonstrated to be of great synthetic potential.^[7] For example, tosylation of aldol adduct **34**, which was obtained in 93% yield by the standard procedure (see

Table 1. Mixed aldol condensation of unsaturated esters with benzaldehyde (**2**).

Entry ^[a]	Ester	Product	Yield [%] ^[b]
1			97
2			90
3			72 ^[c]
4			88 ^[d]
5			70 ^[e]
6			97
7			88
8			80
9			40 ^[f]
10			86

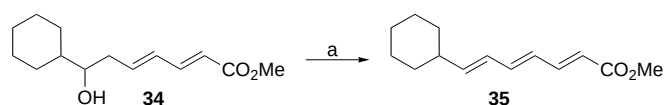
[a] The reaction was performed using aldehyde (1 equiv), ester (2 equiv), ATPH (3.3 equiv), and LTMP (2.3 equiv) in toluene/THF (1/1) at -78°C for 30 min. [b] Yield of isolated, purified products. [c] Diastereomeric ratio = 76:24. [d] Diastereomeric ratio = 70:30. [e] Diastereomeric ratio = 66:33. [f] An unidentified isomer was also obtained (23%).

Table 2. Mixed aldol condensation of various aldehydes with conjugated esters.^[a, b]

aldehyde \ ester	1	4
 22	 26 (87%)	 27 (90%)
 23	 28 (90%)	 29 (87%)
 24	 30 (22%)	 31 (42%)
 25	 32 (71%)	 33 (50%)

[a] The reaction was performed using aldehyde (1 equiv), ester (2 equiv), ATPH (3.3 equiv) and LTMP (2.3 equiv) in toluene/THF (1/1) at -78°C for 30 min. [b] The yields in parentheses are of isolated, purified products.

Experimental Section), followed by elimination with DBU at 90°C gave (2E,4E,6)-trienoate **35**, one of the synthetic intermediates for asukamycin,^[7a] in 90% yield (6E:6Z = 97:3, Scheme 2).



Scheme 2. A rapid route to **35**, the synthetic intermediate of asukamycin. Reagents and conditions: a) 4-methylbenzenesulfonyl chloride (TsCl, 2.0 equiv), 4-(dimethylamino)pyridine (DMAP, 5.0 equiv), CH_2Cl_2 , 25°C , 18 h, 90%; 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 5 equiv), toluene, 90°C , 5 h, >99%.

In summary, a directed access to δ -hydroxy- α,β -unsaturated esters and their derivatives using two different ATPH–carbonyl complexes in place of carbonyl compounds themselves has been demonstrated. Such a strategy has proven to be quite general with respect to both the aldehyde component and the unsaturated esters. Moreover, this method should be of practical value as a straightforward route to important classes of synthetic intermediates such as highly polyenic esters, and thus potentially useful for a short-step convergent approach to complex natural products.^[8]

Experimental Section

The reaction of methyl crotonate (**1**) with benzaldehyde (**2**) is representative. To a solution of ATPH (1.65 mmol) in toluene (6.0 mL) was added **1** (106 μL , 1.0 mmol) and **2** (51 μL , 0.50 mmol) at -78°C under argon. After the mixture was stirred for 20 min, LTMP—generated by treatment of a solution of 2,2,6,6-tetramethylpiperidine (194 μL , 1.15 mmol) in THF (6.0 mL) with a 1.60 M solution of *n*BuLi (0.72 mL, 1.15 mmol) in hexane at -78°C for 30 min—was transferred by a steel cannula to the solution of ATPH-**1** and ATPH-**2** in toluene at -78°C . The reaction mixture was stirred at this temperature for 30 min and quenched with aq NH_4Cl , and the resulting suspension was filtered through a celite pad. The filtrate was extracted with diethyl ether. The organic layer was dried over Na_2SO_4 and

concentrated, and the residue was purified by column chromatography on silica gel (diethyl ether/hexane 1/2 $\rightarrow$ 1/1 $\rightarrow$ 5/1) to give **3** (99.2 mg, yield 97%) as a colorless liquid. 2,6-Diphenylphenol could be isolated in more than 90% yield before the aldol product came off the column. The obtained aldol adduct **3** showed identical spectral and analytical data with those reported in the literature.^[9]

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Axial and Equatorial Hydrogen Bonds in the Tetrahydropyran...HCl Dimer**

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Hydrogen-bond complexes are formed in the gas phase by collisional association of the components upon adiabatic expansion of a high-pressure gas mixture through a nozzle into a vacuum. When the frozen dimers are in collisionless expansion, their rotational spectrum can be observed by conducting pulsed-microwave Fourier transform spectroscopy on the jet within a Fabry–Perot cavity. We report here the first observation of the axial and equatorial hydrogen-bond complexes formed in the gas phase between tetrahydropyran (THP) and hydrogen chloride. The properties of the dimers, especially their geometry, can be readily determined from the rotational spectrum.

Gas-phase molecular structures for binary hydrogen-bonded complexes have traditionally been obtained with molecular beam Fourier transform microwave spectroscopy.^[1, 2] Virtually all of the dimers B...HX (where the acceptor atom in B is O, S; and X=F, Cl, Br, I) characterized so far contain monomers B with two equivalent nonbonding electron pairs. As a result of the investigation of a large number of complexes, it has become possible to enunciate some simple rules^[3] for predicting geometries based on the nonbonding electron pair model of the acceptor molecule B. The hydrogen atom in HX is electrophilic and is assumed to seek the most nucleophilic site of B, which will be along the direction of the nonbonding electron pairs. We have recently studied hydrogen-bonded complexes of five-membered heteroatomic rings such as tetrahydrothiophene...HCl,^[4] tetrahydrothiophene...HF,^[5] and tetrahydrofuran...HCl.^[6] In accordance with these electrostatic rules, two equivalent equilibrium conformations having pyramidal configurations at O or S have been found.

The six-membered ring THP has two nonequivalent lone electron pairs at the O atom in the axial and equatorial positions. We therefore expect two different hydrogen bonds for the THP...HCl complex (Figure 1). To confirm these expectations a detailed experimental investigation of THP...HCl has been carried out by molecular beam Fourier transform microwave spectroscopy.^[7] The rotational spectrum is very sensitive to molecular conformation, so the axial and equatorial forms can be independently studied, just as in the case of a mixture of stable, nonreactive species.

Trial rotational constants of the complexes were estimated from the structures of the monomers THP^[8, 9] and HCl,^[10] according to the above models. The geometrical parameters

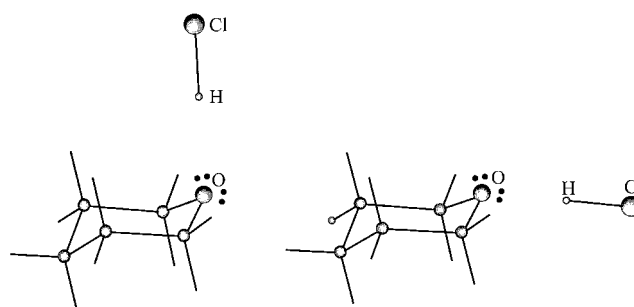


Figure 1. Representation of the two different hydrogen bonds expected in the THP...HCl complex: axial (left) and equatorial (right).

of the hydrogen bond found for the related tetrahydrofuran...HCl^[6] dimer were used to describe the separation and relative orientation of the subunits. The vector sum of the electric dipole moments of the component molecules predicts a strong μ_a -type spectrum and a weak μ_c -type spectrum with the pattern of a nearly prolate asymmetric rotor. Initially argon was used as the carrier gas and the observed spectrum consisted of groups of μ_a -type, R-branch lines separated by about 1200 MHz, which accounts for the B + C value of the axial form. We scanned wide ranges of frequency, and no line presumably belonging to the equatorial conformer was detected. In the hope of observing the spectrum of the equatorial form, a further search was performed with helium as carrier gas. Thus, new bands of weaker intensity appeared along with those attributed to the axial form, and they were readily assigned to the equatorial dimer. In addition to the ground-state rotational spectra of the most natural abundant isotopomers THP...H³⁵Cl and THP...H³⁷Cl, spectra were also measured for both axial and equatorial conformers of the THP...D³⁵Cl species. All the transitions exhibit a characteristic quadrupole hyperfine structure. It arises from the interaction of the electric quadrupole moment of ³⁵Cl or ³⁷Cl with the electric field gradient at the Cl nucleus that couples the Cl nuclear spin angular momentum **I** with angular momentum resulting from the overall rotational **J** to give the total angular momentum **F** = **I** + **J**.

The transition frequencies of each isotopomer were fitted in an iterative least-squares analysis^[11] based on the Hamiltonian in Equation (a), where **H_R** is the operator associated with the energy of the semirigid rotor given in the I^r representation of the A-reduced Watson Hamiltonian by Equation (b).^[12] The coefficients A, B, and C are the rotational constants, and Δ_J , Δ_{JK} , Δ_K , δ_J , and δ_K are the quartic centrifugal distortion constants. Hamiltonian **H_Q** describes the interaction of the Cl nuclear electric quadrupole moment with the electric field gradient at the Cl nucleus through the tensors **Q**(Cl) and **V**(Cl) according to Equation (c).^[13] The extent of the hyperfine splittings is described in terms of the elements of the quadrupole coupling tensor, which are related to the electric field gradient at the Cl nucleus by Equation (d) (α , β to be permuted over the principal inertial axes *a*, *b*, and *c*).

$$H = H_R + H_Q \quad (a)$$

$$H_R = A P_x^2 + B P_y^2 + C P_z^2 - \Delta_J P^4 - \Delta_{JK} P^2 P_z^2 - \Delta_K P_z^4 - 2 \delta_J P^2 (P_x^2 - P_y^2) - 2 \delta_K [P_z^2 (P_x^2 - P_y^2) + (P_x^2 - P_y^2) P_z^2] \quad (b)$$

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$$\mathbf{H}_Q = -\frac{1}{2}Q(\text{Cl}):V(\text{Cl}) \quad (\text{c})$$

$$\chi_{a\beta} = -\left(\frac{eQ}{h}\right)\frac{\partial^2 V}{\partial a \partial \beta} \quad (\text{d})$$

The spectroscopic constants are reported in Table 1. The magnitudes of the planar moments $P_b = \frac{1}{2}(I_a - I_b + I_c)$ which depend on only the b coordinates are also included. Only one of the three possible off-diagonal elements of the nuclear quadrupole tensor χ_{ac} was necessary to produce the high quality of the root mean square (rms) deviation of the fits, suggesting, but not proving, that $\chi_{ab} = \chi_{bc} = 0$.

Several arguments indicate that the axial and equatorial hydrogen-bond complexes have C_s symmetry. The high degree of invariance of P_b among the two complexes and their isotopomers shows that the H and Cl atoms lie in the ac plane of the dimers. Additionally, the near identity of these quantities and the planar moment $P_b = 94.944 \text{ amu } \text{\AA}^2$ ^[9] of the THP monomer is strong evidence that the ac principal inertial planes of the complexes are also symmetry planes. The small differences may be attributed to changes in the zero-point motion on formation of the complexes. The conclusion is then that axial and equatorial THP...HCl complexes have C_s symmetry. This conclusion is also consistent with the need to include only the off-diagonal element χ_{ac} in the fits and with the fact that the ratios $^{35}\chi_{bb}/^{37}\chi_{bb} = 1.2688(10)$ for the axial complex and $^{35}\chi_{bb}/^{37}\chi_{bb} = 1.270(6)$ for the equatorial complex are in good agreement with the theoretical value $^{35}Q/^{37}Q = 1.26878$.^[14] From Equation (d) and considering that $^{35}\text{Cl}/^{37}\text{Cl}$ substitution does not affect the electric field gradient along the b axis, which is normal to the symmetry plane, the ratio $^{35}\chi_{bb}/^{37}\chi_{bb}$ only depends on the quadrupole moment ratio $^{35}Q/^{37}Q$.

On the basis of the above results, the rotational parameters given in Table 1 were used to determine the structures of the dimers. We proceeded by assuming that the structures of THP^[8] and HCl^[10] are unperturbed upon formation of the complexes. The rotational constants were found to have a small dependence on the position of the hydrogen atom, so initially a collinear arrangement of the O...H-Cl system was

considered. A first fit was then performed to determine the O...Cl distance and the angle ϕ between the O...Cl line and the line bisecting the C-O-C angle (Figure 2). The position of the hydrogen atom is defined by the angle α_{az} between the a

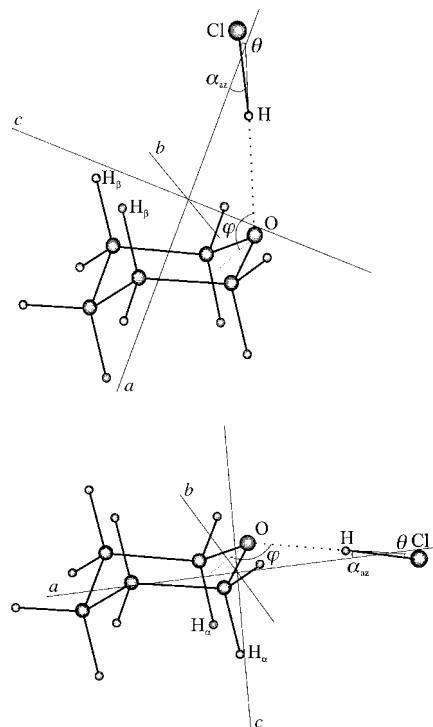


Figure 2. Structure of the axial (top) and equatorial conformers (bottom) of tetrahydropyran...HCl with relevant planes and angles defined.

inertial axis and the HCl bond (z). This angle can be determined from the value of the quadrupole coupling constants, taking into account its remarkable sensitivity to the direction of the HCl axis in the dimer. Thus, the quadrupole coupling tensor can be transformed to its principal axis system by rotation of 31.11° and 11.25° around the b axis for the axial and equatorial forms, respectively.

Table 1. Spectroscopic constants for axial and equatorial tetrahydropyran...HCl complexes.

Parameter	THP...H ³⁵ Cl	THP...H ³⁷ Cl	THP...D ³⁵ Cl	THP...H ³⁵ Cl	Equatorial THP...H ³⁷ Cl	THP...D ³⁵ Cl
A [MHz]	2942.868(49) ^[a]	2939.423(58)	2937.361(52)	4605.689(23)	4605.378(33)	4599.112(24)
B [MHz]	1106.43074(28)	1074.72568(32)	1102.37779(30)	863.49935(27)	836.95830(38)	860.20399(30)
C [MHz]	1061.59199(27)	1032.79626(30)	1058.55392(29)	757.56335(25)	737.05928(30)	755.20535(26)
Δ_J [kHz]	1.57477(91)	1.5041(10)	1.5458(10)	0.51530(64)	0.49212(75)	0.51302(72)
Δ_{JK} [kHz]	0.10304(53)	0.109(12)	0.082(14)	4.6203(77)	4.369(30)	4.180(12)
Δ_K [kHz]	-36.2(23)	[-36.2] ^[b]	[-36.2] ^[b]	[0.0] ^[b]	[0.0] ^[b]	[0.0] ^[b]
Δ_J [kHz]	-0.1052(14)	-0.0964(16)	-0.1047(16)	0.04582(53)	0.04234(51)	0.04389(53)
Δ_K [kHz]	[0.0] ^[b]	[0.0] ^[b]	[0.0] ^[b]	3.936(82)	3.65(12)	3.609(89)
P_b [amu Å ²] ^[c]	95.5113(15)	95.5111(18)	95.5158(16)	95.78601(47)	95.78899(67)	95.78487(50)
χ_{aa} [MHz]	-29.7191(37)	-23.6826(51)	-30.1721(42)	-46.5757(67)	-36.741(62)	-47.1913(77)
χ_{bb} [MHz]	24.5061(63)	19.315(10)	24.9027(74)	24.619(12)	19.377(85)	25.004(12)
χ_{cc} [MHz]	5.2130(63)	4.367(10)	5.2694(74)	21.956(12)	17.364(85)	22.187(12)
$ \chi_{ac} $ [MHz]	33.15(50)	25.83(63)	33.66(64)	14.20(14)	11.154(94)	14.63(45)
σ [kHz] ^[d]	1.7	1.6	1.6	1.5	1.3	1.5
N ^[e]	211	168	171	232	145	182

[a] Standard errors in parentheses are given in units of the last digit. [b] This parameter was kept fixed in the fit. [c] Conversion factor 505379.1 MHz amu Å². [d] Standard deviation of the fit. [e] Number of fitted frequencies.

These values can be taken as a good approximation to the α_{az} angles. In a subsequent least squares fit for each conformer, the fitted geometries were constrained so that they reproduce the above α_{az} angles. The results are listed in Table 2. The

Table 2. Structural parameters of axial and equatorial tetrahydropyran...HCl complexes; see Figure 2.

Parameter	Axial	Equatorial
$r(\text{O} \cdots \text{Cl}) [\text{\AA}]$	3.133(10) ^[a]	3.021(10)
$r(\text{O} \cdots \text{H}) [\text{\AA}]$	1.851(16)	1.740(15)
$r(\text{Cl} \cdots \text{H}_\beta) [\text{\AA}]$	3.41(3)	–
$r(\text{Cl} \cdots \text{H}_a) [\text{\AA}]$	–	3.83(3)
$\phi [^\circ]$	124.1(10)	136.(2)
$\varphi [^\circ]$	125.9(13)	138.4(13)
$\theta [^\circ]$	4.(4)	5.(3)
$\alpha_{\text{az}} [^\circ]$	31.11(18) ^[b]	11.25(11) ^[b]

[a] Standard errors in parentheses are given in units of the last digit.

[b] Values corresponding to the parent isotopic species.

derived values for the hydrogen-bond distance $r(\text{O} \cdots \text{H})$, the angle φ formed between the line bisecting C–O–C angle and the line of the $\text{O} \cdots \text{H}$ bond, and the nonlinearity angle θ are also included.

The observed structures of both conformers can be understood through a simple electrostatic model involving the interaction of the axial and equatorial nonbonding electron pairs on O with the electrostatic region ^+H of the Lewis acid HCl. Thus, we can relate the experimental angles φ to the simple nonbonding pair model of the acceptor molecule THP. On the other hand, the nonlinearity of the system $\text{O} \cdots \text{H} \cdots \text{Cl}$ involved in the hydrogen bond is very small for both axial and equatorial forms (see θ values in Table 2). In this way, we show how the direction of the HCl molecule in the $\text{THP} \cdots \text{HCl}$ complexes acts as a probe for the direction of the nonbonding electron pairs on THP. The angle φ was found to be 12.5° larger for the equatorial form.

Another interesting point is the conformational preference of the axial and equatorial hydrogen-bond complexes. Rough calculations of the dipole moment gave similar values for the a component of the dipole moment, so the fact that the rotational spectra of only the axial form was detected with argon as carrier gas is strong evidence that the axial conformer is the most stable one. In contrast, a preference for the equatorial form is found if we accept that the shortening of the $\text{O} \cdots \text{H}$ distance (of about 0.1 Å) for the equatorial conformer can be related to an increase in bond strength of the primary hydrogen bond.

Usually, the stability of a certain conformer can be explained in terms of a balance between the repulsive and attractive atom–atom interactions. In a monosubstituted cyclohexane the equatorial conformer was always found to be the most stable one (i.e. with $\text{F}^{[15]}$ and $\text{Cl}^{[16]}$), and the lower stability of the axial conformer is ascribed to the steric repulsion between the substituent and the β -axial H atoms of the ring. However, in $\text{THP} \cdots \text{HCl}$ complexes, the distances between the HCl subunit and the ring hydrogen atoms are much longer. In this way, the repulsive interactions are less important, and the conformational preference may be controlled by other second-order interactions, such as a secondary hydrogen bond between ^-O of HCl and the nearest H

atoms of the ring. The large value of the φ angle for the equatorial form causes ^-O to be sufficiently far from the ring hydrogen atoms to make any attraction small. Thus, the nearly zero value of θ for the equatorial conformer provides evidence of a nonsignificant secondary interaction. On the other hand, the calculated distance between Cl and the H atoms of the β -methylene groups is 3.41(3) Å for the axial form (see Figure 1), which is very close to the secondary hydrogen bond length found for the related 2,5-dihydrofuran...HCl (3.28 Å)^[17] and tetrahydrofuran...HCl complexes (3.22 Å).^[6] Thus, the small nonlinearity found for the axial conformer does not exclude secondary interactions which may stabilize the axial conformer.

Experimental Section

The molecular beam Fourier transform microwave spectrometer in the range 6–18 GHz used in this study has already been described elsewhere.^[18] All samples are commercially available and have been used without further purification. Gas mixtures of 1–2 % tetrahydropyran and ≈ 4 % hydrogen chloride or deuterium chloride in argon or helium at a total pressure of about 1.5 bar were pulsed into the evacuated Fabry–Perot cavity. Suitably delayed microwave pulses of 0.2 μs duration and 40 mW peak power induce then a macroscopic polarization in the complexes. The subsequent molecular emission signal was digitized in the time domain, and the frequencies were determined after Fourier transformation of the 8k data points with a sample interval of 40 ns. The accuracy of frequency measurements is estimated to be better than 5 kHz.

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K₆Pb₈Cd: A Zintl Phase with Oligomers of Pb₄ Tetrahedra Interconnected by Cd Atoms**

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Until recently, large deltahedral clusters were known in the solid state only for the boron group.^[1] For Group 14, such clusters with more than four atoms were characterized only in solution or in compounds crystallized from such solutions with the aid of cryptated alkali metal counteranions.^[2] The recently reported phases A₄E₉ and A₁₂E₁₇ (A = K, Rb, or Cs; E = Si, Ge, Sn, or Pb) contain isolated E₉⁴⁻ deltahedra and extend the range of existence of large clusters of this group to the solid state.^[3] According to Wade's rules for counting electrons,^[4] the charge on a deltahedral cluster of a Group 14 element is independent of its nuclearity, and therefore large clusters carry relatively small negative charges. Hence, only a few cations are needed to balance the charge, and they may not adequately shield and separate the larger clusters. In solutions and in crystals grown therefrom the clusters are separated by large organic cations, cryptated alkali metal cations, and/or solvent molecules. In "neat" solids,^[5] however, the available cations are of limited dimensions, and alternative ways to resolve the problem must be found. One such method is to increase the charge on the cluster and therefore the number of required counteranions by substitution with an atom of an electron-poorer element. This approach was used in an attempt to substitute a germanium atom of Ge₉⁴⁻ by zinc and led to the synthesis of Cs₆Ge₈Zn.^[6] In this compound two eclipsed germanium tetrahedra are interconnected by the zinc atom, which caps a face of each tetrahedron and is thus surrounded by six germanium atoms in a trigonal prismatic arrangement. The analogous attempt to replace a lead atom in Pb₉⁴⁻ by cadmium led to the synthesis of the title compound K₆Pb₈Cd, which contains oligomers of cadmium-linked Pb₄ tetrahedra.

Isolated Pb₉⁴⁻ clusters have been structurally characterized in K₄Pb₉ and Cs₄Pb₉.^[3b, c] Since substitution of one lead atom by a cadmium atom would result in a cluster charge of 6-, a reaction designed to produce K₆[Pb₈Cd] was carried out.^[7] The formula of the resulting compound corresponds to the reaction stoichiometry, but its structure is entirely different from that intended.^[8] The compound does not contain nine-atom deltahedral clusters. Instead, it contains isolated lead tetrahedra and isolated oligomers of four lead tetrahedra interconnected by cadmium atoms. In the solid state, isolated tetrahedral anions are found in many compounds.^[9] All binary compounds of the alkali metals with Group 14 elements A₄E₄ (except for C), BaSi₂,^[9] Na₂In and Na₂Tl,^[10] and the mixed-cation ternary phases K₃LiSi₄, Cs₂Na₂Ge₄, K₇LiSi₈, Rb₇NaGe₈, and K₇NaGe₈,^[11] contain such tetrahedra. More recently,

one-dimensional polymers of tetrahedra linked by gold atoms were characterized in A₃[M₄Au] (M = Sn or Pb; A = K, Rb, or Cs) and K₄[(TlSn₃)Au].^[12] Each tetrahedron in these infinite chains is bound to two gold atoms by two *trans* edges, and each gold atom is bonded to two tetrahedra (to two orthogonal edges).

K₆Pb₈Cd contains both isolated tetrahedra, like those in K₄Pb₄ as well as parts of chains (Figure 1). The latter consist of four lead tetrahedra that are linked by three bridging cadmium atoms (Figure 2). The oligomer

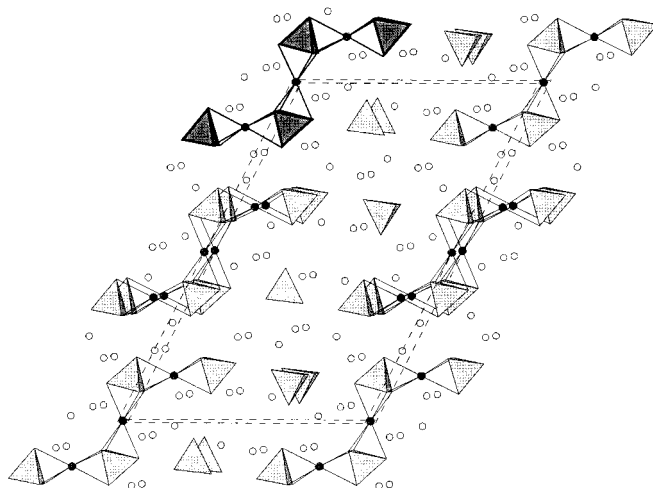


Figure 1. A polyhedral representation of the structure of K₆Pb₈Cd along the *b* axis (*c* is horizontal) of the C-centered monoclinic unit cell (outlined). The isolated Pb₄ tetrahedra and the oligomers of (Pb₄)₄Cd₃ are clearly visible. Filled and open circles represent Cd and K, respectively. The Cd–Pb bonds are indicated by solid lines. One oligomer (see Figure 2) is emphasized.

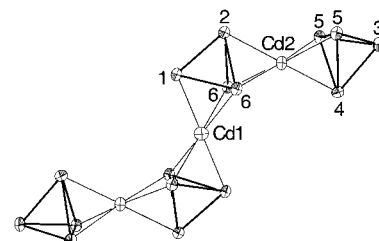


Figure 2. An ORTEP plot of the (Pb₄)₄Cd₃ oligomer (90% probability thermal ellipsoids). The numbers correspond to the lead atoms. Selected distances [Å]: Cd1–Pb1 3.086(2), Cd1–Pb6 3.127(2), Cd2–Pb2 3.129(4), Cd2–Pb6 3.157(3), Cd2–Pb4 3.101(4), Cd2–Pb5 3.064(3), Pb1–Pb2 3.025(3), Pb1–Pb6 3.200(2), Pb6–Pb2 3.153(2), Pb6–Pb6 3.344(3), Pb4–Pb3 3.024(3), Pb4–Pb5 3.249(2), Pb5–Pb3 3.020(2), Pb5–Pb5 3.273(3).

Pb₄CdPb₄CdPb₄CdPb₄ differs from the gold-containing chains in that the Pb₄ tetrahedra are coordinated to the cadmium atoms through faces as η³ ligands. The connectivity is similar to that in the zinc-connected dimers of Ge₄ tetrahedra in Cs₆Ge₈Zn with the difference that the germanium tetrahedra are eclipsed while the lead tetrahedra are staggered.^[6] This results in trigonal-antiprismatic (octahedral) geometry at the cadmium center as opposed to the trigonal-prismatic coordination geometry of the zinc atom. The oligomer has an inversion center at the central cadmium atom, Cd1, and pseudo-inversion centers at the other two cadmium atoms, Cd2. The faces that are coordinated to cadmium are larger

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than the other faces of the tetrahedra. The average Pb–Pb distances are 3.228(3) Å for the former, and 3.022(3) Å for the latter. The same effect is observed in Cs₆Ge₈Zn and indicates relatively strong covalent bonding between the tetrahedra and the interconnecting atoms (see below). The average Cd–Pb distances are virtually identical for Cd1 and Cd2 (3.113(2) and 3.112(2) Å, respectively) and compare well with the Pauling single-bond length of 2.884 Å.^[13] The oligomers are stacked along the *b* axis, and Pb5 and Pb6 form “chains” along this axis (Figure 2). The interatomic distances within these chains alternate, shorter within the oligomers and longer between them. The openness of the capped faces results in relatively short interoligomer distances (Pb6–Pb6 3.568(3), Pb5–Pb5 3.639(3) Å). These are comparable with the corresponding intraoligomer distances of 3.344(3) and 3.273(3) Å for the same pairs of atoms. The result is a significant interaction between the oligomers, which leads to essentially metallic behavior (see below).

Another way to look at these unique oligomers is to regard them as coordination compounds of cadmium with two η³ ligands. The tetrahedra act as mono- or bidentate ligands by using one or two of their four faces, respectively. This suggests that branched structures in which three or even four faces are used might be also possible. Similarly, polydentate tetrahedra coordinated through edges to more than two transition metals can be envisioned as well.

The electronic structure of K₆Pb₈Cd was studied by extended Hückel calculations (potassium excluded).^[14] The average bond overlap population (BOP) of 0.192 for the Cd–Pb bonds compares well with that of the Pb–Pb bonds in the capped faces (0.146) and indicates significant covalency. This is in agreement with calculations on Ge₈Zn, which showed highly covalent Zn–Ge bonds with a BOP of 0.299.^[6] The interactions between the oligomers are quite covalent,

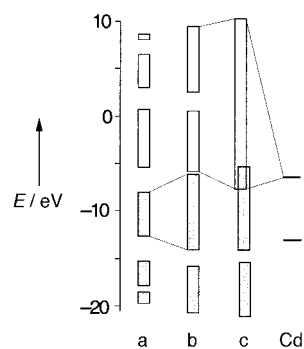
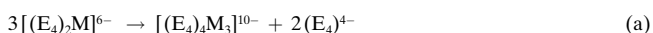


Figure 3. Schematic representation of the results from extended Hückel calculations on well-separated oligomers without Cd atoms (a), the oligomers at the real distances without Cd (b), and the real oligomers with inclusion of the Cd atoms (c). The interactions between the oligomers cause broadening of the valence band (a→b), while the Cd–Pb interactions lead to broadening of the conduction band and its overlap with the filled states (b→c). The energy levels of cadmium are shown on the right.

with an average BOP of 0.171 for the interoligomer Pb6–Pb6 and Pb5–Pb5 bonds. Due to the specific position of the Pb5 and Pb6 atoms, the bond overlap population is larger than that of the intraoligomer bonds even though the interatomic distances are longer. The interactions between the oligomers and between cadmium and the tetrahedra lead to a density of states with no gap at the Fermi level. Both calculations on the Pb sublattice only (all tetrahedra included) and on the real structure but with larger interoligomer separations show well-defined gaps between the valence and conduction bands (Figure 3). Additionally, the calculations show that the interac-

tions between the oligomers lead to broadening mainly of the valence band (Figure 3a, b), while the introduction of cadmium–lead interactions results in broadening of the conduction band (Figure 3c). Consequently the compound is expected to be metallic (and appears to be so^[15]) not due to a partially filled band, but because of overlap between bands.^[16]

Finally, an interesting comparison can be made between the title compound and Cs₆Ge₈Zn. Both compounds have the same stoichiometry but one consists exclusively of bridged dimers only, while the other is composed of bridged tetramers and isolated tetrahedra. This reminds one of a disproportionation reaction of the type (a).



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- [8] Single crystals were selected, mounted in glass capillaries, and checked for singularity on a CAD4 single-crystal diffractometer. Data from one of them (barlike, 0.22 × 0.08 × 0.06 mm) were collected with monochromated MoK_α radiation at 21 °C (ω/2θ scans, 2θ_{max} = 50°, a hemisphere). The data were corrected for Lorentzian and polarization effects and for absorption (ψ scans). The structure was solved by direct methods in the space group *C2/m* and refined on *F*² (SHELXTL 5.0). The lattice parameters were refined from a Guinier powder pattern with Si as an internal standard. Crystal data: *a* = 28.80(1), *b* = 6.912(2), *c* = 18.576(8) Å, β = 117.05(3)°, *Z* = 6; ρ_{calcd} = 6.065 g cm⁻³; μ = 631.64 cm⁻¹; residual electron density +4.56/–4.16 e Å⁻³ at 0.67/1.15 Å from Pb2/Pb1, respectively. The refinement of all atoms with anisotropic thermal parameters converged to *R*1 = 0.0631, *wR*2 = 0.1524 (*I* > 2σ(*I*)) for 1977 observed reflections and 128 variables. Further details of the crystal structure investigations may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de) on quoting the depository number CSD-410552.
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- [15] The crystals have metallic luster and appearance, and two-probe conductivity measurements showed virtually only contact resistance, that is, the same resistance as when the probes are short-circuited.
- [16] While the manuscript was being reviewed, we synthesized and characterized the isostructural $\text{Rb}_6\text{Pb}_8\text{Cd}$ ($a = 29.398(7)$, $b = 7.142(1)$, $c = 19.052(9)$ Å, $\beta = 116.18(2)^\circ$). Although the tetramers are better separated by the larger Rb cations, this compound is also predicted to be metallic. The extent of the broadening of the valence band due to interligand interactions is smaller, but since the Cd–Pb interactions are virtually the same as in $\text{K}_6\text{Pb}_8\text{Cd}$ the conduction band is wide enough to overlap with the lower bands.

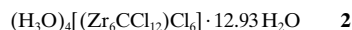
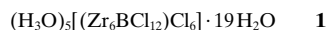
Isolation of Reduced Zirconium Chloride Clusters $[(\text{Zr}_6\text{CCl}_{12})\text{Cl}_6]^{4-}$ and $[(\text{Zr}_6\text{BCl}_{12})\text{Cl}_6]^{5-}$ from Acidic Aqueous Solution**

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Because reduced zirconium compounds are easily oxidized to Zr^{IV} products, no aqueous-solution chemistry of reduced zirconium compounds (oxidation state ≤ 3) is known.^[1, 2] However, a rich solid-state chemistry of centered zirconium halide clusters $[(\text{Zr}_6\text{ZX}_{12})\text{X}_6]^{n-}$ ($\text{Z} = \text{H, Be to N, Al to P, Mn to Ni}$; $\text{X} = \text{Cl, Br, I}$), in which reduced zirconium forms metal–metal bonds, was developed by Corbett et al. in the 1980s and early 1990s.^[3–7] In a preliminary investigation of the dissolution of these cluster-based phases, it was assumed that, as good reducing agents, all $(\text{Zr}_6\text{ZX}_{12})$ -based clusters would undergo oxidation and solvolysis with water, alcohols, and acetone.^[8]

We recently found that solutions of $\text{Rb}_5\text{Zr}_6\text{Cl}_{18}\text{B}$ in methanol at room temperature are stable indefinitely^[9] and that an aqueous solution of $\text{Rb}_5\text{Zr}_6\text{Cl}_{18}\text{B}$ exhibited the characteristic red color of the $[(\text{Zr}_6\text{B})\text{Cl}_{12}]^+$ ion for several hours at room temperature. This led us to more closely examine the reactivity of these zirconium chloride clusters in water. Here we report the isolation of the two reduced zirconium compounds **1** and **2** from aqueous media. We also

present preliminary electrochemical and NMR data for $[\text{Zr}_6\text{ZCl}_{12}]^{m+}$ clusters ($\text{Z} = \text{C}, m = 2$; $\text{Z} = \text{B}, m = 1$) in aqueous solution.



The cluster unit of **1**, $[(\text{Zr}_6\text{BCl}_{12})\text{Cl}_6]^{5-}$, is centered on the cell origin and has perfect O_h symmetry, with Zr–Zr and Zr–B distances of 3.2519(8) and 2.2994(5) Å, respectively. These distances are consistent with those previously reported for B-centered clusters with 14 cluster bonding electrons (CBEs).^[9–12] When crystals of **1** are redissolved in deoxygenated water or methanol, the ^{11}B NMR spectra of the solutions show sharp signals, a further indication that the clusters are not oxidized. Hence, the cluster bears a charge of -5 , and a requisite number of counteranions is required for charge balance. A fragment of the crystal structure of **1** is presented in Figure 1 a.

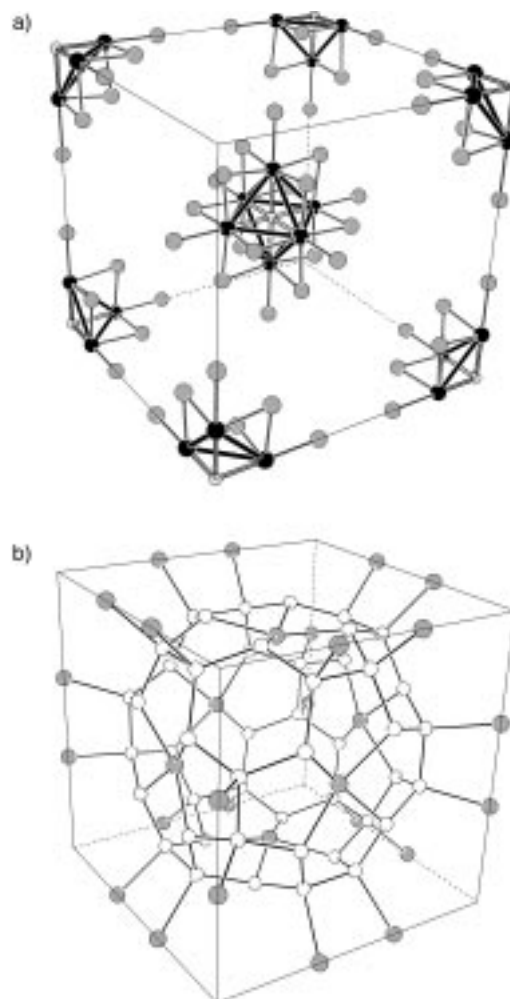


Figure 1. a) $[(\text{Zr}_6\text{BCl}_{12})\text{Cl}_6]^{5-}$ clusters in **1**. Two cluster fragments at the corners of the cube (foreground and background) have been omitted (black circles: Zr, smaller circle: B, shaded circles: Cl). b) The water cage that surrounds the central cluster depicted in a). The shaded circles represent the terminal chloride ligands of the clusters, and the open circles the oxygen atoms of lattice water molecules. Hydrogen bonds are indicated by lines.

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An interesting aspect of the crystal structure of **1** is an $\text{H}_2\text{O}/\text{H}_3\text{O}^+$ network that partitions the crystal into cages that surround each $[(\text{Zr}_6\text{BCl}_{12})\text{Cl}_6]^{5-}$ cluster. This network is cross-linked by hydrogen bonds between oxygen atoms of the lattice water and terminal chloride ligands of the clusters (Figure 1b). A unique oxygen atom is located on a mirror plane (48*k* site), and 48 equivalent oxygen atoms in the cubic cell form eight slightly puckered hexagons with $\text{O}\cdots\text{O}$ distances of 2.743(4) Å within each six-membered ring. Each oxygen atom within a hexagon is connected to a like atom in a neighboring hexagon by a $\text{O}\cdots\text{O}$ (2.810(7) Å) interaction, and the $\text{O}\cdots\text{Cl}$ distance to the terminal chlorine atoms of the clusters is 3.200(3) Å. These $\text{O}\cdots\text{O}$ and $\text{O}\cdots\text{Cl}$ distances lie in the range expected for hydrogen-bonding interactions. If all 72 of the $\text{O}\cdots\text{O}$ linkages and half (24 of 48) of the $\text{O}\cdots\text{Cl}$ (2) links are hydrogen-bonded, then 96 hydrogen atoms are involved in hydrogen bonds, and the hydrogen atoms of the 48 water molecules in a unit cell are all accommodated. Although some of these hydrogen atoms were located in subsequent difference Fourier syntheses, none was included in the final refinement. The $\text{O}\cdots\text{O}$ distances are nearly equal to those of ice and therefore reveal little about the likely locations of any remaining protons required to balance the cluster charge. We assume that such additional protons that are present are bound to oxygen and are disordered over otherwise unoccupied $\text{O}\cdots\text{Cl}$ linkages.^[13]

In **2**, $[(\text{Zr}_6\text{CCl}_{12})\text{Cl}_6]^{4-}$ clusters are ensconced within hydrogen-bonded cages of water and protonated water molecules. The respective mean Zr–Zr and Zr–C distances of 3.236(2) and 2.29(2) Å are consistent with those of carbon-centered clusters with 14 CBEs.^[14, 15] The water network in **2** differs substantially from that of **1**. The presence of additional (acidic) protons can be inferred from the existence of short $\text{O}\cdots\text{O}$ contacts; these range up to 2.92(2) Å, but the two shortest $\text{O}\cdots\text{O}$ distances (2.425(6) Å and 2.53(1) Å) are close to those of $(\text{H}_5\text{O}_2)^+$.^[16] There are four such sites per cluster, and charge balance is achieved by inclusion of hydronium ions as the sole counteranions. On one site, about which the oxygen atoms of four water molecules are tetrahedrally arranged at a distance of about 2.85 Å, partial occupancy by water was indicated. Residual electron density in this position (3.5 e Å^{-3}) was modeled by assuming partial occupation by oxygen atoms (refined at 46 %).^[17]

Preliminary electrochemical studies on the boron- and carbon-centered clusters in aqueous media were performed. The cyclic voltammogram of the boron-centered cluster in 12 M HCl exhibits a well-defined one-electron wave at $E_{1/2} = -0.028\text{ V}$ (relative to SHE), which can be attributed to the redox couple for 13/14 CBEs. A second oxidation wave at 0.3 V is an irreversible multielectron process that is undoubtedly associated with cluster decomposition. The cyclic voltammogram of a solution of the carbon-centered cluster in 12 M HCl showed no sign of a reversible oxidation process; cluster decomposition was observed above 0.5 V. The fact that these oxidation processes are observed near to or more positive than SHE indicates that both the B-centered and C-centered clusters are weaker reducing agents than was generally assumed, at least insofar as simple one-electron reactions are concerned. In a broader study of centered

zirconium clusters in basic AlCl_3 /1-ethyl-3-methylimidazolium chloride melts, we found that the oxidation potentials of the $[(\text{Zr}_6\text{ZCl}_{12})\text{Cl}_6]^{4-}$ (Z = Be, B, C) cluster anions are within 0.25 V of that of $[(\text{Nb}_6\text{Cl}_{12})\text{Cl}_6]^{4-}$ and only weakly dependent on the identity of the interstitial atom Z.^[18] In light of these results, the failure of $\text{Zr}_6\text{ZCl}_{12}$ -based clusters to directly reduce protons to H_2 , even in strongly acidic solutions, is understandable. Interpretation of our voltammetric experiments must be considered as qualitative at this time because the precise ligation of the $[(\text{Zr}_6\text{BCl}_{12})^+]$ -based species responsible for the electrode reactions in aqueous solution is uncertain, in spite of the information provided by NMR spectroscopy (see below).

In the absence of strong oxidants, the pH value and the concentration of free chloride ions strongly influence the stability of the B- and C-centered clusters in solution. $\text{Rb}_5\text{Zr}_6\text{Cl}_{18}\text{B}$ dissolves rapidly and completely in deoxygenated water to form a weakly acidic red-orange solution, the ^{11}B NMR spectrum of which is shown in Figure 2a. We

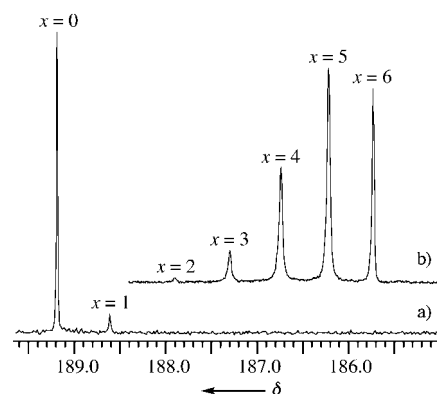


Figure 2. ^{11}B NMR spectra of $\text{Rb}_5\text{Zr}_6\text{Cl}_{18}\text{B}$ dissolved in deoxygenated water at 20 °C (a) and in a 12 M aqueous solution of LiCl at -5 °C (b). Labeling ($x = 0$ –6) indicates the number of terminal chloride ligands in $[(\text{Zr}_6\text{BCl}_{12})\text{Cl}_x(\text{H}_2\text{O})_{6-x}]^{1-x}$.

attribute the intense singlet at $\delta = 189.2$ to the aquo ion $[(\text{Zr}_6\text{BCl}_{12})(\text{H}_2\text{O})_6]^+$, the predominant species in solution.^[9] The acidity of this ion ($\text{p}K_a \approx 4.4$) implies that the fraction of the complex present as the conjugate base $[(\text{Zr}_6\text{BCl}_{12})(\text{H}_2\text{O})_5(\text{OH})]$ is significant ($\approx 10\%$). This solution is unstable, and within several hours it bleaches completely with observable evolution of H_2 . When $\text{Rb}_5\text{Zr}_6\text{Cl}_{18}\text{B}$ is dissolved in a dilute aqueous solution of NaOH, immediate decomposition occurs with vigorous bubbling and rapid loss of color. Solutions of the B-centered cluster in aqueous 12 M LiCl retain their characteristic red color for weeks at room temperature. The ^{11}B NMR spectrum of such a solution at -5 °C shows five singlets (Figure 2b) for a series of $[(\text{Zr}_6\text{BCl}_{12})\text{Cl}_x(\text{H}_2\text{O})_{6-x}]^{1-x}$ complexes ($x = 2$ –6).^[19]

Although the mechanism of cluster decomposition in water is not completely understood, we speculate that the first step involves the movement of terminal hydroxo ligands into bridging (inner) positions. In the presence of excess chloride ion and/or protons, the formation of clusters with bound hydroxide should become unfavorable. This is a result of the competition of chloride ions for terminal binding sites and the

lower Brønsted acidity of $[(\text{Zr}_6\text{BCl}_{12})\text{Cl}_x(\text{H}_2\text{O})_{6-x}]^{1-x}$ anions compared with $[(\text{Zr}_6\text{BCl}_{12})(\text{H}_2\text{O})_6]^+$. Thus, cluster decomposition is suppressed in acidic, chloride-rich solutions. In earlier studies conducted in polar organic solvents, the stability of $[\text{Zr}_6\text{ZCl}_{12}]^{m+}$ clusters was often perceived to be precarious. Our results suggest that this reactivity results from the sensitivity of the clusters to hydroxide ions generated by the deprotonation of adventitious water by “innocent” ligands that were intended to coordinate to the clusters.

For the first time, the stability of reduced zirconium compounds in aqueous media has been demonstrated. The $[\text{Zr}_6\text{ZCl}_{18}]^{n-}$ ($\text{Z} = \text{C}$, $n = 4$; $\text{Z} = \text{B}$, $n = 5$) clusters are thermodynamically stable with respect to oxidation by water or protons, even in strongly acidic solutions, but are readily decomposed in basic media, in which hydroxide ions may be present.

Experimental Section

$\text{Na}_2\text{Zr}_6\text{Cl}_{15}\text{B}$, $\text{Rb}_5\text{Zr}_6\text{Cl}_{18}\text{B}$, and $\text{Rb}_4\text{Zr}_6\text{Cl}_{18}\text{C}$ were synthesized in high-temperature solid-state reactions.^[11, 14, 15]

1: $\text{Na}_2\text{Zr}_6\text{Cl}_{15}\text{B}$ (0.01 g, 0.009 mmol) and concentrated hydrochloric acid (1.0 mL, 12 M) were mixed and stirred for 10 min under a nitrogen atmosphere, and the mixture was centrifuged to separate undissolved starting material from the red solution. Cooling the solution for 2 d at -20°C gave red crystals. When 0.10 g of $\text{Rb}_5\text{Zr}_6\text{Cl}_{18}\text{B}$ (0.062 mmol) was dissolved in 10.0 mL of concentrated hydrochloric acid (12 M) under the same conditions, about 60 mg of red polycrystalline solid (ca. 60% yield) was recovered by cooling the red solution to -20°C for 2 d.

2: A mixture of $\text{Rb}_4\text{Zr}_6\text{Cl}_{18}\text{C}$ (0.10 g, 0.065 mmol) and concentrated hydrochloric acid (10.0 mL, 12 M) was sealed in an ampoule, stirred for 10 min, then centrifuged to separate undissolved starting material from the orange-red solution. Most of the starting material remained undissolved. Cooling the solution to -20°C for 2 d gave **2** as a red crystals (20 mg, ca. 20% yield). Single crystals suitable for X-ray structure analysis were obtained by cooling a solution prepared as described above for two weeks.

X-ray structure analysis: Data for **1** and **2** were collected at -60°C on a Siemens (Bruker) SMART CCD diffractometer with a LT-2 low-temperature apparatus. In each case, a crystal was mounted immediately after removal from the mother solution. Crystals were coated with Apiezon-T grease, mounted on the tip of glass fibers, and immediately inserted into the low-temperature nitrogen stream of the diffractometer for data collection. Lorentz, polarization, and multiscan absorption corrections were applied. The structures were solved by direct methods and refined by full-matrix least-squares algorithms (SHELXS-97).^[20] Crystal data for **1**: red plates, crystal dimensions $0.05 \times 0.15 \times 0.15$ mm, cubic, space group $Im\bar{3}m$, $Z = 2$, $a = 13.8749(7)$ Å, $V = 2671.1(2)$ Å³, $\rho_{\text{calcd}} = 2.031$ g cm⁻³, $\mu = 2.085$ mm⁻¹, 8929 measured reflections, 337 independent reflections ($R_{\text{int}} = 0.0364$), 19 parameters, $R(F) = 0.0218$ and $R_w(F^2) = 0.0561$ for 252 reflections with $I > 2\sigma(I)$, residue electron density 0.550 e Å⁻³. Crystal data for **2**: red plates, crystal dimensions $0.05 \times 0.1 \times 0.1$ mm, tetragonal space group $I4_1cd$, $Z = 8$, $a = 16.2557(3)$, $c = 31.385(1)$ Å, $V = 8293.4(4)$ Å³, $\rho_{\text{calcd}} = 2.413$ g cm⁻³, $\mu = 2.663$ mm⁻¹, 25689 measured reflections, 3044 independent reflections ($R_{\text{int}} = 0.0334$), 199 parameters, $R(F) = 0.0211$ and $R_w(F^2) = 0.0604$ for 1585 reflections with $I > 2\sigma(I)$, residual electron density 0.693 e Å⁻³. Further details of the crystal structure investigations may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository numbers CSD-410844 and -410485.

Cyclic voltammetry was carried out with a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, IN). A glassy carbon disk electrode served as a working electrode, the counterelectrode was platinum wire, and the reference electrode was AgCl/Ag in a 3 M aqueous solution of NaCl.

Keywords: cluster compounds • NMR spectroscopy • solid-state structures • zirconium

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- [13] When $\text{Rb}_5\text{Zr}_6\text{Cl}_{18}\text{B}$ was used in place of $\text{Na}_2\text{Zr}_6\text{Cl}_{15}\text{B}$ as the cluster precursor, a compound crystallized with essentially the same structure as **1** but with a slightly different composition. The lattice parameter was slightly larger ($a = 13.8962(3)$) and a residual peak (ca. 5 e Å⁻³) in the structural refinement appeared in the center of the hexagon of oxygen atoms. This peak was modeled with a variable rubidium occupancy in a crystallographic refinement, which yielded the composition $[\text{Rb}_{0.44}(\text{H}_2\text{O})_{4.56}][(\text{Zr}_6\text{BCl}_{12})\text{Cl}_6] \cdot 19.44\text{H}_2\text{O}$. Atomic absorption analysis also indicated the presence of rubidium.
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- [17] We have isolated three compounds with this structure: $(\text{H}_3\text{O})_4[(\text{Zr}_6\text{CCl}_{12})\text{Cl}_6] \cdot 12.93\text{H}_2\text{O}$ (**2**), $(\text{H}_3\text{O})_4[(\text{Zr}_6\text{BCl}_{12})\text{Cl}_6] \cdot 12.97\text{H}_2\text{O}$ (**3**), and $(\text{H}_3\text{O})_4[(\text{Zr}_6\text{BCl}_{12})\text{Br}_6] \cdot 13.13\text{H}_2\text{O}$ (**4**). The isoelectronic boron-containing compounds **3** and **4** were formed by inadvertent air oxidation of HCl solutions containing B-centered clusters. Compounds **3** and **4** have structures that are unambiguously tetragonal with $P4_2/mnm$ space group symmetry (**3**: $a = 11.5375(2)$, $c = 15.7169(4)$ Å; **4**: $a = 11.7288(6)$, $c = 15.931(1)$ Å). However, the diffraction data of **2** exhibit what superficially appears to be a tetragonal $\sqrt{2} \times \sqrt{2} \times 2$ superlattice, but is likely the result of a subtle twinning of a lower symmetry superlattice that we have not yet determined. The space group and refinement results we have given in the experimental section for **2** must be regarded as provisional, as evidenced by the many systematic absence violations in the weak superlattice reflections. Alternative refinements in $I4_1md$ and $I4_1$ yielded very similar results, but they were not of superior quality. The superlattice is of little chemical significance and of only modest structural significance; the differences between the structure of the C-centered cluster and the two B-centered compounds seem to lie in distortions of part of the water network. Distortions in this part of the structure may be present in all three compounds, but long-range ordering of such distortions is observed only in **2**. Further details will be given in a subsequent publication.
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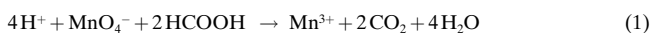
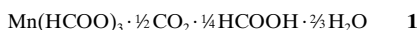
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Manganese(III) Formate: A Three-Dimensional Framework That Traps Carbon Dioxide Molecules**

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Of the first-row transition metals, manganese has a particularly appealing coordination chemistry due to its rich redox behavior, as demonstrated by the variety of structures and oxidation states in polynuclear manganese complexes.^[1] Manganese(III) is of particular interest owing to the large magnetic anisotropy associated with its d^4 electron configuration, which results in high magnetoresistance and superparamagnetic-type behavior in polynuclear compounds.^[2] For the synthesis of manganese(III) complexes, the reaction between permanganate and manganese(II) has been extensively used following the pioneering work of Christou et al.^[3] In a different approach, Lis et al.^[4] explored the reduction of KMnO_4 by 90 % formic acid. They reported the synthesis and solid-state structure of $\text{K}_2[\text{Mn}^{\text{II}}(\text{H}_2\text{O})_2][\{\text{Mn}_3^{\text{III}}\text{O}(\text{HCOO})_9\}_2]$, and claimed to have obtained “manganese(III) formate” by using more concentrated acid. Although the possibility of isolating “manganese(III) formate” has been questioned,^[5] this compound was also synthesized from MnO_2 by Weinland and Stark and was originally formulated as $\text{Mn}_3(\text{HCOO})_9 \cdot 2\text{H}_2\text{O}$.^[6]

Given our interest in the structures and electronic properties of manganese(III) complexes,^[7] we synthesized “manganese(III) formate” as reported by Lis et al. by treating KMnO_4 with 95 % formic acid at 0°C and obtained red cubes of a compound with the composition **1**. The three-dimensional polymeric framework of $\text{Mn}(\text{HCOO})_3$ moieties in **1** acts as a host for carbon dioxide molecules produced by the reaction (1).



The presence of carbon dioxide in the solid material was first discovered by room-temperature FT-IR spectroscopy on powdered samples and subsequently confirmed by single-crystal spectroscopy. In both experiments the sharp, split asymmetric C–O stretching band was reproducibly detected at 2340 and 2362 cm^{-1} . The rotational lines typical of CO_2 in the gas phase were not resolved, and this indicates partially hindered rotation, presumably due to host–guest interactions, and excludes contamination of the sample by atmospheric CO_2 . The vibrational spectra also indicated the presence of HCOOH and H_2O molecules in the lattice.

A single-crystal X-ray investigation of **1** at 180 K revealed that each manganese ion is connected to its nearest neighbors by six bridging formate ligands (Figure 1). The resulting three-dimensional $\text{Mn}(\text{HCOO})_3$ framework is rather open and

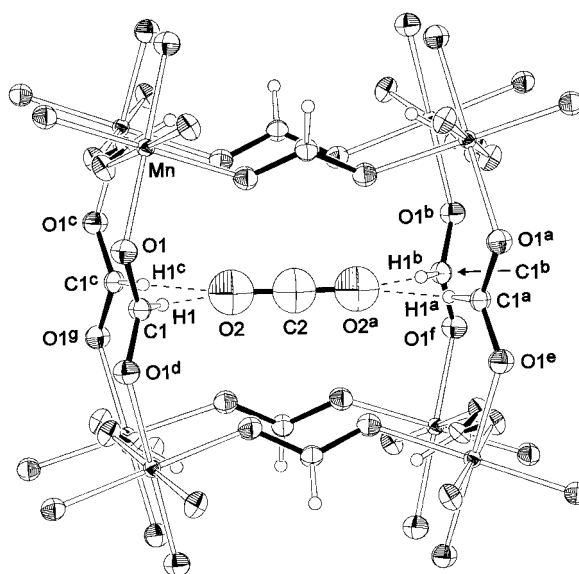


Figure 1. ORTEP plot of the cage with mmm symmetry in the crystal structure of **1** (30 % probability thermal ellipsoids). The disordering of the CO_2 molecule is not shown. Selected distances [$\text{\AA}$] and angles [$^\circ$]: Mn–O1 2.001(3), C1–O1 1.244(3), H1 $\cdots$ O2 2.66(7); O1–Mn–O1 90.0(2), O1–C1–O1^d 123.4(5), H1 $\cdots$ O2–C2 123.1(2), C1–H1 $\cdots$ O2 177.4(2).

contains two types of cages with mmm and $m\bar{3}$ symmetry. The residual electron density in the two types of cage was consistent with CO_2 and HCOOH guests, respectively. Each carbon dioxide molecule ($\text{C2–O2 } 1.16(1)\text{ \AA}$)^[8] interacts with the CH groups of the uncharged $\text{Mn}(\text{HCOO})_3$ framework through four C–H $\cdots$ O–C–O contacts (Figure 1). The water molecules are presumably highly disordered and thus evaded X-ray detection.

While several organometallic compounds containing carbon dioxide ligands are known,^[9] weakly bonded CO_2 is exceptionally rare in solid-state structures that have been investigated by single-crystal X-ray techniques.^[10, 11] In particular, host–guest interactions involving ionic coordination polymers are to our knowledge unprecedented.

An intriguing feature of the structure is the symmetry at the metal centers. The metal ion lies on a S_6 axis and its coordination environment is almost perfectly octahedral, in contrast with the well-known tendency of Mn^{III} ions to

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undergo appreciable Jahn–Teller distortions. An absorption band in the near IR at 10900 cm⁻¹ might correspond to the $x^2 - y^2 \leftarrow z^2$ transition of tetragonally distorted Mn^{III}.^[7, 12] As a possible explanation, we suggest that **1** belongs to a noncubic crystal system and that the observed cubic symmetry arises from threefold merohedral twinning (see Experimental Section).

A further indication of the presence of Jahn–Teller distorted Mn^{III} is provided by the magnetic properties. The χT product increases with decreasing temperature, and above 40 K it follows a Curie–Weiss law with a ferromagnetic θ of about 19 K and a Curie constant close to that expected for a high-spin d⁴ ion (Figure 2).^[7] The sharp peak at 27 K in the

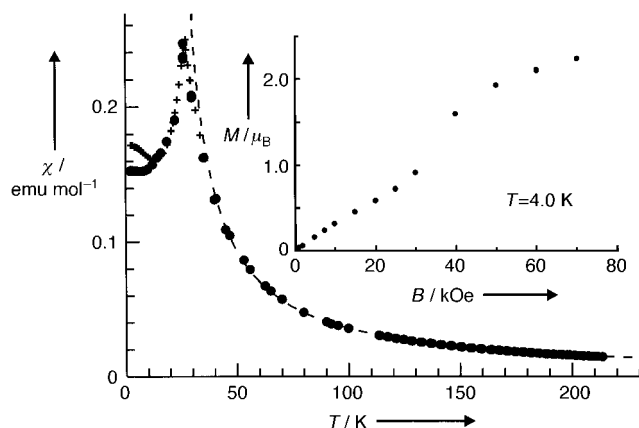


Figure 2. Plot of molar magnetic susceptibility versus temperature for **1** at 1 kOe (●) and 165 Oe (+), and Curie–Weiss plot for $C = 2.91 \text{ emu K mol}^{-1}$ and $\theta = 18.7 \text{ K}$ (---). The inset shows the molar magnetization at 4 K as a function of applied magnetic field.

plots of χ versus T and χT versus T is indicative of an antiferromagnetic phase transition. The overall magnetic behavior can be rationalized in terms of a layered structure with ferromagnetic intralayer interactions and weaker antiferromagnetic interlayer interactions. Axial–equatorial exchange pathways that involve empty and singly occupied orbitals of Jahn–Teller distorted Mn^{III} may provide the required intralayer coupling.^[13, 14] The sudden increase of the slope in the plot of M versus B at 40 kOe (inset of Figure 2) is characteristic of a spin-flip transition and is therefore consistent with the presence of antiferromagnetic interlayer interactions. However, the expected saturation value of $4 \mu_B$ for $S = 2$ is not achieved even at 70 kOe. The absence of saturation might be due to the large magnetic anisotropy of manganese(III) or to the presence of a second and stronger antiferromagnetic interaction.

Experimental Section

Recrystallized KMnO₄ (0.125 g, 0.79 mmol) was added over 1 h to a stirred mixture of HCOOH (8 mL) and distilled water (0.4 mL) at 0 °C to give a red-brown solution. Stirring was continued at 0 °C for an additional 3 h, and the filtered solution was allowed to stand at 25 °C for one week. Red cubes of composition **1** and a colorless solution were recovered. The crystalline product is stable over several months if kept in the mother liquid at 4 °C. Elemental analysis was performed on a microcrystalline sample that was washed with ethanol and diethyl ether and dried under a stream of dinitrogen: found: C 19.41, H 2.13, Mn 22.7; calcd for **1**: C 19.12, H 2.07, Mn

23.32; UV/Vis/near IR (reflectance, 298 K): $\tilde{\nu}_{\text{max}} = 10900, 22000 \text{ cm}^{-1}$; FT-IR (KBr pellets, 298 K): $\tilde{\nu} = 3380, 2894, 2362, 2340, 1728, 1645, 1584, 1334, 1200, 1128, 769, 761, 645, 606 \text{ cm}^{-1}$ (unaltered after 12 h at 0.1 mmHg and 298 K); thermogravimetric analysis (10 K min⁻¹, 300–600 K): continuous weight loss up to thermal decomposition to Mn(HCOO)₂ · x H₂O ($x \approx 0.5$) at ~480 K.

Crystal data for **1**: C_{3.75}H_{4.83}MnO_{8.17}, $M_r = 235.52$, crystal dimensions $0.2 \times 0.2 \times 0.2 \text{ mm}$, cubic, space group $Im\bar{3}$, $a = b = c = 11.736(2) \text{ Å}$, $V = 1616.5(5) \text{ Å}^3$, $Z = 8$, $\rho_{\text{calcd}} = 1.935 \text{ g cm}^{-3}$, $\rho_{\text{expt}} = 1.934(5) \text{ g cm}^{-3}$; MoK α radiation, $\lambda = 0.71069 \text{ Å}$, $\omega/2\theta$ scans, $2\theta_{\text{max}} = 59.9^\circ$, $T = 180(2) \text{ K}$; 4966 reflections collected, of which 451 were independent. The data were corrected for Lorentzian and polarization effects and absorption ($\mu = 1.649 \text{ mm}^{-1}$, min./max. transmission 0.75/0.99, ψ scans). The structure was solved by direct methods (SIR92^[15]) and refined on $|F^2|$ by full-matrix least-squares techniques with 449 reflections and 35 parameters (SHELXL-93^[16]) to give final $R1 = 0.0520$, $wR2 = 0.1264$. The formate hydrogen atom was added in an idealized position, and the C–H distance was refined. The CO₂ molecule was modeled over two equally populated positions related by a 0.4-Å shift along the common O–C–O axis (total occupancy 2/3 CO₂ per mmm cage). Maximum and minimum electron density 0.754 and -0.428 e Å^{-3} . The optical anisotropy of microcrystals of **1** indicates that the symmetry of the crystal lattice is intrinsically noncubic. However, the diffraction patterns of larger crystals exhibit cubic symmetry. A refinement in the orthorhombic space group $Immm$ was also attempted, but neither improvement nor symmetry lowering was obtained. We suggest that the cubic diffraction symmetry is due to threefold merohedral twinning along [111], which explains the apparent absence of Jahn–Teller distortion at the metal sites. The unusual 2/3 occupancy of the mmm cage by CO₂ molecules is also consistent with this interpretation. Removal of the threefold axis leads to three inequivalent mmm cages, and two of them may be preferentially occupied as a result of Jahn–Teller distortion. 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-102134. 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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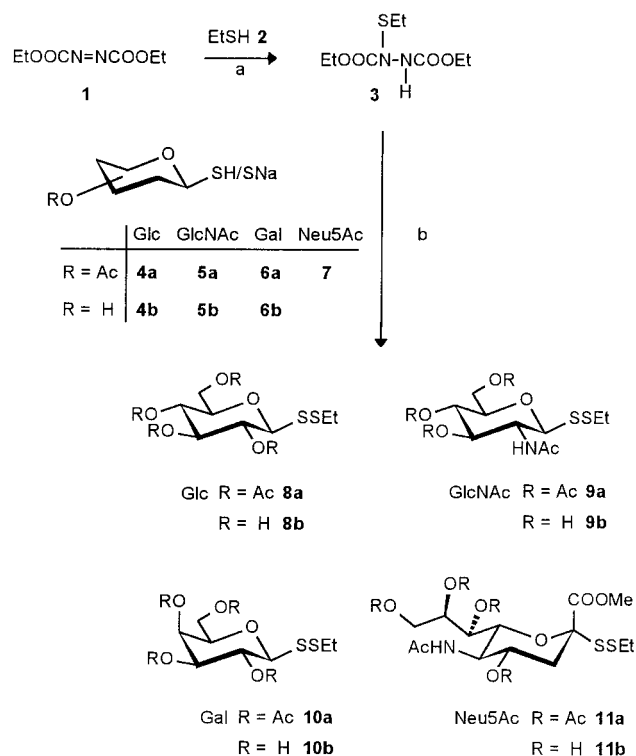
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Solid-Phase Synthesis of Thio-oligosaccharides**

Gerd Hummel and Ole Hindsgaul*

Thioglycosides are of special interest in enzyme-inhibition studies because they are much more stable to the action of glycosidases than O-glycosides and can thus inhibit enzymatic hydrolysis.^[1] Thio-oligosaccharides, which contain sulfur in the glycosidic linkage, have previously been synthesized by a variety of methods including S_N2 displacement of a leaving group by a 1-thioglycopyranose derivative,^[2] substitution of a glycosyl halide by a sugar thiolate,^[3] Lewis acid catalyzed glycosylation between a glycosyl acceptor with an SH group and a glycosyl donor,^[4] or Michael addition of 1-thiosugars to α,β -conjugated systems.^[5] These methods generally require several steps and result in low overall yield. Furthermore, the formation of unsaturated side products, loss of stereochemistry at the anomeric center, and thiol oxidation products complicate the isolation of the desired product.^[6] We present here a new and efficient method for the solid-phase synthesis of thio-oligosaccharides. The key feature of this method is that a highly reactive nucleophilic sugar-1-thiolate *without protecting groups* is used as the nucleophile for coupling to trifluoromethanesulfonate (triflate)-activated glycosides. The required free thiolate is generated on the solid phase from its protected unsymmetrical ethyl disulfide.

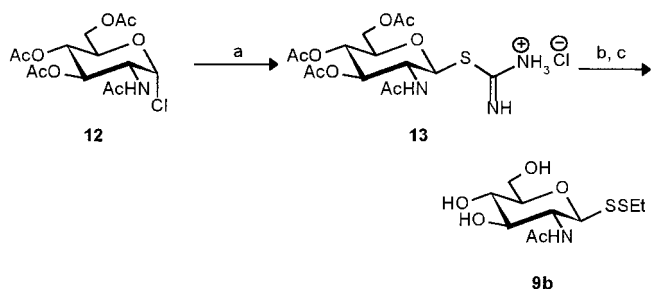
Diethyl-*N*-(ethylsulfanyl)hydrazodicarboxylate (**3**) was prepared by the reaction of diethylazodicarboxylate (**1**) and ethanethiol (**2**) in dichloromethane (Scheme 1).^[7] Compound **3** was isolated as a colorless syrup in 76 % yield after column chromatography. The thiol groups of readily available pro-



Scheme 1. a) CH_2Cl_2 , 20 °C, 2 h, 75 %; b) for R = Ac: CH_2Cl_2 , THF or CH_3CN ; for R = H: MeOH or MeOH/ H_2O , 20 °C, 5 min, 100 %. Ac = acetyl.

tected or unprotected 1-thioaldoses (Glc, GlcNAc, Gal, Neu5Ac)^[8] were selectively protected by using **3** in dichloromethane (**4a**, **5a**, **6a**, **7**) or in methanol (**4b**, **5b**, **6b**). The corresponding sugar disulfides were obtained in quantitative yields. The unprotected disulfides, obtained either directly from **4b**, **5b**, and **6b** or by deacetylation of **8a**, **9a**, **10a**, and **11a** with sodium methoxide in methanol, were used directly for immobilization on the solid phase.

For the preparation of larger quantities of 1-dithioethylglycosides an alternative reaction sequence was developed (Scheme 2, shown for GlcNAc). 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- α -D-glucopyranosyl chloride (**12**)^[9] was heated



Scheme 2. a) $\text{SC}(\text{NH}_2)_2$, acetone, reflux, 15 min, 82 %; b) EtSSEt, MeOH, NEt_3 , 20 °C, 2 h, 89 %; c) NaOMe, MeOH, 20 °C, 2 h, then H^+ , IR-120, 96 %.

with thiourea in acetone for 15 min under reflux to give **13** in 89 % yield. Subsequent treatment of **13** with diethyl disulfide in methanol and triethylamine followed by deacetylation with sodium methoxide in methanol afforded the unprotected disulfide **9b** in 85 % yield.

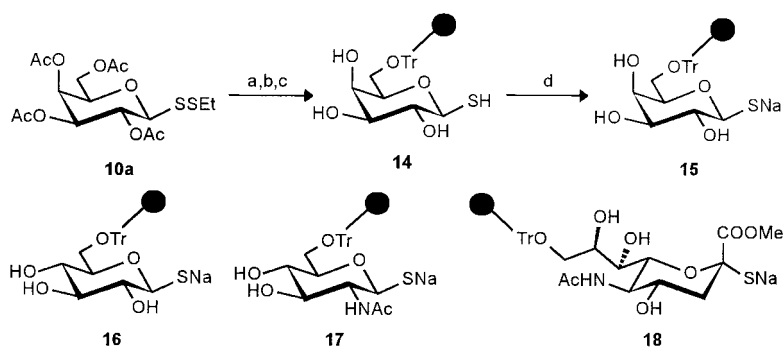
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[**] This work was supported by a grant from Synsorb Biotech. Inc.

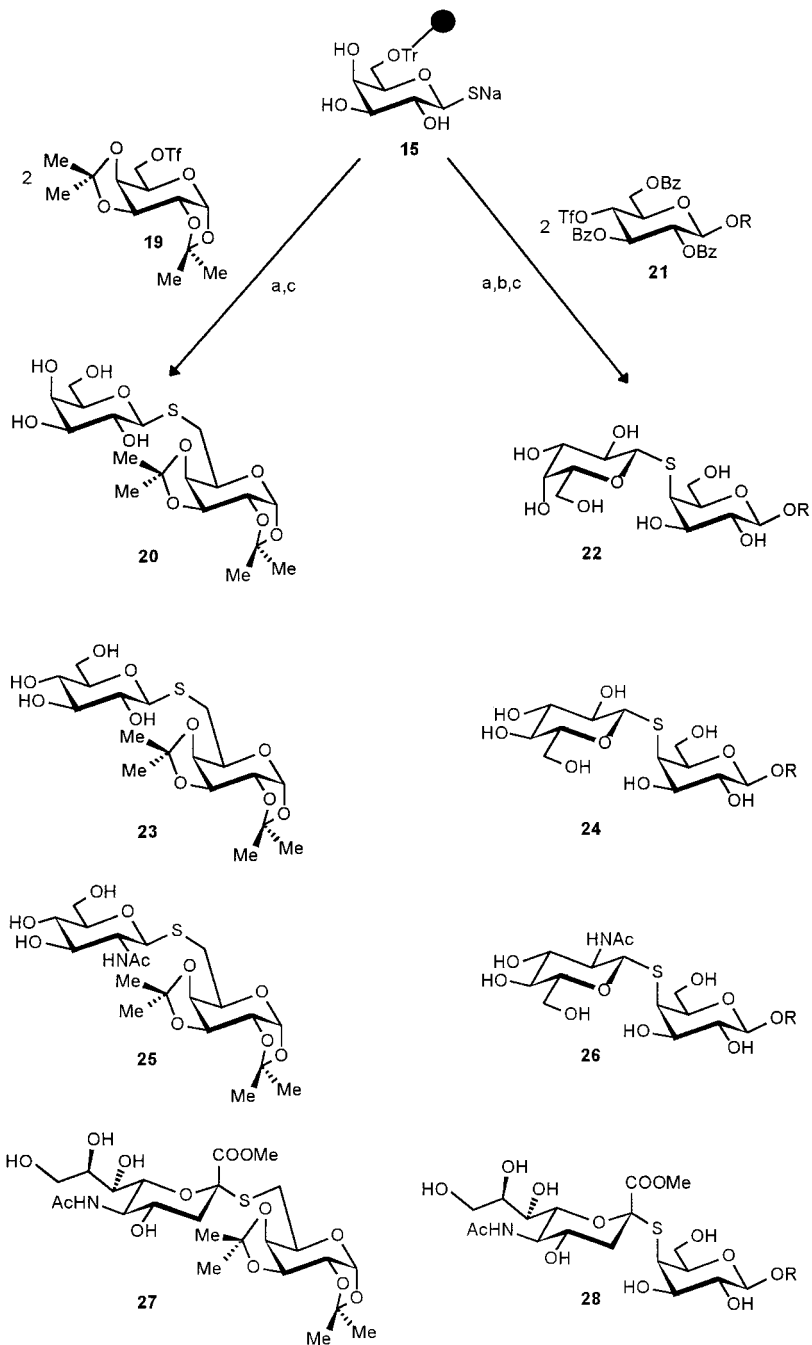
Each of the unprotected sugar disulfides (**8b**, **9b**, **10b**, and **11b**) was immobilized on a trityl chloride derivatized polystyrene resin (0.95 mmol g⁻¹) (shown for Gal, Scheme 3).^[10] The loading of the disulfides on the solid support was determined by elemental analysis (sulfur content) and ranged from 0.56 to 0.70 mmol g⁻¹ depending on the glycoside. The free thiol function was generated on the solid support by reduction of the disulfide with dithiothreitol (DTT) in a mixture of THF, MeOH, and Et₃N (10:2:1). The progress of the reduction could be easily monitored by IR spectroscopy directly on crushed beads ($\tilde{\nu}(\text{S-H}) \approx 2555 \text{ cm}^{-1}$). Attempted reaction of the immobilized thiol **14** with 6-*O*-triflate **19** (see Scheme 4) did not produce the desired thioglycoside, therefore, the nucleophilicity of **14** was enhanced by treatment with NaOMe/THF (Scheme 3).

The resulting thiolate **15** was coupled with triflate **19** after addition of a complexing agent ([15]crown-5 or Kryptofix 221) in THF (Scheme 4, shown for galactose). After 16 h the resin was washed and the thioglycoside **20** was cleaved from the resin by treatment with 2% TFA in CH₂Cl₂ (76% yield; for NMR and MS data see Table 1).^[11] The same reaction with the 4-*O*-triflate **21** gave the corresponding thioglycoside **22** in 64% yield;^[11] reactions of the triflates **19** and **21** with the immobilized sugar thiolates **16**–**18** led to the corresponding Glc, GlcNAc, and Neu5Ac thioglycosides **23**–**28** in similar yields. The structures of all thioglycosides were confirmed by ¹H NMR spectroscopy and mass spectrometry after adsorption on reverse-phase silica gel, washing with water, and elution with methanol.^[11]

To demonstrate the suitability of this method for the construction of larger thio-oligosaccharides, the 1-dithioethyl galactose derivative **29** was synthesized in five steps from **10b**. Glycosylation was performed in the same manner as described in Scheme 4 with triflate **29** and the immobilized thiolate **16** (Scheme 5) to give the immobilized disaccharide **30**. After removal of the benzoyl groups on the resin by using sodium methoxide in THF, the anomeric disulfide was reduced with DTT, deprotonated (NaOMe/THF), and again treated with triflate **29** in the presence of [15]crown-5. After removal of the benzoyl protecting groups with sodium methoxide in THF and cleavage from the resin, trisaccharide **32** was obtained in 92% yield based on **16** (NMR and MS data are given in Table 1). This high yield con-



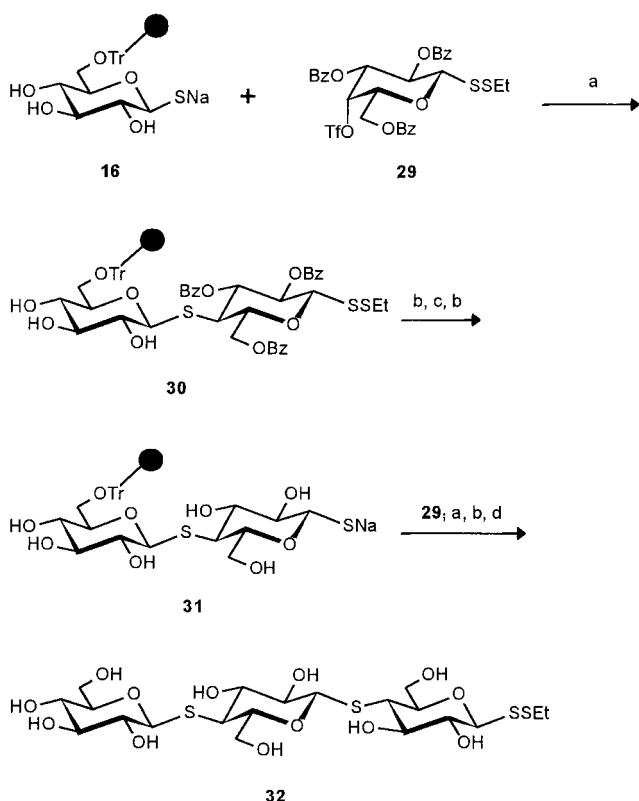
Scheme 3. a) NaOMe, MeOH, 20 °C, 2 h; b) trityl chloride resin, pyridine, 4-dimethylamino-pyridine (DMAP), 60 °C, 48 h; c) DTT, THF, MeOH, Et₃N, 20 °C, 18 h; d) NaOMe, THF, 20 °C, 2 h. Tr = trityl = triphenylmethyl.



Scheme 4. a) [15]Crown-5, THF, 20 °C, 16 h; b) NaOMe, MeOH, THF; c) trifluoroacetic acid (TFA), CH₂Cl₂, 20 °C. **21**, **22**, **24**, **26**, **28**: R = octyl, Bz = benzoyl, Tf = trifluoromethylsulfonyl.

Table 1. Selected ^1H NMR signals (360 MHz, CD_3OD) and MS data of **20**, **22–28**, and **32**.

20 : $\delta = 4.35$ (d, 1H, $J_{1,2} = 9.7$ Hz; 1b-H, 1c-H), 5.45 (d, 1H, $J_{1,2} = 5.0$ Hz; 1a-H); MS: m/z : 461.2 [$M^+ - \text{Na}$]
22 : $\delta = 4.18$ (d, 1H, $J_{1,2} = 7.6$ Hz; 1b-H), 4.42 (d, 1H, $J_{1,2} = 9.6$ Hz; 1a-H); MS: m/z : 493.2 [$M^+ - \text{Na}$]
23 : $\delta = 4.40$ (d, 1H, $J_{1,2} = 9.6$ Hz; 1b-H), 5.45 (d, 1H, $J_{1,2} = 5.0$ Hz; 1a-H); MS: m/z : 461 [$M^+ - \text{Na}$]
24 : $\delta = 4.18$ (d, 1H, $J_{1,2} = 7.6$ Hz; 1a-H), 4.48 (d, 1H, $J_{1,2} = 9.7$ Hz; 1b-H); MS: m/z : 493.2 [$M^+ - \text{Na}$]
25 : $\delta = 4.55$ (d, 1H, $J_{1,2} = 10.3$ Hz; 1b-H), 5.45 (d, 1H, $J_{1,2} = 5.0$ Hz; 1a-H); MS: m/z : 502.2 [$M^+ - \text{Na}$]
26 : $\delta = 4.16$ (d, 1H, $J_{1,2} = 7.6$ Hz; 1a-H), 4.70 (d, 1H, $J_{1,2} = 10.4$ Hz; 1b-H); MS: m/z : 534.3 [$M^+ - \text{Na}$]
27 : $\delta = 1.31, 1.33, 1.39, 1.49$ ($4 \times \text{s}$, 12H; CH_3), 1.80 (dd, 1H, $J_{3a,4} = 11.3$, $J_{3a,3e} = 12.8$ Hz; 3b-H _a), 1.98 (s, 3H; NCOCH_3), 2.75 (dd, 1H, $J_{3e,4} = 4.6$, $J_{3e,3a} = 12.8$ Hz; 3b-H _e), 3.67 (s, 3H; COOCH_3), 4.31 (dd, 1H, $J_{1,2} = 5.0$, $J_{2,3} = 2.4$ Hz; 2a-H), 4.61 (dd, 1H, $J_{2,3} = 2.4$, $J_{3,4} = 7.9$ Hz; 3a-H), 5.42 (d, 1H, $J_{1,2} = 5.0$ Hz; 1a-H); MS: m/z : 604.2 [$M^+ - \text{Na}$]
28 : $\delta = 0.88$ (m, 3H; CH_3), 1.28 (m, 10H; Octyl), 1.56 (m, 2H; OCH_2CH_2), 1.99 (s, 3H; NCOCH_3), 1.79 (dd, 1H, $J_{3a,4} = 11.3$, $J_{3a,3e} = 12.8$ Hz; 3b-H _a), 1.97 (s, 3H; NCOCH_3), 2.75 (dd, 1H, $J_{3e,4} = 4.6$, $J_{3e,3a} = 12.8$ Hz; 3b-H _e), 3.67 (s, 3H; COOCH_3), 4.20 (d, 1H, $J_{1,2} = 7.6$ Hz; 1a-H); MS: m/z : 636.3 [$M^+ - \text{Na}$]
32 : $\delta = 1.30$ (t, 3H, $J = 7.4$ Hz; CH_3), 2.83 (q, 2H, $J = 7.4$ Hz; SCH_2), 4.31 (d, 2H, $J_{1,2} = 9.3$ Hz; 1b-H, 1c-H), 4.40 (d, 1H, $J_{1,2} = 9.5$ Hz; 1a-H); MS: m/z : 635.1 [$M^+ - \text{Na}$]



Scheme 5. a) [15]Crown-5, THF, 20 °C, 16 h; b) NaOMe, MeOH, THF; c) DTT, THF, MeOH, Et_3N ; d) TFA, CH_2Cl_2 , 20 °C.

firmly that O-glycosidic bond cleavage from the resin was in fact responsible for the lower yields of about 70% found for compounds **20–28**.

In conclusion, a highly efficient method for the synthesis of thio-oligosaccharides on the solid-phase has been described.

All glycosides were obtained stereoselectively and in high yield. Side products arising from elimination of the triflates could be easily removed by washing the resin after glycosylation. The protection of the anomeric thiol function as an ethyl disulfide proved to be compatible with common carbohydrate reaction conditions and served as an ideal protective group.

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- The resin is commercially available from Novabiochem. Immobilization was performed by using pyridine as solvent in the presence of 4-dimethylaminopyridine (DMAP) for 48 h at 60 °C.
- Yield of isolated product. The isopropylidene groups of **20**, **23**, **25**, **27** and the O-glycosidic bonds of unprotected **22**, **24**, **26**, **28** were partly cleaved under acidic conditions. Cleaving the benzoyl-protected thiodisaccharides from the resin afforded the corresponding disaccharides in quantitative yields since the presence of the electro-negative benzoyl esters stabilizes the glycosidic linkages. The use of more acid labile linker systems is currently under investigation.

Characterization of Ligand Binding by Saturation Transfer Difference NMR Spectroscopy**

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The difference between a saturation transfer spectrum and a normal NMR spectrum provides a new and fast method (saturation transfer difference (STD) NMR spectroscopy) to

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screen compound libraries for binding activity to proteins. STD NMR spectroscopy of mixtures of potential ligands with as little as 1 nmol of protein yields 1D and 2D NMR spectra that exclusively show signals from molecules with binding affinity. In addition, the ligand's binding epitope is easily identified because ligand residues in direct contact to the protein show much stronger STD signals. For example, the binding specificity of Lewis^b-hexasaccharide to *Aleuria aurantia* agglutinin (AAA) can be mapped to the two fucosyl residues.

Identification of the binding activity and characterization of the binding epitope of ligands to proteins is important in biochemical and pharmaceutical research. The advantage of NMR-based techniques in comparison to other screening methods in use today, such as ELISA, RIA, Biacore, or immunoblotting, is the possibility of directly identifying the binding component from a mixture of potential ligands. Any small molecule in solution reversibly interacting with the binding domain of a protein shows characteristic differences in its relaxation behavior and molecular mobility from those molecules having no affinity for the protein. Transferred NOE spectra,^[1] diffusion- or relaxation-edited NMR spectra,^[2] as well as ¹⁵N chemical shift studies^[3] were used to identify substances with binding affinity from compound mixtures. The factors that reduce the utility of NMR methods for high-throughput screening are relatively long acquisition times and the dependence on ligand excess. An additional drawback of the ¹⁵N chemical shift technique is the need for large quantities of valuable ¹⁵N-labeled biomolecules and the limitation on the protein size to < 30 kDa.^[3] Spin diffusion experiments have to date been used in a variety of ways such as the study of motional properties of proteins,^[4] the effect of H₂O suppression on NMR spectra,^[5] and the study of ligand–protein interactions.^[6]

Macromolecules, like proteins, consist of a large system of protons tightly coupled by dipole–dipole interactions.^[7] The longitudinal relaxation rate R_1 of protons in proteins is dominated by the cross relaxation rate σ_{intra} . Selective saturation of a single protein resonance will result in a rapid spread of the magnetization over the entire protein (spin diffusion). Intermolecular transfer of magnetization from protein to ligand (σ_{inter}) leads to progressive saturation of the ligand (Figure 1). The only limitation imposed on molecules in the mixture is that they must not be affected by the selective saturation pulse. The NOE pumping method^[2b] also relies on the transfer of polarization from the receptor to the bound compound. In contrast to the NOE pumping method, STD NMR spectroscopy does not require a diffusion filter, and the NOE mixing (pumping) time is replaced by the selective saturation time. These factors make STD NMR spectroscopy more sensitive and flexible in its applications. STD NMR spectroscopy should enable the identification of ligand binding to proteins with dissociation constants K_D between 10^{-3} and 10^{-8} .^[8]

The efficiency of the STD NMR technique is shown by the binding of *N*-acetylglucosamine (GlcNAc) to wheat germ agglutinin (WGA). Other studies have shown that the lectin receptor has an affinity for this ligand.^[9] We composed a library containing six nonbinding saccharides additional to the binding monosaccharide GlcNAc. Figure 2A shows the 1D

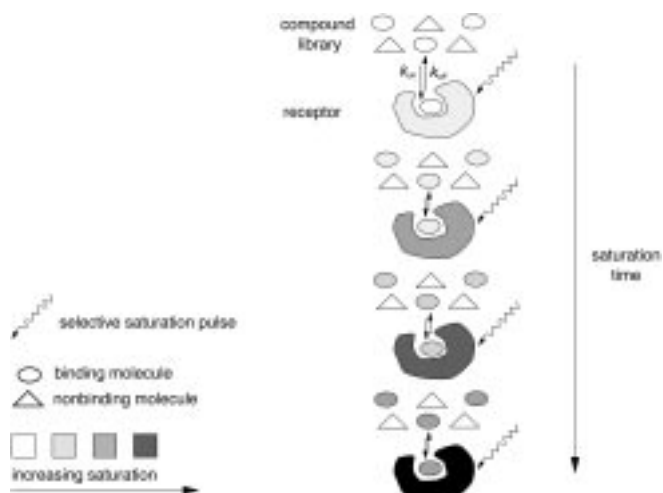


Figure 1. Scheme displaying the effect of a selective saturation pulse on the protein envelope to binding (ellipse) and nonbinding molecules (triangle). The selective pulse saturates only a few protein resonances. This saturation is spread over the entire protein by intramolecular saturation transfer (spin diffusion) indicated by the shading of the receptor. Resonances of small molecules are not directly affected by the selective pulse. Only those ligand molecules interacting with the receptor are saturated by intermolecular saturation transfer. Through chemical exchange these saturated ligands are transferred into solution where they are detected. The molecules represented here by triangles do not bind to the protein and thus do not become saturated. The degree of saturation of the binding molecules lag behind the degree of saturation of the receptor as indicated by the decreased shading of the binding molecule because the ligands normally remain in the binding site only for a short time.

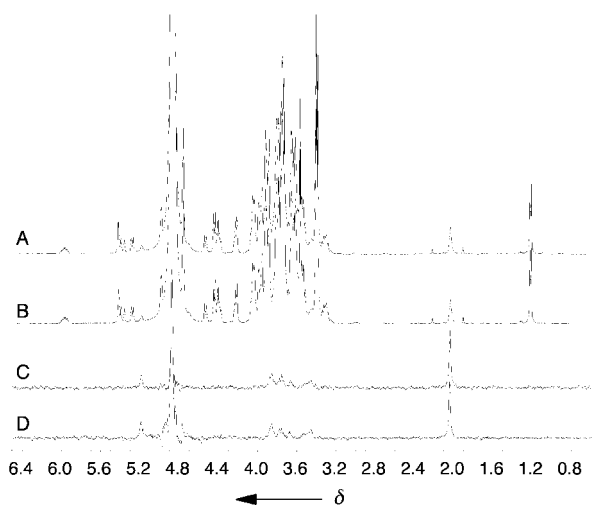


Figure 2. Characterization of ligands with binding affinity with the 1D STD NMR spectroscopic method. A) 1D ¹H NMR spectrum of a mixture of seven saccharides (1 mm of α -D-GlcNAc, α -D-Man-OMe, α -D-Gal-OMe, α -L-Fuc-OMe, β -D-Gal-(1 $\rightarrow$ 4)- β -D-Glc-OMe, β -D-Gal-(1 $\rightarrow$ 4)- β -D-Glc-O-Allyl, D-raffinose) and WGA recorded with off-resonance irradiation at $\delta = 30$, and B) with on-resonance irradiation at $\delta = 10$. C) The corresponding STD spectrum (spectrum (A) minus spectrum (B)). D) Reference spectrum showing a STD NMR spectrum obtained from WGA and GlcNAc alone without the six nonbinding saccharides. The spectra (C) and (D) were obtained by collecting 128 scans for the on- and off-resonance spectra each and internal subtraction from each other by phase cycling. Only resonances of GlcNAc remain in the STD NMR spectra (C) and (D). The concentration of WGA in solution was 45 μ M (binding sites), the excess of the ligands was about 20:1. A pulse train of 40 Gaussian bell-shaped selective pulses of 50 ms duration separated by a 1 ms delay were used to saturate the protein. A T_2 relaxation filter consisting of a 70 ms spin lock pulse was applied to remove protein background signals resulting also in reduced signal intensities in (C) and (D).

^1H NMR spectrum^[10] of the compound library recorded with off-resonance irradiation at $\delta = 30$. Figure 2B shows the 1D ^1H NMR spectrum of the same sample with selective saturation of the protein by a cascade of shaped pulses with on-resonance irradiation at $\delta = 10$. One can clearly see that the binding compound cannot be identified by observing changes of intensity between the spectra (A) and (B) due to extreme overlap in the hump region (ring protons) (Figure 2). However, in the difference spectrum given in Figure 2C even signals of the hump region may be assigned, making identification of GlcNAc possible. For reference, the STD NMR spectrum in Figure 2D was recorded with a sample containing WGA in the presence of only GlcNAc. The spectra in Figures 2C and 2D are identical proving that all signals from non-binding compounds are completely eliminated in Figure 2C.

The dependence of the degree of saturation on ligand excess was determined by titration of GlcNAc relative to WGA (Figure 3A). The signal-to-noise ratio determined

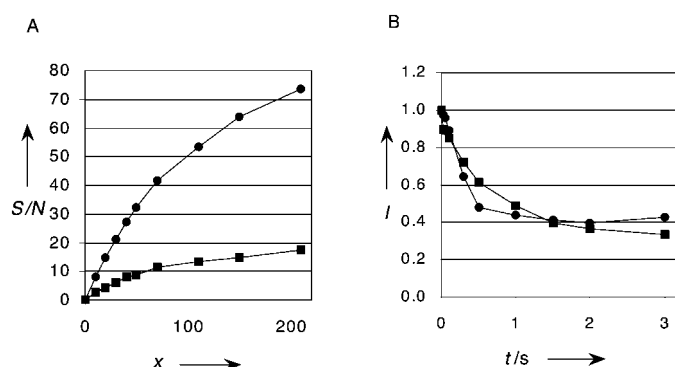


Figure 3. A) Signal-to-noise ratio (S/N) obtained from STD NMR spectra as a function of the molar excess (x) of GlcNAc relative to WGA for selected resonances ($\bullet$ N -acetyl group, $\blacksquare$ α -H1). The sample contained 22 nmol WGA. The spectra were recorded at 290 K with off-resonance irradiation at $\delta = 20$ and on-resonance irradiation at $\delta = 7$. Maximum saturation is not even reached at a 200-fold molar excess of ligand. B) Integral value (I) as a function of saturation duration (t) of selected α -L-Fuc-OMe resonances ($\bullet$ O -methyl group, $\blacksquare$ H6-Fuc group). The sample contained 28 nmol of AAA and a 30-fold molar excess of ligand. The spectra were recorded at 310 K with selective irradiation of the protein at $\delta = 7$. Saturation of signals increases to about 2 s leveling out at about 60% reduction of signal intensity.

from difference spectra of the anomeric α -H1 proton and the N -acetyl group increases beyond an excess of 210:1. The intensity of the STD NMR signal increases as a function of ligand excess as long as ligand molecules having received no or little saturation bind to the receptor. Therefore, high turnover rates result in a larger effect at higher ligand-to-protein ratios. Slow dissociation rates will yield smaller STD NMR signals and, thus, reduce sensitivity.

Figure 3B indicates the dependence of the STD NMR signal intensity on the duration of saturation. The saturation profile shows that intra- and intermolecular saturation transfer is very efficient since a 100 ms irradiation period leads to a decrease in signal intensity of ligand resonances by about 15%. The maximum degree of saturation under these conditions of about 60% is reached at a saturation time of about 1.5 s. More important in this context is that prolonged irradiation of the protein does not lead to a decrease of the STD NMR signal intensity. The high degree of saturation

(60%) even at a ligand excess of 30:1 makes STD NMR spectroscopy very sensitive and, therefore, only very small amounts of protein are required. In sum, the degree of saturation of ligands depends on the size of the protein, the offset, and the duration of the on-resonance irradiation, the dissociation rate constant k_{off} , and the excess of ligand.

Screening of the library containing 22 nmol (400 μg) of WGA and a 20-fold ligand excess by 1D STD NMR spectroscopy is possible in two minutes. The sensitivity limits of the method became apparent when we successfully observed GlcNAc binding to as little as 1 nmol of WGA (18 μg) from a mixture of 200 nmol GlcNAc (44 μg) and α -L-Fuc-OMe (36 μg) each within about one hour on a 500 MHz spectrometer. Using higher field spectrometers will make the method even more efficient since sensitivity and spin diffusion increase with field strength.

To identify more complex components from a library additional experiments, that is 2D NMR spectra, are necessary. The saturation transfer effect is applicable to virtually any form of NMR experiment. This allows us to record 2D experiments that reveal structural information of the ligand interacting with the protein. Here, we demonstrate the benefits of STD NMR spectroscopy by means of a TOCSY difference spectrum (Figure 4) with the same NMR sample

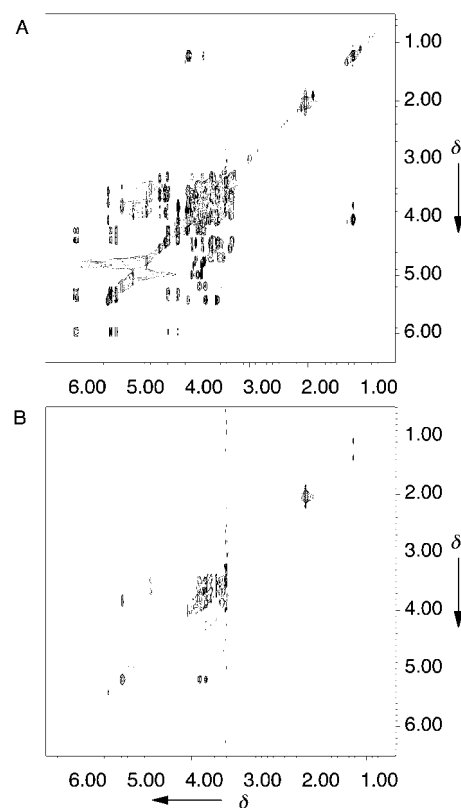
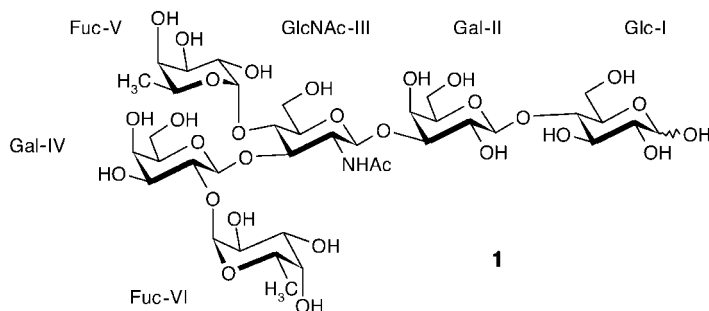


Figure 4. Characterization of ligands with binding affinity with the 2D STD NMR spectroscopic method. A) TOCSY spectrum of a mixture of seven saccharides and WGA recorded with off-resonance irradiation at $\delta = 20$. B) STD TOCSY spectrum obtained by subtraction of a spectrum recorded with on-resonance irradiation at $\delta = 7$ from spectrum (A). The on- and off-resonance TOCSY spectra were recorded at 290 K with 200 increments in t_1 and 32 transients each. Only resonances of GlcNAc remain in the STD TOCSY spectrum. The two spectra were recorded with t_1 increments interlaced and processed and phased identically before subtraction.

used in the 1D experiment. The spectrum in Figure 4A shows the TOCSY spectrum of the compound mixture recorded with off-resonance irradiation. Figure 4B shows the STD TOCSY spectrum containing only cross peaks originating from the binding component GlcNAc. The recording of 2D NMR spectra allows unambiguous identification of complex ligand molecules directly from mixtures.

To test the STD NMR spectroscopic method with a larger ligand we recorded TOCSY spectra of Lewis^b-hexasaccharide (lacto-*N*-difucosylhexaose I, **1**) in presence of the fucose-



binding lectin AAA.^[11] The resonances of the individual sugar residues of the Lewis^b-hexasaccharide show very different intensities (Figure 5). Only the fucose residues are located in direct contact to the protein and, thus, obtain a high degree of saturation directly from the protein (Figure 5B). The other sugar residues, namely Gal-II, GlcNAc-III and Gal-IV, show only about 60 % of the saturation since they can only obtain saturation through the directly interacting fucoses (Figure 5C). The Glc-I residue shows an even smaller effect of about 30 % saturation relative to that of the fucosyl residues (not shown). Therefore, binding epitope and neighboring residues can easily be mapped by STD NMR spectroscopy. This holds true for all ligands where the transfer of magnetization outside the binding epitope goes through a “bottle-neck” such as peptide^[12] or glycosidic linkages. The analysis shown here was made with 10 nmol of recoverable protein and 1 μ mol of ligand. Therefore, STD NMR spectroscopy seems to be a viable alternative to, for example, mapping of binding epitopes by determining the affinity of a large number of short overlapping peptides by ELISA.

In conclusion, STD NMR spectroscopy is a new member of bioaffinity NMR methods^[1a,b] which allow detection and identification of binding molecules directly from mixtures. It is insensitive to ligand excess and eliminates the risk of detecting false positives, since only saturation transferred to the ligand through the protein is detected. With STD NMR spectroscopy there are few limitations to the size of the ligand molecules as long as the receptor can be selectively excited. Compound mixtures containing substances in variable concentrations and of different sizes can therefore be efficiently screened. Furthermore, there is no limit to the size of the protein. In fact, there is an increase of sensitivity with the size of protein due to a more efficient inter- and intramolecular saturation transfer. In contrast to the determination of structure–activity relationships (SAR) by NMR spectroscopy this method does not yield information on the receptor site of the protein. STD NMR spectroscopy should also be appli-

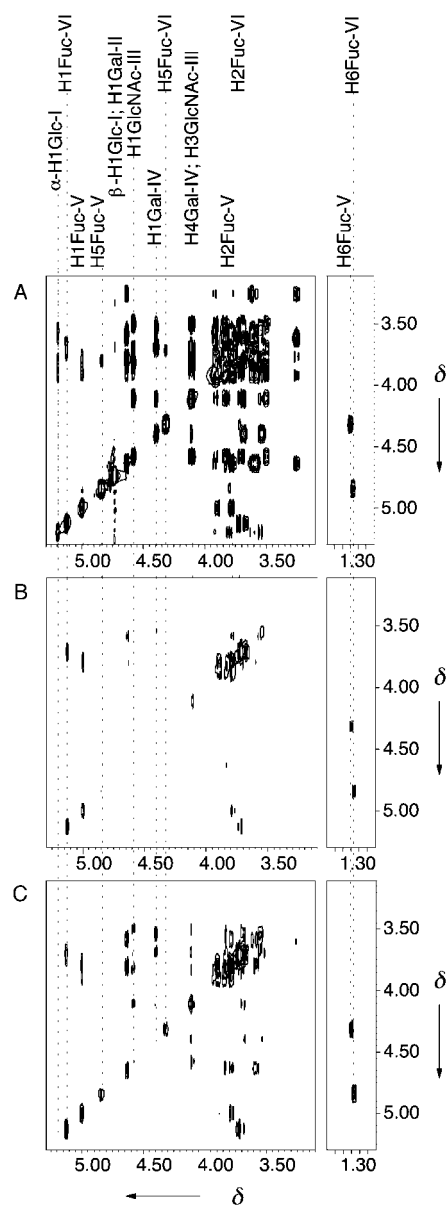


Figure 5. STD NMR TOCSY spectra of Lewis^b-hexasaccharide demonstrating epitope mapping. A) TOCSY spectrum of **1** without protein. B) STD TOCSY spectrum of **1** in the presence of the protein AAA (10 μ M binding sites) showing only the intensive signals of fucosyl residues V and VI. C) STD TOCSY spectrum at about 60 % level of that in (B) showing in addition to the fucosyl cross peaks from (B) cross peaks originating from Gal-II, GlcNAc-III, and Gal-IV. The full STD TOCSY exhibiting also the even less intensive signals of Glc I at about 30 % of the intensity of cross peaks in (B) is not shown. The on- ($\delta=10$) and off-resonance ($\delta=30$) TOCSY spectra were recorded at 300 K with 200 increments in t_1 and 40 transients each.

cable to tighter binding ligands although the absolute effect will be smaller than with faster exchanging ligands since fewer ligands are saturated and exchanged into solution. Very valuable is the fact that most 1D or nD experiments can be modified in such a way that the STD NMR method is applicable, for example TOCSY, COSY, NOESY, and inversely detected ¹³C or ¹⁵N spectra.

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A Solution-Phase Chemical Approach to a New Crystal Structure of Cobalt**

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Cobalt has long been known to have two crystal structures—close-packed hexagonal (hcp) and face-centered cubic (fcc). While both phases can coexist at room temperature, the fcc structure is thermodynamically preferred above 450 °C and the hcp phase is favored at lower temperatures.^[1] For small particles, however, the fcc structure appears to be preferred even below room temperature.^[2] The existence of fcc and hcp cobalt was first reported by Hull^[3] in 1921 after analyzing diffraction patterns of metallic powders prepared by several methods. Krainer and Robitsch^[4] reported observing new diffraction lines in samples prepared by spark erosion of bulk cobalt surfaces, but the structure was never fully established. Kajiwar et al.^[5] observed several new lines in the diffraction pattern of cobalt nanoclusters prepared by plasma evaporation and the subsequent condensation of the metal, but attributed these lines to a form polymorphous to the two known structures. Similar results are reported by Leslie-Pelecky et al.^[6] with cobalt particles prepared by the reduction of cobalt salt in solution with metallic lithium. A recent report by Respaud et al.^[7] provides some evidence for a new structure of cobalt present in small cobalt clusters produced by the decomposition of organometallic precursors, but the structure was not identified. Herein we report and identify a new stable structure for elemental cobalt.

The fcc and hcp phases of cobalt are close-packed structures that differ only in the stacking sequence of atomic planes in the 111 direction. Low activation energy for formation of stacking faults often leads to the formation of both phases in the same sample under high-temperature crystallization techniques, such as melting–crystallization and evaporation–condensation. Low-temperature solution chemistry methods, on the other hand, often yield exclusively one phase of cobalt.

We used thermal decomposition of octacarbonyldicobalt in solution in the presence of trioctylphosphane oxide (TOPO) as a coordinating ligand to prepare cobalt nanoclusters. This method provides a “clean” route for the preparation of the material since elemental cobalt is the only nonvolatile product of the reaction: $[\text{Co}_2(\text{CO})_8] \rightarrow 2\text{Co} + 8\text{CO}$. The resulting powder was highly susceptible to magnetic fields generated by a small permanent magnet, suggesting that it consisted of metallic cobalt. Transmission electron microscopy (TEM) showed that the powder consisted of roughly spherical

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particles with an average diameter of 20 nm and a 15 % size distribution. Later experiments showed that the size of the crystals is not limited to this range. In fact, injection at higher temperature yields regular polyhedron-shaped faceted crystals as large as 0.3 μm and possibly even larger (Figure 1). Elemental analysis gave the following

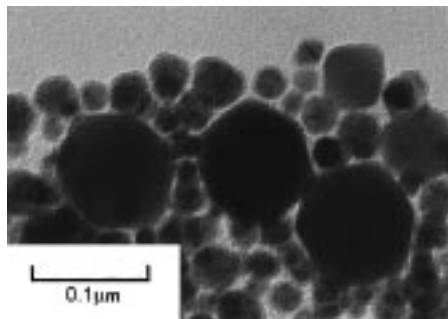


Figure 1. TEM image of cobalt crystals with the new ϵ -cobalt structure.

atomic percentage: Co 83%, O 11%, other elements (C, H) 6%. The presence of oxygen indicates surface oxidation of the cobalt particles, corresponding to a coverage of one to two monolayers of cobalt oxide for particle sizes in the 20 nm range. The other elements, we believe, come from residual organic solvents adsorbed on the particle surfaces. The absence of phosphorus in the elemental analysis indicates that TOPO was completely removed during washing of the particles. It should be emphasized here that freshly synthesized cobalt nanoparticles are extremely reactive towards oxidation. In fact, rapid exposure to air results in immediate oxidation accompanied by a red glow. Therefore, even simply washing the particles in organic solvents inevitably leads to surface oxidation by residual moisture and dissolved air. The resulting oxide layer appears to passivate the surface of the particles and considerably reduce the speed of further oxidation.

Figure 2a shows an X-ray powder diffraction pattern of the reaction product.^[8] This pattern does not correspond to either of the two known structures of cobalt. In fact, neither the positions of the peaks nor their intensity distribution correspond to known cobalt phases. After detailed analysis it was concluded that this pattern corresponds to a new, previously unknown phase of cobalt, which we call ϵ -cobalt. This structure is cubic (space group $P4_32$) with a unit cell parameter $a = 6.097 \pm 0.001 \text{ \AA}$. The unit cell structure is similar to that of β -manganese (a high-temperature phase of manganese).^[9] It contains 20 cobalt atoms, which are divided into two types: twelve atoms of Type I and eight atoms of Type II (Table 1). These two types of atoms differ in their local coordination. Unlike an ideal close-packed structure, which has twelve nearest neighbors, ϵ -cobalt has only three nearest neighbors for Type I and two nearest neighbors for Type II atoms (Table 2). This results in a structure for ϵ -cobalt that is less dense than both the hcp and fcc structures. The calculated density of ϵ -cobalt is 8.635 g cm^{-3} , compared to 8.788 g cm^{-3} and 8.836 g cm^{-3} for the fcc and hcp structures,

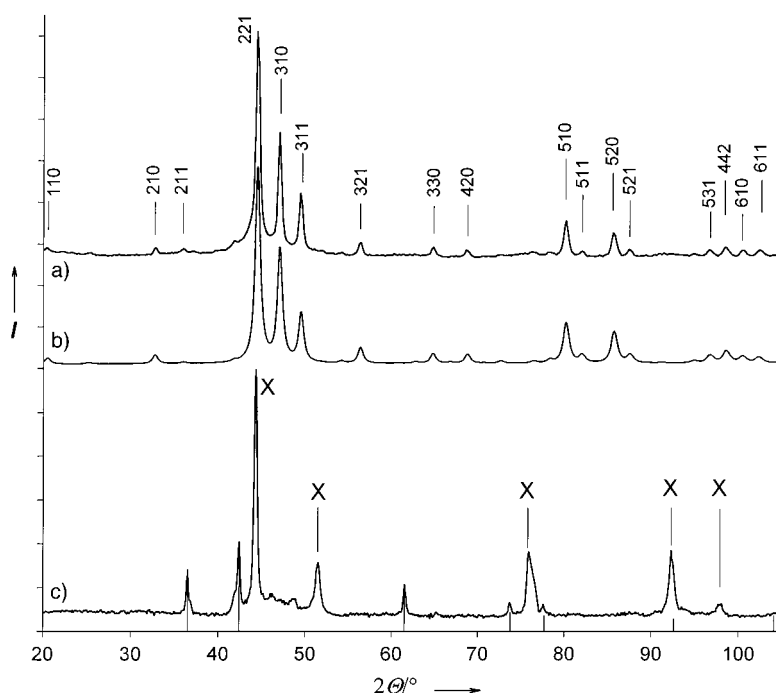


Figure 2. X-ray powder diffraction pattern of the ϵ -cobalt structure: a) experimental; b) calculated; c) the sample shown in a) after being heated to 500 °C, peaks corresponding to fcc cobalt are denoted by X, peaks corresponding to CoO are marked with a stick spectrum.

Table 1. Fractional atomic coordinates in the unit cell of ϵ -cobalt structure.^[a]

	<i>x</i>	<i>y</i>	<i>z</i>
Type I			
1	<i>v</i>	<i>v</i>	<i>v</i>
2	$v + \frac{1}{2}$	$\frac{1}{2} - v$	$1 - v$
3	$1 - v$	$v + \frac{1}{2}$	$\frac{1}{2} - v$
4	$\frac{1}{2} - v$	$1 - v$	$v + \frac{1}{2}$
5	$\frac{3}{4} - v$	$\frac{3}{4} - v$	$\frac{3}{4} - v$
6	$v + \frac{1}{4}$	$\frac{1}{4} - v$	$v + \frac{3}{4}$
7	$\frac{1}{4} - v$	$v + \frac{3}{4}$	$v + \frac{1}{4}$
8	$v + \frac{3}{4}$	$v + \frac{1}{4}$	$\frac{1}{4} - v$
Type II			
9	$1 - u$	$u + \frac{3}{4}$	$\frac{3}{8}$
10	$\frac{1}{2} - u$	$\frac{3}{4} - u$	$\frac{5}{8}$
11	<i>u</i>	$u + \frac{1}{4}$	$\frac{1}{8}$
12	$u + \frac{1}{2}$	$\frac{1}{4} - u$	$\frac{7}{8}$
13	$\frac{3}{8}$	$1 - u$	$u + \frac{3}{4}$
14	$\frac{5}{8}$	$\frac{1}{2} - u$	$\frac{3}{4} - u$
15	$\frac{1}{8}$	<i>u</i>	$u + \frac{1}{4}$
16	$\frac{7}{8}$	$u + \frac{1}{2}$	$\frac{1}{4} - u$
17	$u + \frac{3}{4}$	$\frac{3}{8}$	$1 - u$
18	$\frac{3}{4} - u$	$\frac{5}{8}$	$\frac{1}{2} - u$
19	$u + \frac{1}{4}$	$\frac{1}{8}$	<i>u</i>
20	$\frac{1}{4} - u$	$\frac{7}{8}$	$u + \frac{1}{2}$

[a] $u = 0.20224$, $v = 0.06361$.^[9]

respectively.^[1] The calculated diffraction pattern for the new phase (Figure 2b) is in excellent agreement with experimental data.^[10] The comparison shows that the experimental pattern is clean and corresponds to a pure ϵ -cobalt structure. The position and intensity of each peak is precisely reproduced and there are no peaks corresponding to either known cobalt phases or cobalt oxide. Numerical values for experimental and calculated peak positions and intensities are shown in Table 3. A model unit cell for ϵ -cobalt is shown in Figure 3.

Table 2. Local coordination for the two types of Co atoms in ϵ -cobalt.

Near neighbor type (number)	Interatomic distance [Å]	Near neighbor type (number)	Interatomic distance [Å]
Type I		Type II	
Type I (3)	2.281(9)	Type I (2)	2.487(2)
Type II (3)	2.487(2)	Type I (2)	2.543(4)
Type II (3)	2.543(4)	Type I (2)	2.587(0)
Type II (3)	2.587(0)	Type II (4)	2.554(6)
Type II (2)	2.580(1)		

Table 3. Assignments of reflections and comparison between observed and calculated values for ϵ -cobalt.

$2\theta_{\text{obs}}$	Obs. intensity	hkl	Cell parameter [Å] ^[a]	$2\theta_{\text{calc}}$	Calcd intensity
32.83	3.3	210	6.1002	32.848	4.5
36.10	0.6	211	6.0946	36.085	0.8
44.57	100	221	6.0989	44.585	100
47.14	55	310	6.0968	47.138	58
49.59	27	311	6.0970	49.590	25
64.88	4	330	6.0975	64.886	5
80.30	22	510	6.0966	80.293	21
82.15	3	511	6.0968	82.147	4
85.84	18	520	6.0966	85.832	19
96.85	4	531	6.0968	96.845	5
98.67	8	442	6.0981	98.694	8
100.52	6	610	6.0984	100.544	4.5
102.40	5	611	6.0980	102.422	4

[a] Average = 6.097 ± 0.001 .

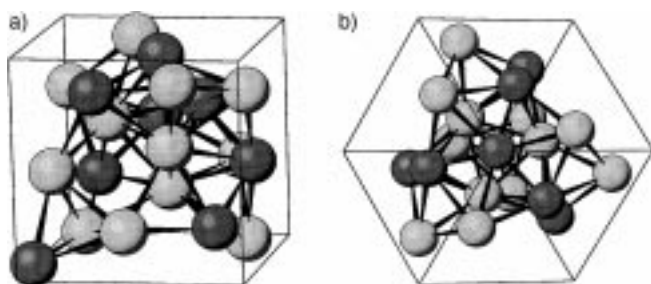


Figure 3. The unit cell of ϵ -cobalt: a) unit cell cube filled with eight atoms of Type I (dark) and twelve atoms of Type II (light); b) 111-projection of the same cube showing threefold symmetry along its main diagonal.

The magnetic moment per atom in ϵ -cobalt, measured as $1.70 \mu_B$, is similar, within our experimental error, to that of the two known structures ($1.75 \mu_B$ and $1.72 \mu_B$ for bulk fcc and hcp cobalt).^[11]

The new ϵ -cobalt phase appears to be metastable under normal conditions. Although stable at room temperature for at least several months, heating the sample shown in Figure 2a to 500°C completely transforms it to the known fcc phase (Figure 2c).^[12] Subsequent cooling does not return the sample to its original ϵ -cobalt structure. The same preparation method but without the presence of TOPO yields nanocrystals with a pure fcc phase. When TOPO is added, ϵ -cobalt is formed along with fcc cobalt. Increasing the concentration of TOPO in the reaction mixture leads to the exclusive formation of ϵ -cobalt (Figure 4). Tight coordination of ligand

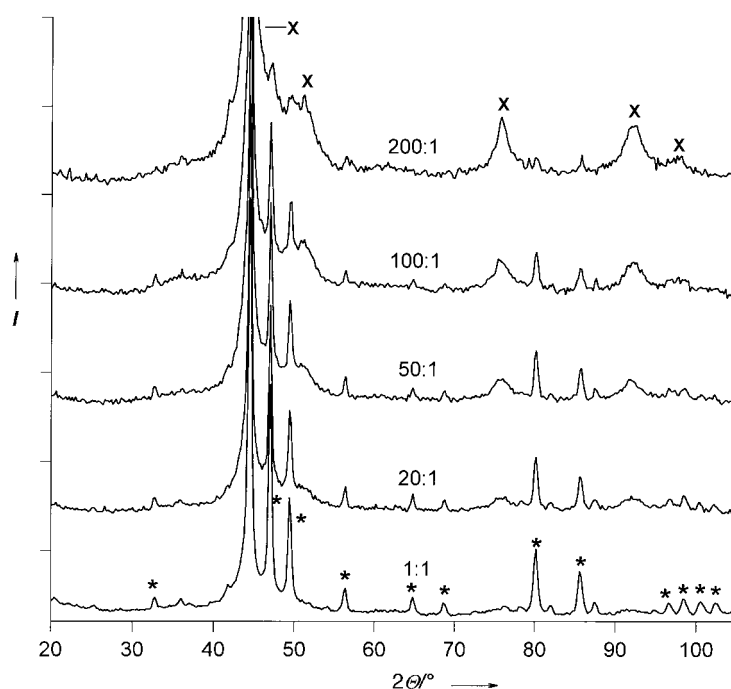


Figure 4. Evolution of the X-ray diffraction pattern of cobalt nanocrystals prepared with different concentrations of TOPO. The numbers shown correspond to the ratio of cobalt atoms to TOPO molecules in the reaction mixture. The gradual change from fcc (peaks denoted by X) to ϵ -cobalt (selected peaks denoted by *) with increasing concentration of TOPO is clearly observed. Sharpening of the peaks corresponding to improved crystallinity of the nanocrystals is also observed.

molecules (TOPO) around the growing crystal and around solubilized cobalt atoms is responsible for changing the energetics of growth in favor of the new, less dense phase. In fact, the particular synthesis of this paper is not unique in producing ϵ -cobalt. Sun and Murray^[13] have recently confirmed our assignment of the structure of ϵ -cobalt in nanocrystals obtained by reducing cobalt salts in solution in the presence of alkylphosphanes as the coordinating ligands. Due to its metastability, ϵ -cobalt may only be accessible by solution-phase approaches, rather than more common techniques, such as varying temperature and pressure.

The discovery of a new phase for a common element is an example of the critical role that ligands and surfactants can play in determining the resulting structure of crystals grown in solution at low temperatures. Such solution-phase reactions are often controlled kinetically rather than thermodynamically, leading to the initial trapping and growth of metastable phases. Similar low-temperature growth conditions may lead to the discovery of previously unknown structures for other elements and for more complex systems. There is a strong correlation between crystal structure and the magnetic properties of cobalt. The anisotropic high magnetic coercivity hcp phase is the preferred structure for permanent magnet applications (recording media, etc), while the more symmetric low coercivity fcc phase is useful for soft magnetic applications. The newly discovered structure of cobalt may display magnetic properties different from the two previously known structures, and this could be scientifically interesting and technologically useful.

Experimental Section

[Co₂(CO)₈] (2.5 g, Strem Chemicals, Inc.) was dissolved in dry toluene (30 mL) under air-free conditions, filtered, and then loaded into a syringe. Trioctylphosphane oxide (TOPO, 5 g, Strem Chemicals, Inc.) was placed in a 100 mL flask and dried under vacuum at 150 °C. The temperature was then brought down to 50 °C, and the solution of [Co₂(CO)₈] was quickly injected into the flask while vigorously stirring under a nitrogen atmosphere. The temperature was slowly increased to 110 °C. This was accompanied by evolution of carbon monoxide and by a color change of the reaction mixture from deep blue to black. After completion of the reaction the heating mantle was removed, and the mixture was allowed to cool to room temperature. The product of the reaction was isolated as a black powder and was thoroughly washed with hexane and methanol and dried under nitrogen.

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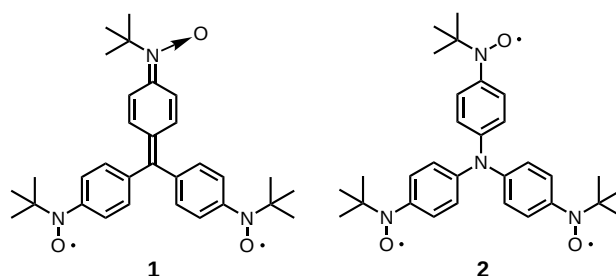
Keywords: clusters • cobalt • structure elucidation • X-ray scattering

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A Triphenylamine Derivative with Three *p*-(*N*-*tert*-Butyl-*N*-oxylamino)phenyl Radical Units and Yet a Doublet Ground State**

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Intramolecular ferromagnetic interactions of electron spins in polyradicals are of current interest in the design of molecular ferromagnets.^[1] Previously, we reported that the oxidation of tris[*p*-(*N*-*tert*-butyl-*N*-hydroxyamino)phenyl]methane with silver(I) oxide gave the quinonoid diradical **1**, which has a robust triplet ground state.^[2] When the methane carbon atom in **1** is replaced with a nitrogen atom to make tris[*p*-(*N*-*tert*-butyl-*N*-oxylamino)phenyl]amine (**2**), a quinonoid struc-



ture requires a zwitterionic structure. Hückel MO calculations show that tris(*p*-methylenephényl) methane, a simplified model of **1**, has two degenerate SOMOs similar to those of trimethylenemethane (TMM).^[3] Since the corresponding amine has one more π electron than the methane, the former is expected to become isoelectronic with the anion radical of the latter and therefore exist as a doublet monoradical. We report here the synthesis, structure, and magnetic properties of **2**.

Triradical **2** was obtained by oxidation of tris[*p*-(*N*-*tert*-butyl-*N*-hydroxyamino)phenyl]amine with Ag₂O. A single crystal for an X-ray diffraction measurement was obtained by slow evaporation of a solution of **2** in *n*-heptane/dichloromethane.^[4] The molecule has no threefold axis passing through the central nitrogen atom (Figure 1). The *p*-phenylene rings are tilted by 29.8°, 34.3°, and 57.3° from the plane formed by C1, C11, and C21. The intramolecular distances between the oxygen atoms of the oxylamino groups are 9.73

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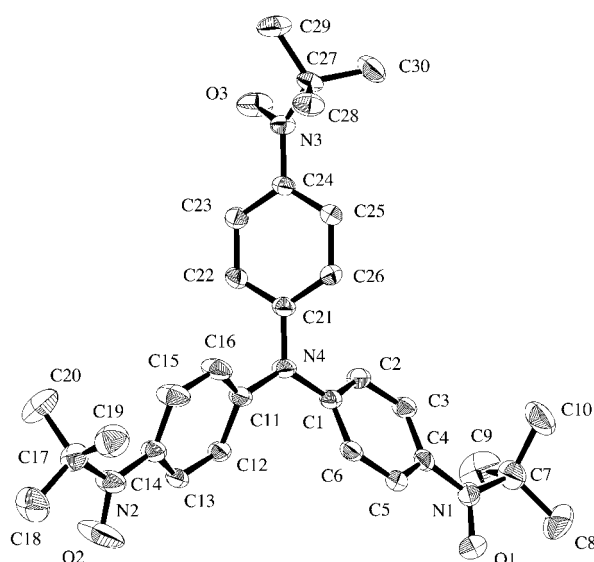


Figure 1. ORTEP drawing of **2** with thermal ellipsoids drawn at the 50% probability level. The hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: N4–C1 1.412(4), N4–C11 1.421(4), N4–C21 1.428(4), N1–O1 1.291(3), N2–O2 1.285(4), N3–O3 1.294(3), N1–C4 1.417(4), N2–C14 1.414(4), N3–C24 1.413(4); C1–N4–C11 122.1(2), C1–N4–C21 118.9(2), C11–N4–C21 118.7(2), O1–N1–C4 116.9(2), O2–N2–C14 116.9(3), O3–N3–C24 116.6(2).

(O1–O2), 11.30 (O2–O3), and 11.41 Å (O3–O1), whereas the shortest intermolecular distance between O1 and O2' of neighboring molecules is 4.84 Å.^[4, 5]

The X-band ESR spectra of **2** in degassed 2-methyltetrahydrofuran (MTHF) were measured in the temperature range 8.5–300 K. At 300 K the ESR spectrum centered at $g = 2.006$ consisted of a septet in a ratio of 1:3:6:7:6:3:1 ($|a_N| = 4.06$ G) due to hyperfine coupling with the three equivalent nitrogen nuclei (Figure 2). This suggests that the exchange interaction is much larger than the hyperfine interaction ($|J| \gg |a_N|$).

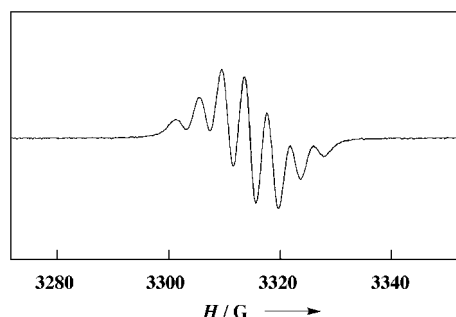


Figure 2. X-band ESR spectrum of **2** (10 mm in MTHF) at 300 K (9.327 GHz).

In a frozen solution **2** showed a large main peak with a very weak outer shoulder in the $g = 2$ region, but no signal arising from the $\Delta m_s = 2$ or 3 transitions. The intensity of the main peak decreased as the temperature was increased in the range 8.5–100 K, which is in good agreement with Curie's law. This result is not inconsistent with a quartet ground state of **2**. However, it is not possible to determine rigorously whether

the ground state is quartet, doublet, or degenerate quartet/doublet.

Figure 3 shows the temperature dependence of the molar paramagnetic susceptibility (χ_{mol}) of **2** at 500 G as a plot of $\chi_{\text{mol}}T$ versus T . The observed $\chi_{\text{mol}}T$ values remained almost

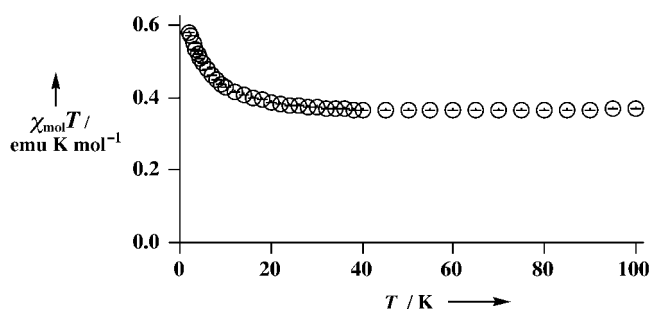


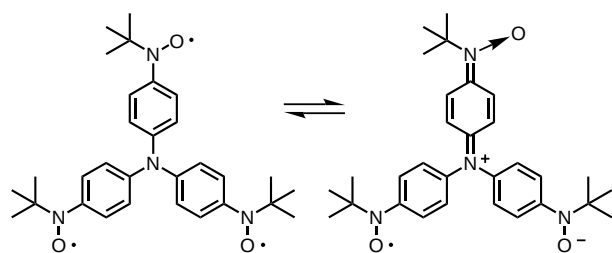
Figure 3. Plot of $\chi_{\text{mol}}T$ versus T for **2** at 500 G.

constant from 100 down to 20 K and are in good agreement with the theoretical value ($0.375 \text{ emu K mol}^{-1}$) expected for an assembly of single isolated spins. As the temperature was decreased below 20 K, the $\chi_{\text{mol}}T$ values increased continuously to a value of $0.58 \text{ emu K mol}^{-1}$ at 2 K.

Further proof of the ground-state spin multiplicity of **2** was furnished by similar magnetic susceptibility measurements on dilute samples (5%) of **2** in poly(vinyl chloride), in which possible intermolecular coupling would be considerably diminished. Under these conditions, the continuous increase in the $\chi_{\text{mol}}T$ values below 20 K was absent, which suggests that there is no intramolecular magnetic interaction populating the quartet state at the cryogenic temperature and that the ground state must be a doublet state.^[6] The doublet–quartet energy gap is estimated to be greater than 100 K ($> 0.2 \text{ kcal mol}^{-1}$).

Ab initio MO calculations at the B3LYP/6-31G level were performed on models in which all three *N-tert*-butyl groups in **2** were replaced with methyl groups and the structures were optimized. The doublet was found to be more stable than the quartet by $0.84 \text{ kcal mol}^{-1}$. Whereas the central amine nitrogen atom does not carry high net spin density in either state (Mulliken spin density of 0.0093 and 0.0314 for the doublet and quartet respectively), it has high α and β spin densities in the 100th magnetic orbital and the 99th doubly occupied MO, respectively, in the doublet ground state. This demonstrates the operation of a superexchange mechanism through the lone pair of electrons on the central nitrogen atom, with two spins on the oxylamino radical centers coupled antiferromagnetically and one spin remaining intact. A superexchange mechanism has been invoked in organic dicarbenes,^[7] but there are none in which the interaction has been demonstrated to be as strong as in **2**. Any contribution of a quinonoid structure for **2** to the equilibrium illustrated in Scheme 1 is thus estimated to be very small.^[8]

In conclusion, the doublet ground state of the triphenyl amine **2** carrying three *N-tert*-butyl-*N*-oxylamino groups in the *para* positions indicates that not only the topology but also the number of the π electrons is important in defining ferromagnetic couplers for molecular-based magnets.^[1]



Scheme 1. Equilibrium of the triradical and zwitterionic structures of **2**.

Experimental Section

Tris[*p*-(*N*-*tert*-butyl-*N*-hydroxyamino)phenyl]amine: To a solution of tris[*p*-(*N*-*tert*-butyl-*N*-hydroxyamino)phenyl]amine (2.0 g, 4.15 mmol) in THF (40 mL) was added *tert*-butyllithium (1.6 M pentane solution, 18.2 mL) at -78°C . The mixture was warmed to 0°C over 10 min and then cooled again to -78°C . 2,2-Dimethylnitrosoethane (1.45 g, 16.6 mmol) was added, and the mixture was warmed to room temperature and stirred for 1 h. Saturated aqueous ammonium chloride and ether were added, and the organic layer was separated, washed with water, dried over MgSO_4 , and evaporated under reduced pressure. The residue was washed with dichloromethane to afford tris[*p*-(*N*-*tert*-butyl-*N*-hydroxyamino)phenyl]amine (0.77 g, 37%) as a white powder. M.p. 153°C (decomp.); ^1H NMR (270 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 8.20$ (s, 3H), 7.92 (d, $J = 8.57$ Hz, 6H), 6.84 (d, $J = 8.57$ Hz, 6H), 1.06 (s, 27H); ^{13}C NMR (67.8 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 145.34, 143.74, 125.29, 122.23, 59.19, 25.99$; FAB MS calcd for $\text{C}_{30}\text{H}_{42}\text{N}_4\text{O}_3$: 506.3257, found: 506.3256.

2: To a solution of tris[*p*-(*N*-*tert*-butyl-*N*-hydroxyamino)phenyl]amine (100 mg, 0.20 mmol) in dichloromethane (30 mL) was added an excess of freshly prepared Ag_2O (ca. 300 mg), and the mixture was stirred for 2 h. The reaction mixture was filtered, and the solvent was removed under reduced pressure at ambient temperature. The residue was subjected to chromatography on aluminum oxide with dichloromethane as eluent and recrystallized from *n*-heptane/dichloromethane (2/1) to give **2** as dark violet crystals (86 mg, 87%). M.p. 190 – 192°C ; FAB MS: m/z 503 $[\text{M}^+]$; elemental analysis calcd for $\text{C}_{30}\text{H}_{39}\text{N}_4\text{O}_3$: C 71.54, H 7.80 N 11.12; found: C 71.44, H 7.79, N 11.08.

Magnetic measurement: Fine crystalline or polymer film samples were mounted in a capsule (Japan Pharmacopoeia NO. 5, $\varnothing 4.5 \times 11$ mm) and measured on a Quantum Design MPMS-5S SQUID susceptometer at 500 G. Corrections for the diamagnetic contribution were made with Pascal's constants.

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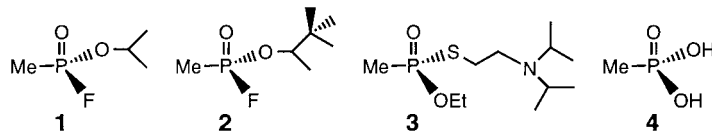
Keywords: ab initio calculations • isoelectronic analogues • magnetic properties • radicals • superexchange mechanism

- [4] Crystallographic data for **2**: $\text{C}_{30}\text{H}_{39}\text{N}_4\text{O}_3$, $M_r = 503.66$, monoclinic, space group Cc , $a = 13.11(1)$, $b = 23.081(5)$, $c = 10.410(2)$ Å, $\beta = 116.80(5)^{\circ}$, $V = 2810(2)$ Å³, $Z = 4$, $\rho_{\text{calc}} = 1.190$ g cm⁻³, $\mu(\text{MoK}\alpha) = 0.77$ cm⁻¹. Data were collected on a Rigaku RAXIS-IV imaging plate area detector at -100°C with graphite-monochromated $\text{MoK}\alpha$ radiation ($\lambda = 0.71071$ Å). The structure was solved with direct methods and refined to $R = 0.039$, $R_w = 0.054$ for 2461 observed reflections ($I > 3.00\sigma(I)$) and 335 parameters. 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-101263. 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).
- [5] Symmetry operation: $x, -y, 1/2 + z$.
- [6] The origin of the ferromagnetic interaction between the neighboring molecules in crystals may be located at O1–C12' and O3–C25' (3.272(4) and 3.131(3) Å, respectively) along the chain made by the molecules aligned in the direction of the c axis.
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Monitoring Chemical Warfare Agents: A New Method for the Detection of Methylphosphonic Acid**

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Issues surrounding chemical warfare agents are currently of great importance with regard to national security and world affairs. The lethal compounds **1** (sarin), **2** (soman), and **3** (VX)



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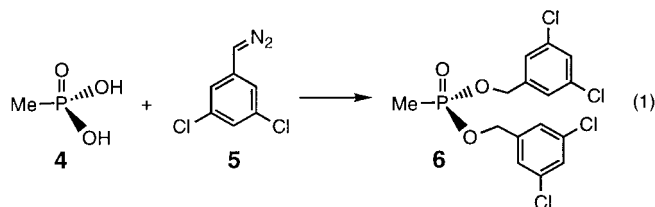
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have been feared as the “nuclear weapons” of the poorer nations because their manufacture is relatively simple and the starting materials are inexpensive and readily available. Recently, sarin was implicated in attacks by Iraq on Kurdish communities^[1] and in the terrorist activity in the Tokyo subway.^[2] Since these and other nerve agents degrade naturally in the environment to methylphosphonic acid (MPA) **4** or can be degraded in the laboratory to MPA, a convenient detection method for this substance is necessary. Therefore, the development of a simple, portable, and inexpensive immunoassay kit would be valuable to monitor various treaty compliances and also during military operations.^[8]

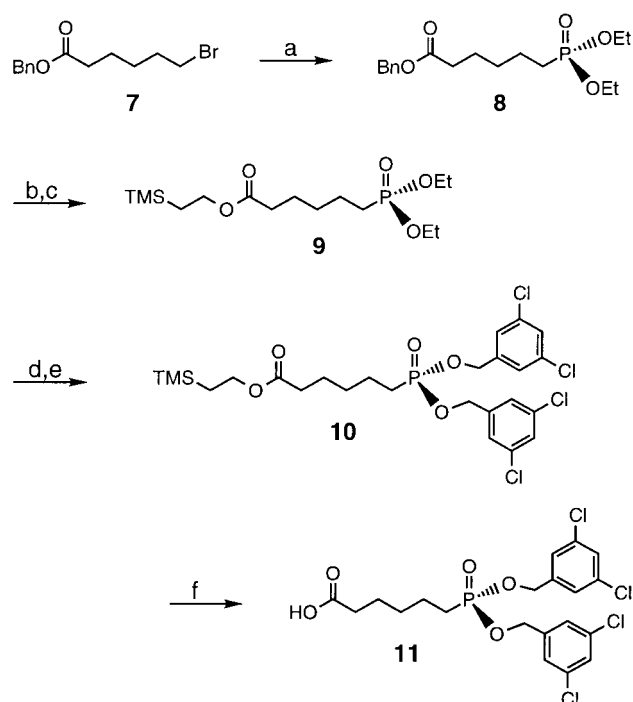
As a result of immunogenicity problems, monoclonal antibodies (mAbs) that bind MPA could not be successfully obtained. Consequently, we reasoned that if MPA were readily derivatized with recognition elements, mAbs that bound the MPA derivative could be elicited by using a structurally congruent, immunogenic hapten. In this way, the presence of MPA itself could be assessed indirectly through formation and detection of the derivative. Since diazomethane rapidly and quantitatively forms methyl esters from either carboxylic or phosphonic acids,^[3] it was anticipated that a more complex diazo compound might also prove useful. We decided to utilize 3,5-dichlorophenyldiazomethane **5** since this compound was prepared in high yield and showed excellent stability.^[4] Moreover, the dichloro-substituted aromatic ring presented a potent epitope as part of the hapten structure.

The best solvent for the esterification of **4** with **5** [Eq. (1)] was found to be dioxane/0.5% water wherein the small amount of water was used to ensure complete solubility of



MPA. Although the yield of isolated and purified bis(3,5-dichlorobenzyl) methylphosphonate **6** was good (67%), the estimated in situ yield was near 80% by visual colorimetric comparison to authentic standard solutions eluted by thin-layer chromatography and stained with cerium molybdate. The remaining 20% of the mass balance was accounted for by an impurity in **5** (10%) and the formation of a by-product, MPA mono(3,5-dichlorobenzyl) ester (10%). The latter was formed since we chose to use only a stoichiometric amount of **5** (2 equiv). Despite the presence of water in the reaction, hydrolysis of the diazo reagent to 3,5-dichlorobenzyl alcohol was not observed.

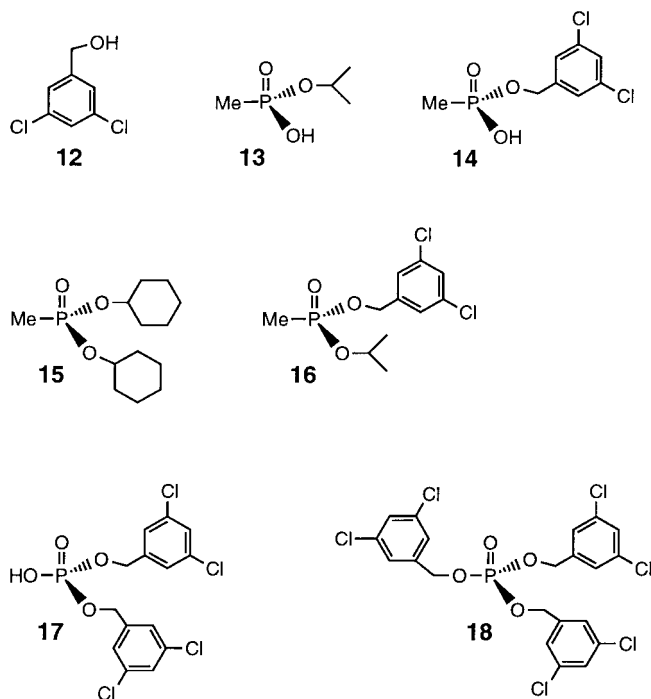
The hapten **11** (CDC) corresponding to the derivative **6** was then prepared (Scheme 1). The CDC was conjugated to the carrier protein keyhole limpet hemocyanin (KLH) and entered into our standard immunization program in mice. An enzyme-linked immunosorbent assay (ELISA) protocol in a competition format^[5] was used during the hybridoma



Scheme 1. Synthesis of the hapten **11** structurally related to the MPA derivative **6**. a) diethyl phosphite (1.2 equiv), NaH (1.2 equiv), DMF, 0°C → 25°C, 2 h; b) H₂, Pd/C, methanol, 25°C, 1 h; c) 2-trimethylsilylethanol (1.2 equiv), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 1.2 equiv), 4-dimethylaminophenol (DMAP, cat), DMF, 25°C, 16 h; d) 1) Me₃SiBr (2.1 equiv), CH₂Cl₂, 25°C, 1 h; 2) oxalyl chloride (3 equiv), DMF (cat.), CH₂Cl₂, 25°C, 1 h; e) 3,5-dichlorobenzyl alcohol (2.1 equiv), NEt₃ (2.5 equiv), DMAP (cat), 25°C, 16 h; f) trifluoroacetic acid (TFA, 5 equiv), 0°C → 25°C, 1 h.

screening and cloning process to increase the likelihood of cloning antibodies with high specificity for **6**. Hence, clones were selected on the basis of the best binding of the free ligand **6** versus a bovine serum albumin (BSA) conjugate of **11**. A panel of 11 mAbs that bound **6** were isolated of which CDC27B4 showed the highest affinity ($K_d \approx 1 \mu\text{M}$). Based on previous work in our laboratory, this apparent K_d was likely up to an order of magnitude higher than the true K_d since competition ELISA tends to overestimate the value. Importantly, CDC27B4 showed excellent specificity when examined for cross-reactivity versus alternate ligands **4**, and **12**–**18**.

At concentrations of 10 μM , no binding of these compounds was detected using competition ELISA. Even at 100 μM of **16**, the derivative of the first breakdown product of sarin, no binding was observed. Given the presence of the 3,5-dichlorophenyl substituent as an anticipated strong haptenic determinant, the specificity was rather exquisite. The results suggested that both rings were recognized during the immune response and were required for binding. Moreover, the antibody was specific for the chemical nature and coordination sphere of the phosphorus species as revealed by the phosphate esters **17** and **18**. Despite the presence of at least two 3,5-dichlorophenyl substituents, the hydroxyl group of **17** and the steric bulk of the extra ring of **18** precluded binding at 10 times the K_d of the derivative **6**. The K_d for these compounds was about 300 μM .



Based on principles of antibody fine specificity and that the ligands tested were structurally related to **6**, there should be no interference from other compounds present at test sites. It was estimated that 170 ± 10 ppb of MPA analyte could be assayed as derivative **6**. A sensitivity comparable to many reported instrumental technologies that are expensive and usually necessitate specialized training. The sensitivity was also similar to other immunoassays developed for chemical warfare agents themselves.^[6] Depending on the confidence limit chosen for detection, we anticipate the derivatization–ELISA procedure will accurately reflect the presence of MPA in an appropriate field sample.

Other methods routinely used for MPA analysis invoked chromatographic and/or spectrometric techniques.^[7] Most of these approaches also required a prior derivatization step and furthermore were generally not amenable for the testing of crude samples. Our method is inexpensive, sensitive, convenient, robust, and would require only a minimum of sample preparation. For instance, a field sample from a suspected chemical warfare agent manufacturing, storage, or deployment site could be obtained by swipe and extracted with dioxane/water or a collected wet sample evaporated and redissolved. A simple filtration step might then be employed followed by addition of the reagent **5** and then assay. Notably, the presence of **5** in a 100-fold molar excess relative to antibody had no detrimental effects on binding. Hence, no other handling or purification steps should be necessary. While refinements are necessary to establish a field kit, we believe derivatization–ELISA can complement other MPA detection methods. These efforts and adaptation to other compounds related to chemical warfare agents are in progress.

Experimental Section

6: Reagent **5** (187 mg, 1 mmol) was added slowly to a solution of MPA (48 mg, 0.50 mmol) in 1,4-dioxane/0.5% water (5 mL). Bubbling was

immediately observed upon addition. The resulting solution was stirred at room temperature for 1 h and then diluted with ethyl acetate (10 mL). After washing with brine (5 mL), the organic layer was dried with MgSO_4 and concentrated to give a yellow solid. The solid was purified using flash chromatography (95/5 $\text{CH}_2\text{Cl}_2/\text{EtOAc}$) affording a white solid (138 mg, 67%).

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[Mn(en)₃][Cr(CN)₆]₂·4H₂O: A Three-Dimensional Dimetallic Ferrimagnet (*T_c* = 69 K) with a Defective Cubane Unit**

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Recently, there has been increasing interest in metal assemblies of ordered networks.^[1–11] One fascinating target in this research area are molecular-based magnets that exhibit

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spontaneous magnetization. Hexacyanometalate ions $[\text{M}(\text{CN})_6]^{n-}$ are often used as building blocks for dimetallic magnetic materials. Two types of dimetallic compounds have been derived from $[\text{M}(\text{CN})_6]^{n-}$: 1) The Prussian-Blue family constructed with a simple metal ion^[6] and 2) complex-based dimetallic assemblies constructed with a complex cation such as $[\text{Ni}(\text{L})_2]^{2+}$ (L = ethylenediamine derivative) and $[\text{M}^{\text{III}}(\text{salen})]^+$ (M = Fe or Mn; salen = *N,N'*-ethylenebis(salicylideneaminate) dianion).^[8–10] Almost all members of the Prussian-Blue family show spontaneous magnetization, and high magnetic ordering temperatures T_c are reported for some of them. However, the magnetostructural correlation for Prussian-Blue compounds is still unclear due to the lack of structural information. Complex-based dimetallic assemblies with one-dimensional (1D) chain, 1D rope ladder, two-dimensional (2D) honeycomb, and 2D square structures have been obtained, and magnetic ordering is recognized only for the 2D network dimetallic assemblies.^[8] The first three-dimensional (3D) dimetallic assemblies with a cubane network, $[\text{Ni}(\text{L})_2]_3[\text{Fe}(\text{CN})_6]\text{X}_2$ (L = ethylenediamine (en) or trimethylenediamine, X = ClO_4^- or PF_6^-)^[9] were recently reported but they showed no magnetic ordering due to the diamagnetic nature of Fe^{II} . Here we report on a dimetallic compound $[\text{Mn}(\text{en})]_3[\text{Cr}(\text{CN})_6]_2 \cdot 4\text{H}_2\text{O}$ that has a 3D network structure extended by the $\text{Cr}^{\text{III}}\text{--CN--Mn}^{\text{II}}$ linkage and that shows magnetic ordering at $T_c = 69\text{ K}$.

The dimetallic assembly $[\text{Mn}(\text{en})]_3[\text{Cr}(\text{CN})_6]_2 \cdot 4\text{H}_2\text{O}$ was obtained as light green crystals by the reaction of $\text{K}_3[\text{Cr}(\text{CN})_6]$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, and ethylenediamine in 2:3:3 molar ratio in an aqueous solution under argon. The X-ray crystal structure analysis^[12] reveals that all the cyanide groups of $[\text{Cr}(\text{CN})_6]^{3-}$ are involved in the coordination to adjacent Mn^{2+} ions. The asymmetric unit consists of a $[\text{Cr}(\text{CN})_6]^{3-}$ ion, three halves of $[\text{Mn}(\text{en})]^{2+}$ ions, and two water molecules. The geometry about Mn1 and Mn2 is pseudo-octahedral with a chelating en ligand and four cyanide nitrogen atoms from adjacent $[\text{Cr}(\text{CN})_6]^{3-}$ units (Figure 1). The Cr–C and Mn–N

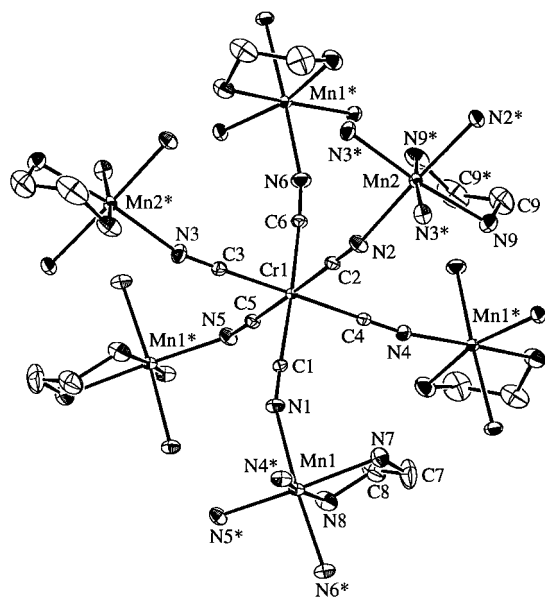


Figure 1. ORTEP drawing of the heptanuclear unit of $[\text{Mn}(\text{en})]_3[\text{Cr}(\text{CN})_6]_2 \cdot 4\text{H}_2\text{O}$ (H_2O are omitted).

bond lengths range between 2.065(3) and 2.080(3) Å and between 2.208(3) and 2.287(3) Å, respectively. The Mn–N–C bond angle ranges between 145.7(3)° and 167.4(3)°. In the lattice, a 3D network structure is formed by the alternate array of $[\text{Cr}(\text{CN})_6]^{3-}$ and $[\text{Mn}(\text{en})]^{2+}$ ions (Figure 2). The network

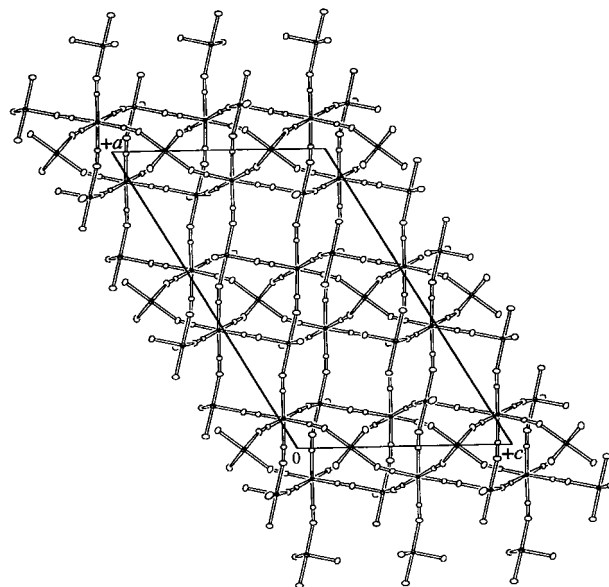


Figure 2. Projection of the 3D network structure onto the *ac* plane (en and H_2O are omitted).

comprises defective cubes with three Cr atoms, three Mn1 atoms, and one Mn2 atom at the seven corners and eight Cr–CN–Mn edges. The shortest Cr···Mn(1), Cr···Mn(2), and Mn(1)···Mn(2) distances are 5.1553(8), 5.2842(8), and 6.659(1) Å, respectively. The lattice water molecules reside in cavities of the network and form hydrogen bonds with the cyano nitrogen atoms (O(1)–H(13)···N(5) and O(2)–H(15)···N(2)).

The magnetic behavior^[13] of the dimetallic compound is shown in Figure 3 in the form of $\chi_M T$ versus T and χ_M versus T plots. The $\chi_M T$ value is $13.08\text{ cm}^3\text{K mol}^{-1}$ ($10.23\mu_B$ per Mn_3Cr_2) at room temperature and decreases with decreasing temperature down to a minimum value of $12.04\text{ cm}^3\text{K mol}^{-1}$ ($9.82\mu_B$) at 156 K. Upon further cooling, the $\chi_M T$ value increases up to a maximum value of $5671\text{ cm}^3\text{K mol}^{-1}$ ($213\mu_B$)

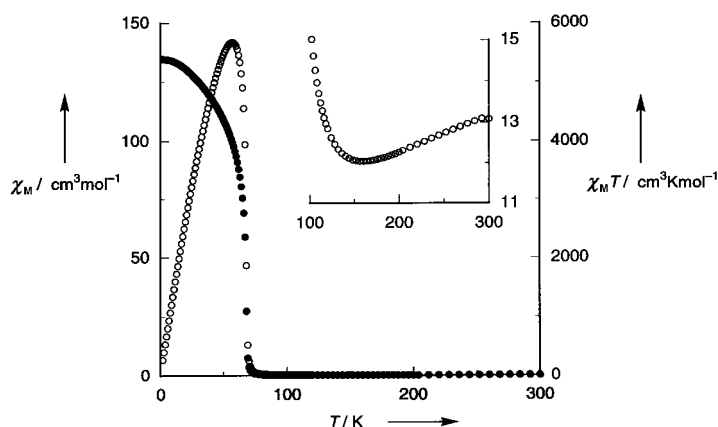


Figure 3. Plots of χ_M (●) and $\chi_M T$ (○) versus T for $[\text{Mn}(\text{en})]_3[\text{Cr}(\text{CN})_6]_2 \cdot 4\text{H}_2\text{O}$ per Mn_3Cr_2 unit. Insert: Expanded view of the minimum region of $\chi_M T$.

at 50 K and then decreases below this temperature. The $1/\chi_M$ versus T plot in the range from 300 to 156 K obeys the Curie–Weiss law with a Weiss constant of -38 K. The negative Weiss constant indicates an intramolecular antiferromagnetic interaction between the adjacent Cr^{III} and Mn^{II} ions through the cyano bridge. The minimum $\chi_M T$ value agrees with the spin-only value of $12.38 \text{ cm}^3 \text{ K mol}^{-1}$ ($9.95 \mu_B$) for antiferromagnetically coupled Mn_3Cr_2 ($S_T = 9/2$). The abrupt increase in $\chi_M T$ around 70 K suggests the onset of three-dimensional magnetic ordering. The decrease of $\chi_M T$ below 50 K results from a saturation of the χ_M value.

Low-field magnetization measurements for the dimetallic compound confirm the long-range magnetic ordering below 69 K to produce a ferrimagnet (Figure 4). The field dependence of magnetization M shows a sharp increase with applied

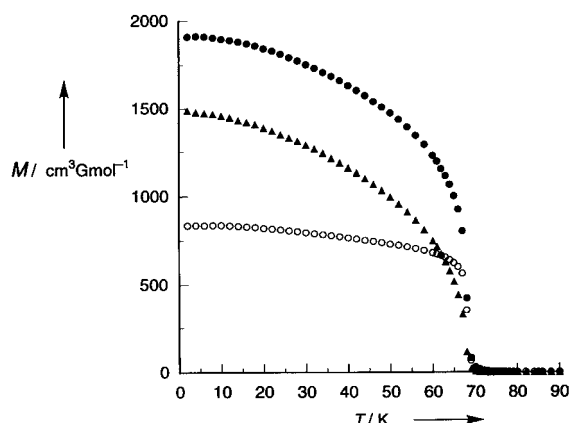


Figure 4. Plots of magnetization M versus T at 5 G: field-cooled magnetization (FCM; ●), remnant magnetization (RM; ▲), zero-field magnetization (ZFCM; ○).

field and rapid saturation. The saturation magnetization $M_s = 8.7 \mu_B$ is close to the expected value of $9 \mu_B$, adding support to the assumption that the adjacent Mn^{II} and Cr^{III} centers are antiferromagnetically coupled (Figure 5). The magnetic hysteresis loop at 2 K (Figure 5, insert) is typical of soft magnets showing a remnant magnetization of $1.01 \times 10^4 \text{ cm}^3 \text{ G mol}^{-1}$ and a weak coercive field $H_c = 28$ G.

It should be noted that $[\text{Mn}(\text{en})]_3[\text{Cr}(\text{CN})_6]_2 \cdot 4\text{H}_2\text{O}$ is similar to one type of Prussian-Blue family, $\text{A}_3^{\text{II}}[\text{B}^{\text{III}}(\text{CN})_6]_2 \cdot$

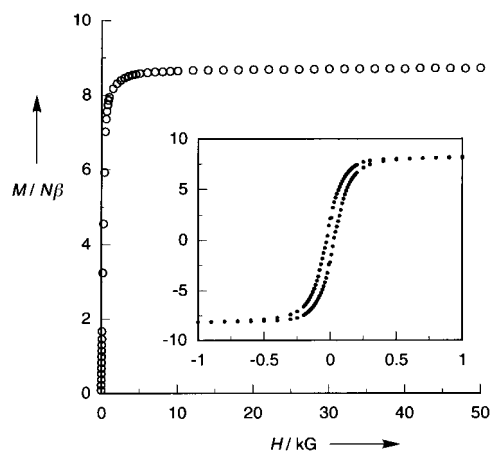


Figure 5. Field dependence of magnetization M ($N\beta$) at 2 K. Insert: hysteresis loop ($M/N\beta$ versus H) at 2 K.

$x\text{H}_2\text{O}$.^[6, 14] We presume that this type of Prussian-Blue analogue has a similar 3D network structure with an $\text{A}(\text{H}_2\text{O})_2^{2+}$ site in place of the $[\text{Mn}(\text{en})]^{2+}$ site in the present compound.

In conclusion, $[\text{Mn}(\text{en})]_3[\text{Cr}(\text{CN})_6]_2 \cdot 4\text{H}_2\text{O}$ is a 3D ferrimagnet showing magnetic ordering below 69 K. To the best of our knowledge, the magnetic phase-transition temperature is the highest among the structurally characterized molecular-based magnets.

Experimental Section

To a solution of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.6 mmol) and en (0.6 mmol) in water (15 mL) was added an aqueous solution (10 mL) of $\text{K}_3[\text{Cr}(\text{CN})_6]$ (0.4 mmol) under argon at room temperature, and the resulting yellow mixture was allowed to stand to give light green prismatic crystals. They were collected by suction filtration and washed with water. All the operations for the synthesis were carried out in the dark to avoid the decomposition of $\text{K}_3[\text{Cr}(\text{CN})_6]$. Yield 53 mg, 32 %. Elemental analysis (%): calcd for $\text{C}_{18}\text{H}_{32}\text{N}_{18}\text{Cr}_2\text{Mn}_3\text{O}_4$: C 25.94, H 3.87, N 30.25, Cr 12.48, Mn 19.78; found: C 25.92, H 3.74, N 30.20, Cr 12.19, Mn 19.49; IR: $\tilde{\nu} = 2152 \text{ cm}^{-1}$ ($\text{C}\equiv\text{N}$).

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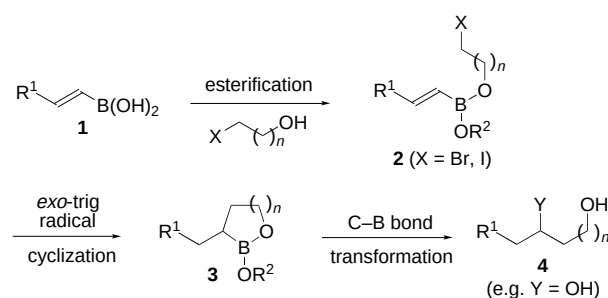
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- [12] Crystal data for $[\text{Mn}(\text{en})_3][\text{Cr}(\text{CN})_6]_2 \cdot 4\text{H}_2\text{O}$ at 298 K: light green prisms, $\text{C}_{18}\text{H}_{32}\text{N}_{18}\text{Cr}_2\text{Mn}_3\text{O}_4$, $M_r = 833.37$, crystal size $0.2 \times 0.2 \times 0.2$ mm, monoclinic, space group $C2/c$ (No. 15), $Z = 4$, $a = 24.505(7)$, $b = 11.323(4)$, $c = 14.810(4)$ Å, $\beta = 120.69(2)^\circ$, $V = 3533(1)$ Å³, $\rho_{\text{calc}} = 1.566$ g cm⁻³, $\mu(\text{MoK}\alpha) = 16.90$ cm⁻¹. Data collection: Rigaku AFC-5S diffractometer with graphite-monochromated $\text{MoK}\alpha$ radiation ($\lambda = 0.71069$ Å). The data were collected using the ω -2 θ scan technique to a maximum 2 θ value of 55.0° at a scan speed of 8.0° min⁻¹ (in omega). The cell parameters were determined by 25 reflections in the 2 θ range of 29.07° ≤ 2 θ ≤ 29.94°. Over the course of the data collection, the standard intensities decreased by 5.5%. Intensity data were corrected for Lorentz polarization, which effected the Ψ scan absorption. The structure was solved by direct methods and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The refinement converges with $R = \Sigma ||F_o| - |F_c|| / \Sigma |F_o| = 0.033$ and $R_w = [\Sigma w(|F_o| - |F_c|)^2 / \Sigma w |F_o|^2]^{1/2} = 0.042$ ($w = 1/\sigma^2(F_o)$) for 3032 reflections ($I > 3.00\sigma(I)$). Further details on the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository number CSD-410501.
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The First Boron-Tethered Radical Cyclizations and Intramolecular Homolytic Substitutions at Boron**

Robert A. Batey* and David V. Smil

Silicon-tethered radical cyclizations, first reported by Nishiyama et al.^[1] and Stork et al.,^[2] are a useful strategy for the construction of C–C bonds. Numerous syntheses have

incorporated these processes, usually for hydroxymethylations of allylic alcohols,^[1–3] and these reactions account for approximately half of the publications in the field of silicon-tethered chemistry. The success of temporary silicon connections in radical chemistry, together with our ongoing interest in developing new reactions of organoboron compounds,^[4] led us to consider employing boron in an analogous tethering role. Narasaka et al. used phenylboronic acid to tether dienes and dienophiles together through O–B–O linkages in Diels–Alder reactions,^[5] but there are no examples of this strategy in free-radical chemistry. Carboni et al. demonstrated both intermolecular free-radical additions to alkenylboranes and radical cyclizations with alkenylboranes.^[6] We now report the first boron-tethered radical cyclizations for the synthesis of 1,3-, 1,4-, and 1,5-diols. The general strategy employs the covalent C–B–O linkage of boronic esters as a tether (Scheme 1).^[7] We envisaged that the ease of synthesis



Scheme 1. General strategy for boron-tethered radical cyclizations of alkenylboronic esters **2**.

of the precursor boronic acids **1** and esters **2** and the synthetic versatility of the C–B bond^[8] in the cyclic products **3** would significantly expand the scope of tethered-radical processes.

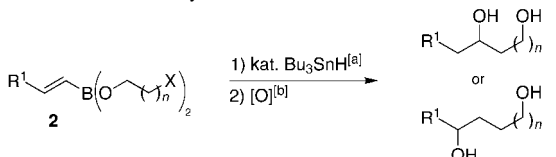
The requisite (*E*)-alkenylboronic acids **1** were obtained in good yields by hydroboration of the corresponding alkynes with the dimethyl sulfide complex of dibromoborane^[9] ($R^1 = \text{alkyl}$) or catecholborane^[10] ($R^1 = \text{aryl}$). Treatment of **1** with 2-bromoethanol, 2-iodoethanol, or 3-bromopropanol in THF in the presence of 4 Å molecular sieves at room temperature over 24 h readily afforded the (*E*)-alkenylboronic esters **2**. The precursors **2** were then subjected to free-radical conditions by using Corey's catalytic tributylstannane method.^[11] Heating **2a–f** (substrates with *n*-alkyl or aryl substituents at the β -alkenyl position) to 55 °C in THF for 48 h, in the presence of the radical initiator dimethyl-2,2'-azobisisobutyrate (DAB),^[12] gave the boracycles **3**, which were not isolated, but immediately oxidized with trimethylamine *N*-oxide (TMANO)^[13] to the 1,3- or 1,4-diols **4a–f** (Table 1), that is, the products of 5- or 6-*exo*-trig radical cyclization, respectively. No products resulting from 6-*endo*-trig or 7-*endo*-trig cyclization were isolated or observed in the crude reaction mixture, and this indicates an *exo:endo* selectivity of greater than 95:5.^[14] Initial attempts to perform these cyclizations in refluxing THF gave lower yields (10–40% below those with optimized conditions) due to competing direct reduction of the C–Br bond of **2**. Ionic reduction of **2** by the sodium cyanoborohydride co-reductant in the absence of radical initiator was demonstrated to be increasingly competitive

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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.

Table 1. Intramolecular cyclization of boronic esters **2** to diols **4** or **5**.

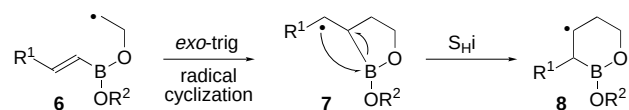


Substrate	R ¹	X	n	Product	Yield [%] ^[c]
2a	Pr	Br	1	4a	81
2b	Bu	Br	1	4b	77
2c	Ph	Br	1	4c	63
2d	<i>p</i> -tolyl	Br	1	4d	65
2e	Pr	Br	2	4e	85
2f	Ph	Br	2	4f	75
2g	Pr	I	1	4a	73
2h	Ph	I	1	4c	68
2i	<i>i</i> Pr	Br	1	5a	83
2j	<i>t</i> Bu	Br	1	5b	67
2k	Chx	Br	1	5c	77

[a] Substrate **2** in THF (0.1 M), Bu₃SnH (0.01 equiv), NaBH₃CN (2.5 equiv), DAB (0.4 equiv), 55 °C, 48 h. [b] Cyclization product in benzene (0.05 M), TMANO (5.0 equiv), 80 °C, 24 h; H₂O, 80 °C, 24 h. [c] Yields of isolated products after column chromatography.

with increasing temperature. An optimal reaction temperature of 55 °C, the operational threshold of the radical initiator, effectively decreases the formation of direct reduction products. Cyclizations can also be conducted with stoichiometric amounts of tributylstannane at high dilution to minimize direct reduction, but the catalytic tributylstannane method, aside from providing higher yields, is more convenient due to the greater ease of product purification. The *Z* analogue of **2b**^[15] also cyclized readily and gave diol **4b** in 72 % yield. Comparable results were obtained with the iodides **2g** and **2h** as the radical precursors. A preliminary attempt to use less than two equivalents of bromoethanol per boronic acid was also made. However, treatment of the diisopropyl esters^[16] of alkenylboronic acids with one equivalent of bromoethanol followed by free-radical cyclization and oxidation resulted in 20–40 % lower yields of the 1,3-diols. The tethered nature of the radical process was unambiguously established by attempting to cyclize 2-hex-1-enyl-[1,3,2]dioxaborolane in the presence of 2-bromoethanol (1:20 and 10:1 ratio) and (*E*)-hexenylboronic acid in the presence of 1-bromo-2-(*tert*-butyldimethylsiloxy)ethane. In both cases, tethering of the reactive species as boronate esters is precluded, and cyclization products were not observed.

Surprisingly, for the substrates **2i–k** with *sec*- or *tert*-alkyl substituents at the β-alkenyl position, 1,4-diols **5** rather than the expected 1,3-diols **4** were isolated (Table 1)!^[17] These results suggest that following 5-*exo*-trig cyclization of **6**, the boracyclic radical intermediate **7** rearranges to radical **8** before trapping an H atom from tributylstannane (Scheme 2). The high selectivity for formation of **4** or **5**, depending upon the substituents, is particularly noteworthy. Although the

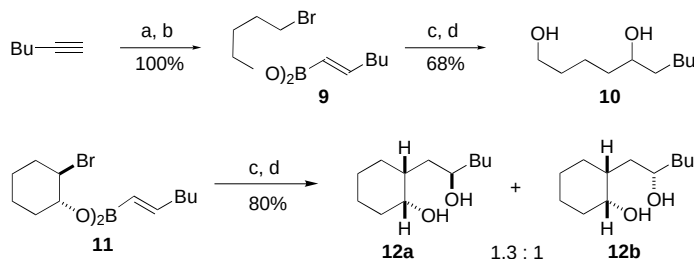


Scheme 2. Tandem radical cyclization and S_{Hi} mechanism leading to 1,4-diols **5** for R¹ = *i*Pr, *t*Bu, and Chx (cyclohexyl).

origin of this effect is not clear, presumably for **2i–k**, the extra steric bulk of R¹ lowers the rate of H atom trapping by the corresponding radicals **7** and facilitates an intramolecular homolytic substitution (S_{Hi})^[18, 19] at boron.^[20, 21] Treatment of **2k** using the standard stoichiometric tributylstannane method (benzene, 55 °C) at higher stannane concentrations gave mixtures of the 1,4-diol **5c** and cyclohexyl-1,3-butanediol (**4g**). The product ratio **5c**:**4g** decreased steadily with increasing concentration of stannane: 7.9:1 (0.1 M), 4.8:1 (0.25 M), 3.3:1 (0.5 M) to 2.6:1 (0.75 M), as the efficiency of H atom trapping by the stannane relative to S_{Hi} rearrangement increases. Reaction of **2j** in the presence of a stoichiometric amount of tributyltin deuteride (0.1 M) gave the 1,4-diol **5b** with a D atom in the 3-position; this confirms that the rearrangement is a radical reaction.

Intermolecular S_{Hi} reactions of carbon-centered radicals at boron centers have been observed in the gas phase,^[22] but to our knowledge this is the first intramolecular S_{Hi} reaction at boron and the first to involve a boronic ester. An alternative mechanism for this transformation involves β scission to give an (RO)₂B[•] radical. We consider that such an unprecedented β scission is unlikely, because the (RO)₂B[•] radical formally has five electrons in the valence shell of boron. Indeed, the energy barriers for the degenerate S_{Hi} rearrangement and the β scission reaction of the (HO)₂BCH₂CH₂[•] radical were calculated as 11.7 and 47.6 kcal mol^{−1}, respectively (MP2/6-31G*//UHF/6-31G*^[23]).

Ring opening of THF with an alkenyldibromoborane provides ready access to the 4-bromobutylboronates **9** without the need for boronic acid intermediates (Scheme 3).^[24] Treatment of **9** under the standard conditions efficiently gave the



Scheme 3. Syntheses of **10** and **12**. a) HBBBr₂·SMe₂, CH₂Cl₂, room temperature; b) THF, room temperature; c) Bu₃SnH (cat.), NaCNBH₃, DAB (cat.), THF, 55 °C; d) TMANO, 80 °C; H₂O, 80 °C.

1,5-diol **10**, which is formed by a rare example of a 7-*exo*-trig radical cyclization.^[25] A preliminary attempt to elucidate the diastereoselectivity of a boron-tethered cyclization was conducted with the boronate **11**, prepared in an analogous manner to **9** by ring opening of cyclohexene oxide (2 equiv) at 0 °C with (*E*)-hexenyldibromoborane. However, treatment of **11** according to the standard protocol led to an inseparable 1.3:1 mixture of the diastereomeric 1,4-diols **12**,^[26] which result from a tandem cyclization and S_{Hi} rearrangement mechanism (Scheme 3).^[27]

We have demonstrated the first boron-tethered free-radical reactions and shown that they are a useful alternative to the widely used silicon-tethered radical processes. In some cases rearranged products are observed that result from a subse-

quent intramolecular $S_{\text{H}}\text{i}$ reaction at boron. Further studies and applications of both boron-tethered free-radical cyclizations and $S_{\text{H}}\text{i}$ reactions at boron are in progress.

Experimental Section

Radical cyclization of **2a**: To a 0.1 M solution of **2a** (1.96 g, 6.00 mmol) in THF was added NaBH_3CN (942 mg, 15.0 mmol), DAB (552 mg, 2.40 mmol), and Bu_3SnH (16 μL , 0.06 mmol) at room temperature under nitrogen. The reaction mixture was stirred vigorously and heated to 55°C for 48 h, during which a fine precipitate formed. After cooling the mixture to room temperature, the solvent was removed in vacuo. The residue was then taken up in dichloromethane (20 mL), filtered, and the solvent removed in vacuo. The residual oil was then dissolved in benzene (120 mL), and TMANO (3.33 g, 30.0 mmol) was added. The light yellow solution was stirred vigorously and heated at reflux for 24 h. Water (20 mL) was then added, and the resulting two-phase system stirred for an additional 24 h at 85°C . After cooling the mixture to room temperature, the layers were separated, and the aqueous layer extracted with dichloromethane (5×15 mL). The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated in vacuo. Purification of the residual oil by column chromatography on silica gel (20% ethyl acetate/hexanes as eluent) afforded **4a**^[28] (642 mg, 4.86 mmol) as a clear, colorless oil (81% yield): ^1H NMR (400 MHz, CDCl_3): δ = 3.70–3.56 (m, 3H), 3.06 (brs, 2H), 1.72–1.57 (m, 3H), 1.50–1.28 (m, 5H), 0.90 (t, J = 7.1 Hz, 3H).

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The Trivalent Neodymium Complex [(C₅Me₅)₃Nd] Is a One-Electron Reductant!**

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Increased selectivity in synthetic chemistry is a continuing goal of chemical research for esthetic, economic, and environmental reasons. Selectivity in metal-based chemistry can often be increased by optimizing steric factors and varying the ligand size. However, the lanthanide metals offer an additional opportunity to optimize steric factors by varying the size of the metal atom. This is possible because the limited radial extension of the 4f valence orbitals^[1] makes these metals chemically similar and there are 15 choices^[2] which vary gradually in size by about 0.015 Å increments.^[3]

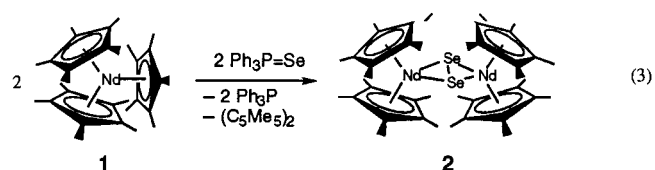
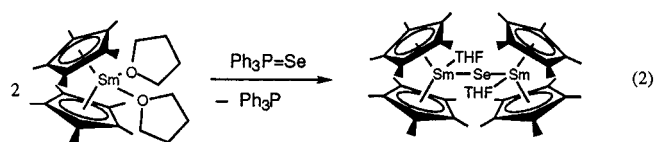
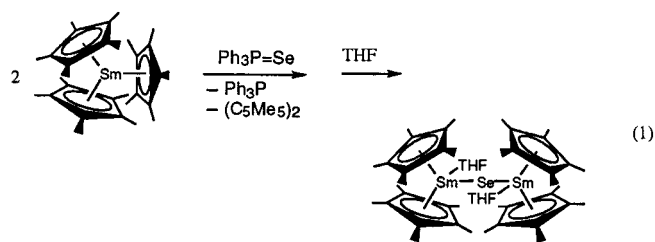
Although optimization of the size of the metal atom is possible in trivalent lanthanide chemistry, since molecular Ln^{III} compounds are known for all of the metals in the series,^[4] it has not been achievable with the reductive chemistry of the divalent lanthanides, since only a few of the lanthanides form molecular compounds containing divalent metals and the reductive behavior of these metals is quite different. Hence the extensive reductive chemistry of Sm^{II}^[5] can only be size-optimized by varying the ligand set and not by choosing another Ln^{II} center of better size. Eu^{II} and Yb^{II} are much weaker reductants and the recently discovered [TmI₂(dme)₃] (dme = 1,2-dimethoxyethane) is much more difficult to handle.^[6] As a result, the extensive one-electron reductive lanthanide chemistry found for Sm^{II} is limited to this element.

However, recently, we have shown^[7] that the sterically crowded trivalent samarium complex [(C₅Me₅)₃Sm]^[8] displays one-electron reductive chemistry which is analogous to the one-electron reductive chemistry of divalent [(C₅Me₅)₂Sm].^[9] For example, analogous reductions of Se=PPh₃ to PPh₃ and Se²⁻ by the trivalent^[7] and divalent^[10] compounds are shown in Equations (1) and (2), respectively. Since (C₅Me₅)₂ is a by-

product of the reduction with the Sm^{III} complex, it appears that one of the (C₅Me₅)⁻ rings in [(C₅Me₅)₃Sm] is the reducing agent. This is reasonable considering the extreme steric crowding in [(C₅Me₅)₃Sm]: the (C₅Me₅)⁻ rings are not close enough to the metal center to be stabilized very well and they can react by reductive pathways to form less sterically crowded products. In comparison, simple (C₅Me₅)⁻ salts such as KC₅Me₅ and C₅Me₅MgCl·THF do not display this reductive reactivity with Se=PPh₃.

The one-electron reduction by the sterically crowded Sm^{III} complex suggested that reduction chemistry could be done with any of the lanthanides if suitably crowded trivalent lanthanide complexes could be synthesized. This would mean that the reductive chemistry of Sm^{II} could be extended to other lanthanides which have different physical properties and potentially more favorable metal sizes. To prove this, it was necessary to demonstrate a reduction reaction with a metal which did not have a readily accessible divalent state, since in the samarium system in Equation (1) there would always be a possibility that the reaction could go through an undetected Sm^{II} intermediate. Fortunately, we recently discovered a high-yield synthetic route to the second crystallographically characterized [(C₅Me₅)₃Ln] complex, [(C₅Me₅)₃-Nd] (**1**),^[11] which allows us to test this concept. It should be noted that Nd^{II} has been reported in solid-state halide systems^[12] and is presumed to form upon potassium reduction,^[13] but no [(C₅Me₅)₂Nd] complex is formed as readily as [(C₅Me₅)₂Sm].

The reaction analogous to that in Equation 1 was examined with neodymium and it was found that [(C₅Me₅)₃Nd] does indeed function as a one-electron reductant: Se=PPh₃ is reduced to Ph₃P and [(C₅Me₅)₂Nd]₂(μ-η²:η²-Se₂). The (C₅Me₅)₂ by-product is consistent with reduction by (C₅Me₅)⁻. Interestingly, the product of this 2:1 (C₅Me₅)₃Nd:Se=PPh₃ reduction is a Se₂²⁻ rather than a Se²⁻ complex as found in Equation 1 and residual [(C₅Me₅)₃Nd] remains at the end of the 2:1 reaction. Accordingly a 1:1 reaction was examined and found to give **2** cleanly in high yield [Eq. (3)].



Complex **2** was characterized by elemental and chemical analysis, NMR and IR spectroscopy, and X-ray crystallography (Figure 1).^[14] The Se-Se distance (2.389(2) Å) is comparable to the range of Se-Se single bond lengths (2.256(6)–2.383(7) Å),^[15] and all the other distances are normal for Nd^{III} metallocenes.^[16]

After complex **2** was isolated, the analogous 1:1 [(C₅Me₅)₃-Ln]:Se=PPh₃ reaction was attempted with samarium and the analogous isostructural and isomorphous complex **3**^[17] was obtained. Hence [(C₅Me₅)₃Sm] can convert Se=PPh₃ to both Se₂²⁻ and Se²⁻ depending upon the stoichiometry. To confirm this, the reaction of **3** with [(C₅Me₅)₃Sm] was examined and

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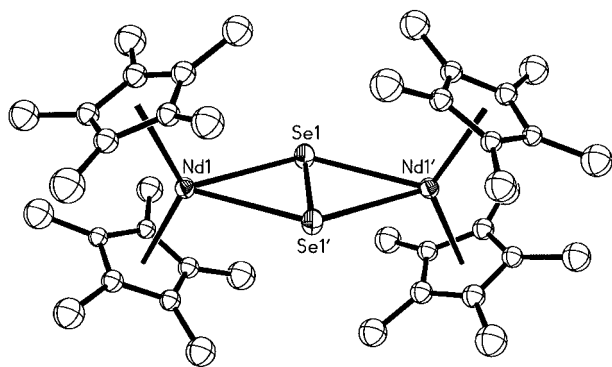
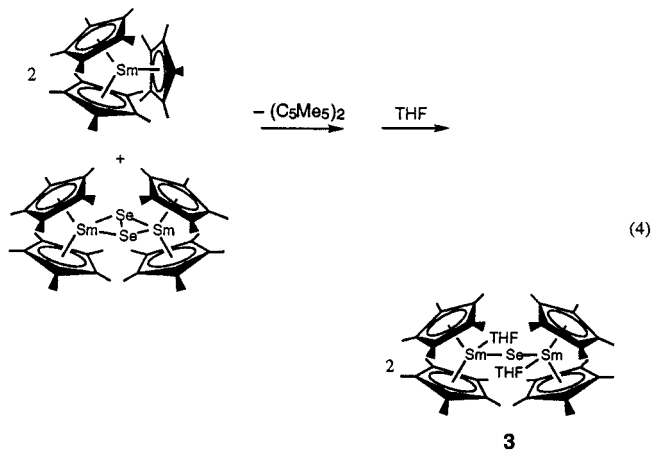


Figure 1. Molecular structure of $[(C_5Me_5)_2Nd]_2(\mu-\eta^2:\eta^2-Se_2)$ (**2**) with the thermal ellipsoid plot drawn at the 50% probability level.

found to form the Se^{2-} product, $[(C_5Me_5)_2Sm(thf)_2(Se)]$, as expected [Eq. (4)]. Since $[(C_5Me_5)_3Sm]$ appears to be a stronger reductant than $[(C_5Me_5)_3Nd]$, this suggests that the reductive reactivity of $[(C_5Me_5)_3Ln]$ complexes can be tunable on the basis of metal size.



These results show that one-electron reduction reactions can be done not only with sterically crowded Sm^{III} complexes, but also with sterically crowded complexes of metals which do not have readily accessible divalent states. It is anticipated that one-electron reduction chemistry can be extended to all of the lanthanides as syntheses of appropriately sterically crowded complexes are developed. This should allow the reductive chemistry previously limited to samarium to be generalized to the rest of the lanthanide series and it should allow optimization of this lanthanide reduction chemistry on the basis of metal size. We also anticipate that investigation of the reductive chemistry of other $[(C_5Me_5)_3Ln]$ complexes will lead to new lanthanide metallocene complexes as demonstrated by the synthesis of **2**. Examination of the reductive chemistry of a range of sterically crowded molecules is clearly warranted to determine the importance of the $(C_5Me_5)^-$ ligand to this chemistry.

Experimental Section

2: In a THF-free glovebox, toluene (10 mL) was added to $[(C_5Me_5)_3Nd]$ (150 mg, 0.272 mmol) and $Ph_3P=Se$ (93 mg, 0.272 mmol) and the reaction mixture was stirred to form an olive green solution. Within 30 min, the solution turned cherry red and gradually a red precipitate appeared. After

the reaction was stirred at ambient temperature for 22 h, the solvent was removed under reduced pressure and the red precipitate was washed with toluene (2×5 mL). The ^1H NMR spectrum of the light green toluene wash contained resonances for Ph_3P and $(C_5Me_5)_2$. The red precipitate was crystallized from hot toluene to yield $[(C_5Me_5)_2Nd]_2(\mu-\eta^2:\eta^2-Se_2)$ (**2**) (91 mg, 68%, m.p. $>260^\circ C$) as dark burgundy crystals. The ^1H NMR spectrum of the crystals was taken at $57^\circ C$ to enhance solubility. ^1H NMR (400 Hz, $[D_6]$ benzene, $57^\circ C$): $\delta = 8.64$ (br. s, C_5Me_5); IR (KBr): $\tilde{\nu} = 2966$ s, 2907 s, 2849 s, 2719 m, 1437 m, 1372 m, 1084 w, 1055 w, 1020 cm^{-1} ; C,H analysis (%): Calcd for $C_{40}H_{60}Nd_2Se_2$: C 48.66, H 6.13; found: C 48.40, H 5.97.

3: $[(C_5Me_5)_3Sm]$ (154 mg, 0.277 mmol) was treated with $Ph_3P=Se$ (101 mg, 0.296 mmol) in toluene (10 mL) as described above. The mixture was initially dark brown and turned clear red after 2 h. After the reaction was stirred overnight, dark red solids were separated by centrifugation, washed with hexanes (5 mL), and dried under vacuum to yield $[(C_5Me_5)_2Sm]_2(\mu-\eta^2:\eta^2-Se_2)$ (**3**) (45 mg, 33%, m.p. $>260^\circ C$). The ^1H NMR spectrum of the crystals was taken at $100^\circ C$ to enhance solubility. ^1H NMR (400 MHz, $[D_8]$ toluene, $100^\circ C$): $\delta = 1.10$ (s, C_5Me_5); IR (KBr): $\tilde{\nu} = 2962$ s, 2906 s, 2851 s, 2720 w, 1453 m, 1372 m, 1093 w, 1025 cm^{-1} ; C,H analysis (%): Calcd for $C_{40}H_{60}Sm_2Se_2$: C 48.06, H 6.05; found: C 47.86, H 5.89. Crystals suitable for X-ray crystallographic analysis were obtained by recrystallization from boiling toluene.

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Regio- and Stereoselective Synthesis of γ -Alkylidenebutenolides by Cyclization of Dilithiated 1,3-Dicarbonyl Compounds with N,N' -Dimethoxy- N,N' -dimethylethane-diamide**

Peter Langer* and Martin Stoll

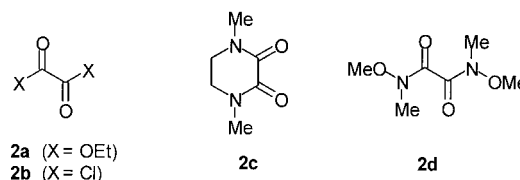
Dedicated to Professor Armin de Meijere on the occasion of his 60th birthday

Numerous natural products, including prominent compounds such as dihydroxerulin, tetrenolin, freelingyne, or pulvinic acid, belong to the pharmacologically important category of γ -alkylidenebutenolides.^[1] Dihydroxerulin, for instance, has proven to be an important nontoxic inhibitor in the biosynthesis of cholesterol,^[2] while tetrenolin exhibits antibiotic activity against gram-positive bacteria.^[3] α -Hydroxy- γ -alkylidenebutenolides are particularly suitable building blocks for natural product synthesis (by means of transition metal-catalyzed coupling and reduction reactions of the corresponding enol triflates). However, it was only recently that an efficient stereoselective route was introduced to obtain a specific member of this class, 5-(2-hydroxyethylidene)-2(5H)-furanone, by stereospecific elimination of L- and D-gulonol-1,4-lactone.^[4] Since a carbohydrate derivative is used as the substrate for this reaction, this method cannot be used to synthesize other

substituted butenolides. Previously reported β -eliminations for the synthesis of α -alkyl-substituted or unsubstituted γ -alkylidenebutenolides proceed with low^[5] stereoselectivity or no stereoselectivity at all.^[6] Wittig reactions of suitable phosphorilides with methoxymaleic anhydrides proceed with undesired regiochemistry and generally unsatisfactory stereoselectivity.^[7] Wittig reactions of alkyl-substituted maleic anhydrides^[8] or other methods^[9] lead only to *E/Z* mixtures of α -alkyl-substituted or unsubstituted γ -alkylidenebutenolides. To our knowledge, no method exists which provides a direct and stereoselective approach to γ -alkylidenebutenolides with a wide range of substitution patterns.

To fill this gap, we investigated the concept of direct cyclization of 1,3-dicarbonyl compounds with the oxalic acid dielectrophiles **2**. Despite the simplicity of this idea, the cyclization of 1,3-dicarbonyl compounds containing a terminal hydrogen atom (e.g. acetylacetone) with oxalyl chloride in the presence of Lewis acids was not successfully carried out until 1990.^[10] The cyclization in this case proceeds through the *central* carbon atom and an oxygen atom of the 1,3-diketone under formation of 4-acyl-5-alkyl-2,3-dioxo-2,3-dihydrofurans. To the best of our knowledge, we present herein the first cyclizations of 1,3-dicarbonyl compounds with oxalic acid dielectrophiles which proceed by attack of a *terminal* carbon atom of the nucleophile. This method allows for a simple regio- and stereoselective route to a series of γ -alkylidenebutenolides. To achieve the desired regioselectivity, the 1,3-dicarbonyl compounds are used in the corresponding ambident dianionic form.^[11, 12]

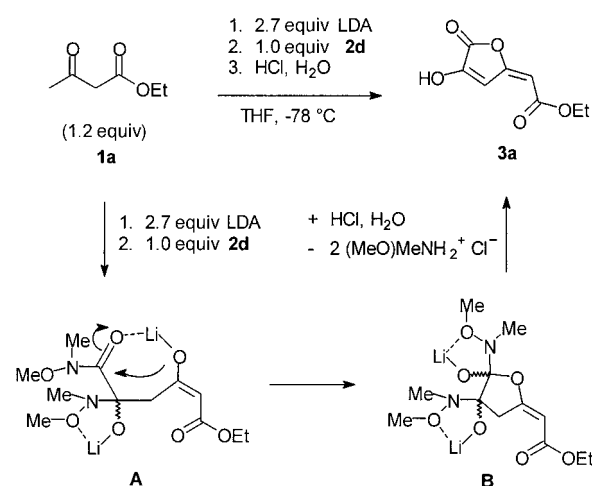
Initial experiments showed that reactions of the dianion of ethyl acetoacetate (**1a**) with diethyl oxalate (**2a**) or oxalyl chloride (**2b**) led to the formation of complex, inseparable mixtures (owing to overaddition, polymerization, or decomposition; Table 1). The reaction of the dianion of **1a** with 1,4-



dimethylpiperazine-2,3-dione (**2c**)^[13] also remained unsuccessful, although **2c** had previously been reported to undergo condensation reactions with two equivalents of monofunctional organolithium compounds. Fortunately, the problem could be solved with the use of the Weinreb amide^[14] N,N' -dimethoxy- N,N' -dimethylethane-diamide (**2d**), which was reported in 1995 and which until now has been used only in condensation reactions with simple monolithium compounds such as phenyllithium.^[14a] Exposure of this Weinreb oxalic amide to the dianion of **1a** induced a cyclization reaction, and the γ -alkylidenebutenolide **3a** was obtained in 75 % yield (Scheme 1). The product was formed both regioselectively (by cyclization of the terminal carbon and the neighboring oxygen atom of the dianion) and with complete stereoselectivity. The *E* configuration of the semicyclic double bond was unequivocally proven with NOE NMR studies of the γ -alkylidenebutenolide **3b**, which was obtained in 73 % yield from the

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Scheme 1. A possible mechanism for the reaction of the dianion of **1a** with **2d**. LDA = lithium diisopropylamide.

Table 1. Optimization of the reaction of the dianion of **1a** with the oxalic acid dielectrophiles **2a–d**.

Entry	2	<i>T</i> [°C]	<i>t</i> [h]	Equiv (1a)	Yield (3a) [%]
1	a	−78→−20	6	1.0	0
2	b	−78→−20	6	1.0	0
3	c	−78→−20	6	1.0	0
4	d	−78→−20	6	1.0	69
5	d	−78→−20	6	2.0	52
6	d	−78→−20	6	1.2	75
7	d	−78→−40	5	1.2	64
8	d	0→−20	4	1.2	0

reaction of **2d** with the dianion of *tert*-butyl acetoacetate (**1b**, Table 2). Optimized yields were obtained by use of 1.2 equivalents of the dianion, addition of the Weinreb oxalamide **2d** to the solution of the dianion at −78 °C, and warming of the reaction solution to room temperature within a period of six hours, followed by subsequent treatment with hydrochloric acid (Table 1). When two equivalents of the dianion were used, **3a** was still obtained in 52 % yield.

According to Harris et al., the reaction of the bis(*N*-methoxy-*N*-methylamide) of a glutaric acid derivative with the dianion of **1b** led to the formation of an open-chain product.^[15a] No conversion was observed, however, in the reaction of the simple *N*-methoxy-*N*-methylacetamide with the monoanion of acetophenone.^[15b] In the case of the cyclization of the bis-Weinreb amide presented here, which we believe is the first reported cyclization of this type, the intramolecular formation of the five-membered ring must be preferred over the formation of any open-chain 1:2 product. The product is probably formed by a regioselective attack of the terminal carbon atom of the dianion onto the substrate and a subsequent cyclization step that also proceeds regioselectively at the neighboring oxygen atom. Our working hypothesis for explaining the regioselectivity of the ring closure is based on the complexation of the lithium atom by both the amide and the enolate oxygen atoms as illustrated in intermediate **A** (Scheme 1). This chelation step brings the amide and the enolate functionalities close together, thus favoring a regioselective ring closure under formation of

intermediate **B**.^[16] Both five-membered chelate complexes of intermediate **B** are subsequently cleaved with hydrochloric acid to form the carbonyl group. An attempt to prepare *E*-configured γ -alkylidenebutenolides by stereospecific elimination at insufficiently low temperatures^[4c] led to a mixture of geometric isomers. The same happens upon treatment of *E*-configured γ -alkylidenebutenolides with chlorosulfonic acid.^[8] These observations strongly support the assumption that the stereoselectivity observed in this study to preferentially form γ -alkylidenebutenolides with *E* configuration is not thermodynamically but instead kinetically controlled.

The reaction of the Weinreb oxalamide **2d** with the dianions of acetylacetone (**1c**), *N,N*-diethyl acetoacetamide (**1d**), and benzoylacetone (**1e**) gave rise to the γ -alkylidenebutenolides **3c**, **3d**, and **3e**, respectively, in generally good yields and with very good stereoselectivities (Table 2).

Table 2. Synthesis of the γ -alkylidenebutenolides **3a–q**.^[a]

3	R ¹	R ²	R ³	Yield [%]
a	H	H	OCH ₂ CH ₃	75
b	H	H	OC(CH ₃) ₃	73
c	H	H	CH ₃	56
d	H	H	N(CH ₂ CH ₃) ₂	63
e	H	H	C ₆ H ₅	57
f	CH ₃	H	OCH ₃	70
g	CH ₂ CH ₃	H	OCH ₂ CH ₃	54
h	H	CH ₃	OCH ₂ CH ₃	71
i	H	CH ₂ CH ₃	OCH ₂ CH ₃	43
j	H	−CH ₂ CH ₂ O−		73
k	H	−CH ₂ CH(CH ₃)O−		60
l	H	−CH ₂ CH(CH ₂ CH ₃)O−		38
m	CH ₃	−CH ₂ CH ₂ O−		20
n	H	−CH ₂ CH ₂ CH ₂ −		75
o	H	−CH ₂ CH ₂ CH ₂ CH ₂ −		23
p	H	−CH ₂ CH ₂ (C ₆ H ₄)−		74
q	H	−(C ₆ H ₄)O−		52

[a] The *E*:*Z* ratio was >98:2 for **3a–e** and **3h–q**. In the cases of **3f** and **3g** the *E*:*Z* ratio was 1:40 and <2:98, respectively.

Although *E*/*Z* selectivities of greater than 98:2 were obtained in three experiments to synthesize the butenolide **3c**, there was one experiment in which an isomeric mixture (5:1) was isolated (probably because higher temperatures were used when adding **2d**). This result provides independent evidence for the *E* configuration of the semicyclic double bond: Analogous to the ¹H NMR data of similar compounds,^[4] the main component (*E*)-**3c** shows a larger chemical shift for the two CH signals than the corresponding signals of the minor component (*Z*)-**3c**. Starting from the R¹- or R²-substituted methyl and ethyl acetoacetates **1f–i** the corresponding substituted butenolides **3f–i** were prepared in good yields and with very high stereoselectivities. In the case of **3f** and **3g** the *Z*-configured butenolides were formed with efficient stereoselectivities. The *Z* configuration of **3g** was unequivocally proven by NOE NMR studies. The change of the

stereoselectivity from *E* to *Z* configuration can be explained by the steric influence of the alkyl substituents in the β -position of the butenolide.

The interesting γ -alkylidenebutenolides **3j–o** could be synthesized easily by using the cyclic 1,3-dicarbonyl compounds **1j–o**: The conversion of the oxalamide **2d** with the dianions of the 2-acetyl- γ -butyrolactones **1j–m** yielded the butenolides **3j–m** in generally good yields and with very high stereoselectivities in favor of the *E* isomers. Likewise, the butenolide **3n** was prepared in good yield starting from 2-acetylcyclopentanone (**1n**). Furthermore, the butenolide **3o** was prepared from 2-acetylcyclohexanone (**1o**). The low yield in this case can be explained by the necessary separation of the regioisomeric minor product, which was formed by the cyclization of the secondary α -carbon atom in the six-membered ring of 2-acetylcyclohexanone. The reaction of **2d** with the dianion of 2-acetyltetralone (**1p**) yielded the butenolide **3p** in good yield and with very high stereoselectivity. Reaction of **2d** with the dianion of 3-acetyl-2,3-dihydrobenzofuran-2-one (**1q**), which to our knowledge has not been reported to date, led to the γ -alkylidenebutenolide **3q**, an analogue of the natural product calycin,^[17] in good yield and with very high stereoselectivity.

Cyclization of the dianions of the dicarbonyl compounds **1a–q** with the Weinreb amide **2d** allowed the regio- and stereoselective preparation of a series of substituted α -hydroxy- γ -alkylidenebutenolides. The yields obtained for the butenolides **3a–q** are quite satisfactory considering that a series of side reactions (e.g. decarbonylation^[14a]) are possible due to the unstable nature of the oxalic acid structure of **2d**.^[18] The reaction presented herein constitutes a significant expansion of the methods known today for the synthesis of γ -alkylidenebutenolides, which are of pharmacological relevance and of importance for natural product synthesis. Moreover, this reaction is convenient to carry out.

Experimental Section

Typical procedure: **3a**: A solution of lithium diisopropylamide (LDA) in THF was prepared by dropwise addition of *n*BuLi (1.44 mL, 3.4 mmol, 2.35 M solution in *n*-hexane) to a solution of diisopropylamine (0.44 mL, 3.4 mmol) in THF (20 mL) at 0 °C. After 15 min of stirring at 0 °C, **1a** (0.19 mL, 1.47 mmol) was added, and the solution was stirred for 45 min at 0 °C. *N,N,N',N'*-Tetramethylethylenediamine (TMEDA; 0.5 mL, 3.4 mmol) was then added at –78 °C followed by a solution of **2d** (220 mg, 1.25 mmol) in THF (4 mL). The temperature of the reaction solution was increased to 0 °C over a period of 5.5 h. The cooling bath was removed, and the reaction mixture was stirred for 30 min at 20 °C. Then 4 mL of a 10% aqueous HCl solution were added and the solution stirred for 10 min, after which another 20 mL of a 10% aqueous HCl solution were added. The aqueous layer was extracted multiple times with THF/diethyl ether (1/3). The combined organic layers were dried (MgSO₄) and filtered, and the solvent was removed in vacuo. Purification of the residue by preparative chromatography (silica gel, diethyl ether/petroleum ether, 1/10 → 1/3) yielded 170 mg of a colorless solid. ¹H NMR ([D₆]acetone, 200 MHz): δ = 1.32 (t, *J* = 8 Hz, 3H, CH₃), 4.22 (q, *J* = 8 Hz, 2H, CH₂), 5.71 (s, 1H, CHCO₂Et), 7.20 (s, 1H, ring CH); ¹³C NMR ([D₆]acetone, 50 MHz): δ = 14.43 (CH₃), 60.94 (CH₂), 98.68 (CHCO₂Et), 108.76 (ring CH), 150.34, 160.74, 164.22, 166.10 (C); MS (EI, 70 eV): 184 (*M*⁺, 17), 156 (20), 139 (69), 69 (100). All new compounds were characterized by spectroscopic methods and high-resolution mass spectra and/or elemental analyses.

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The Parent Hexacarbaborane *arachno*-C₆B₆H₁₂ and a Methylated Pentacarbaborane *arachno*-CH₃C₅B₇H₁₂: Domains of Incipient Hydrocarbon Behavior within Borane Clusters**

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Hydrocarbons have as a structural basis domains of the diamond or graphite lattice, or combinations thereof, with peripheral bonds to hydrogen. The structural basis of boron hydrides are hollow triangulated polyhedral clusters, the surfaces of which consist of boron-linked BH units. Mixed hydrides will have intermediate structures, and there is considerable interest in which type of structure will emerge or be suppressed as the carbon-to-boron ratio varies. Will there be a sudden jump from borane cluster to hydrocarbon skeleton behavior with increasing carbon content, or will separate domains of hydrocarbon and borane behavior gradually emerge in the same compound? Such crossover behavior might be expected when the carbon-to-boron ratio is approximately unity.

Recently, the highly substituted carborane C₆B₆H₆Et₆ (**1**) was reported to have the twelve-vertex polyhedral drum structure **I**.^[1] This is formally an *arachno* structure derived by the removal of two polar six-connectivity vertices from the closed fourteen-vertex 1:6:6:1 D_{6d} stack **II**. By contrast, we now report that the unsubstituted parent species C₆B₆H₁₂ (**2**) has a quite different μ -6,9-(CH=CH)-*arachno*-5,6,8,10-C₄B₆H₁₀ structure (Figure 1), in which an *arachno* ten-vertex C₄B₆ polyhedral cluster domain is bridged by an ethylene group with hydrocarbon character (structure **III**). This is also formally a twelve-vertex *arachno* polyhedral structure, and it can be derived from an alternative closed fourteen-vertex structure, namely, the 2:2:2:4:2:2 C_{2v} stack **IV**, by the removal of two six-connectivity vertices.

Compound **2** was obtained, along with the previously reported tetracarbaboranes^[2] and other carboranes, as a product of the reaction of acetylene with 4,5-C₂B₇H₉ in Et₂O in the presence of 0.1 equivalents of 1,8-di(methylamino)-naphthalene under forcing conditions (repeated supply of

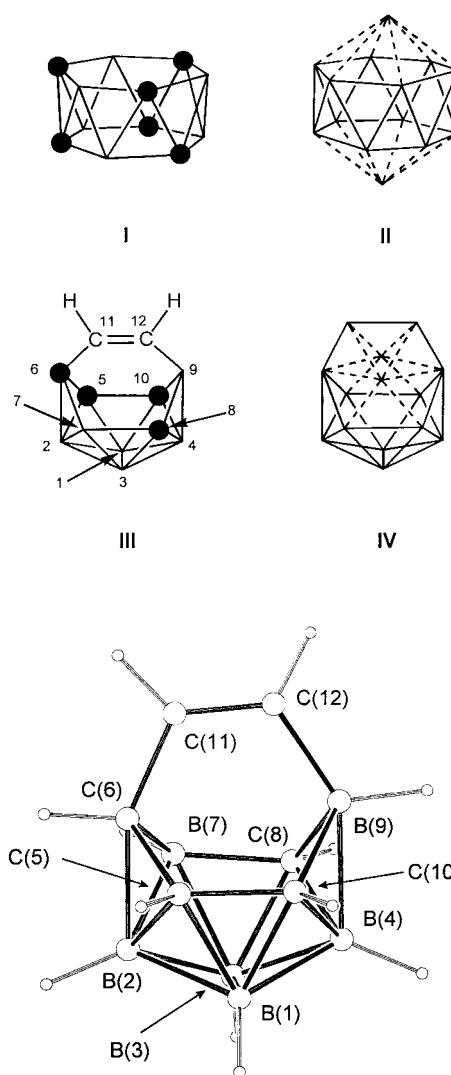


Figure 1. MP2/6-31G* energy-minimized molecular structure of **2**. Selected interatomic distances [Å]: C(5)–C(6) 1.551, C(6)–B(7) 1.773, B(7)–C(8) 1.576, C(8)–B(9) 1.760, B(9)–C(10) 1.739, C(10)–C(5) 1.497, C(6)–C(11) 1.507, B(9)–C(12) 1.575, C(11)–C(12) 1.337.

acetylene, 120 °C, 48 h). This contrasts to the room-temperature reaction with methyl propionate which led to ten-vertex tricarboranes.^[3] The constitution of **2** was established by comparison of its NMR spectroscopic data with ab initio/IGLO calculations at the II/IGLO/MP2/6-31G* level.^[4, 5] Other structures, including asymmetrical drum polyhedra analogous to **I**, did not match. The compound is an example of an unsubstituted, parent *arachno*-C₆B₆H₁₂ carborane. Other isomers may be feasible. The contrast between structures **I** and **III** illustrates that the structural crossover is critically dependent on the cluster substituents and the boron-to-carbon ratio of the skeleton. It will also depend on the relative positioning of the boron and carbon atoms.

A second new twelve-vertex product isolated from the reaction, namely, the first cluster pentacarbaborane CH₃C₅B₇H₁₂ (**3**), is in accord with these perceptions. The structure of **3** (Figure 2) was also established by comparison of its NMR data with ab initio/IGLO calculations at the II/IGLO/MP2/6-31G* level.^[4, 5] Compound **3** has a lower

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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.

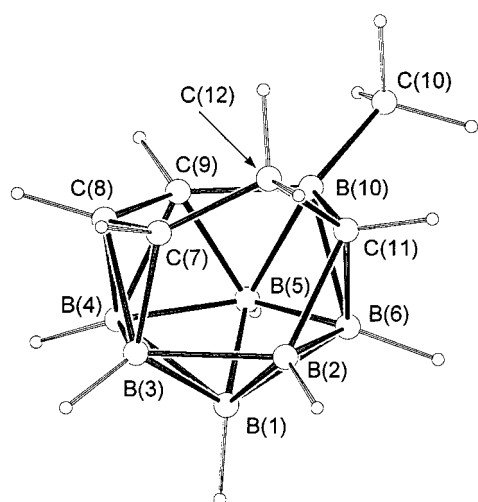
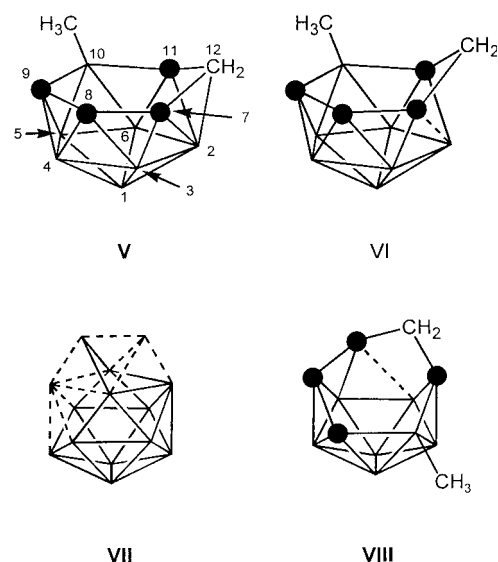


Figure 2. MP2/6-31G* energy-minimized molecular structure of **3**. Selected interatomic distances [Å]: C(7)–C(8) 1.484, C(8)–C(9) 1.531, C(9)–B(10) 1.630, B(10)–C(11) 1.650, C(11)–C(12) 1.532, C(12)–C(7) 1.505, B(2)–C(12) 2.304, B(2)–C(7) 2.222.

skeletal carbon-to-boron ratio than the hexacarbaborane **2** and one substituent bound to boron, and the cluster concomitantly has more polyhedral character. It resembles the twelve-vertex structure **V**, but is perhaps better regarded as 10-CH₃-μ-7,11-(CH₂)-*arachno*-7,8,9,11-C₄B₆H₁₀, an eleven-vertex *arachno*-C₄B₇ cluster with a CH₂ bridge as in **VI**; that is, the hydrocarbon domain consists of the one-carbon CH₂ unit rather than the two-carbon C₂H₂ unit of **2**. In principle the skeleton of **3** is also of twelve-vertex *arachno* geometry and is derived from the 2:2:2:4:2:2 C_{2v} stack by the removal of two nonadjacent atoms, as in **VII**. However, there is also some additional connectivity lengthening (hatched lines in **VI** and **VIII**), and the B(2)–C(7) separation is long (2.22 Å), so that B(2)–B(3)–C(7)–C(12)–C(11) is effectively an incipient five-membered open face (Figure 2).

The alternative view **VIII** and the C₆B₆H₁₂ structure **III** represent successive stages in the emergence of the dicarbon hydrocarbon domain as the skeletal carbon-to-boron ratio increases. Conversely, the progression from **III** to **VIII** represents successive stages in the incorporation of an acetylenic unit into a borane framework and may have mechanistic implications. As suggested in Scheme 1, the products may result from C₂ insertion into the ten- and eleven-vertex carboranes **4** and **5**, which are present in the reaction mixture. The formation of **3** would involve extrusion of the original CH₂ vertex into an exoskeletal position.

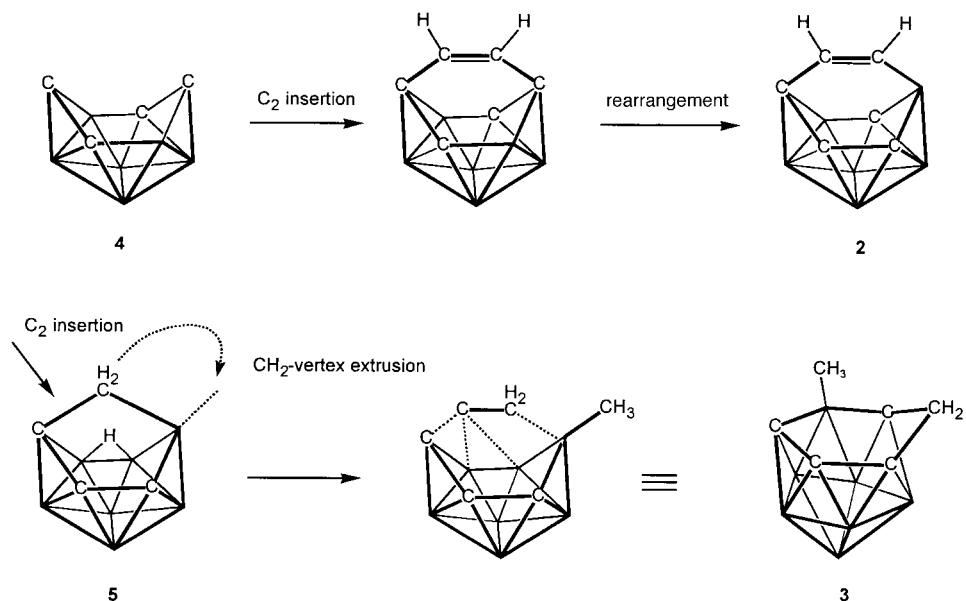
Compounds **2** and **3** are the first unsubstituted hexacarbaborane and the first twelve-



vertex cluster pentacarbaborane, respectively, and they illustrate new aspects of the crossover between open hydrocarbon skeletons and closed borane-type clusters. Attempts to isolate the other high-carbon carboranes that are present in trace quantities as reaction products are underway.

Experimental Section

arachno-4,5-C₂B₇H₁₃ (5 mmol) was treated with 1,8-di(methylamino)naphthalene (0.5 mmol) and an excess of acetylene in 30 mL of Et₂O (stainless steel vessel, 120 °C, repeated supply of acetylene, 48 h), followed by filtration, evaporation of the solvent, and extraction of the residue with a mixture of 5% aqueous HCl (20 mL) and hexane (30 mL). The hexane layer was evaporated, and the residue subjected to repeated preparative HPLC separation in hexane. The separation of this complex carborane mixture resulted in the isolation of a large number of fractions. The following carboranes have so far been isolated in pure form: *nido*-C₄B₆H₁₀ (1%),^[2] *nido*-C₄B₇H₁₁ (2%),^[2] **2** (3%), *arachno*-C₄B₇H₁₃ (10%),^[2] and **3** (4%) (in order of increasing *k'* values); they were identified by NMR spectroscopy. The new carboranes **2** and **3** were isolated as white waxy solids that are stable under nitrogen.



Scheme 1. Proposed mechanisms for the formation of **2** and **3**.

NMR data for **2**: experimental (CDCl₃, 294–297 K): $\delta(^{11}\text{B})$ ($\delta(^1\text{H})$; $^1J_{\text{BH}}$): BH(1) – 30.5 (+1.20; 164 Hz), BH(2) + 0.4 (+3.17; 171 Hz), BH(3) – 29.6 (+1.74; 165 Hz), BH(4) + 4.5 (+2.75; 156 Hz), BH(7) + 3.8 (+3.53; 170 Hz), BH(9) – 22.8 (+2.28; 121 Hz); CH signals at $\delta(^1\text{H})$ = +7.12, +6.82, +2.92 (2H, accidental coincidence), +2.75, +2.49, and +2.13; calculated (II/IGLO//MP2/6-31G*):^[4,5] $\delta(^{11}\text{B})$ ($\Delta\delta$): B(1) – 29.8 (–0.7), B(2) + 2.4 (+2.0), B(3) – 31.6 (+2.0), B(4) + 8.5 (–4.0), B(7) + 5.7 (–1.9), and B(9) – 24.1 (+1.3); IR (KBr): $\tilde{\nu}$ = 3059, 2987 cm^{–1} (CH=CH ?); MS (70 eV): m/z (%): 150 (25) [M^+], 148 (100) [$M^+ - \text{H}_2$].

NMR data for **3**: experimental (CDCl₃, 294–297 K): $\delta(^{11}\text{B})$ ($\delta(^1\text{H})$; $^1J_{\text{BH}}$): BH(1) – 39.7 (+0.52; 148 Hz), BH(2) + 32.4 (+5.04; 170 Hz), BH(3) – 31.1 (+0.61; 160 Hz), BH(4) + 0.4 (+3.53; 170 Hz), BH(5) – 5.5 (+2.70; J not measurable due to overlap), BH(6) – 7.9 (+1.81; 157 Hz), BCH₃(10) – 5.2 (CH₃ at +0.28); CH signals at $\delta(^1\text{H})$ = +3.05, +2.90, +2.67, +1.87, +1.39 and +1.25; calculated (II/IGLO//MP2/6-31G*):^[4,5] $\delta(^{11}\text{B})$ ($\Delta\delta$): B(1) – 42.1 (–2.4), B(2) + 33.9 (+1.5), B(3) – 31.5 (–0.4), B(4) – 2.5 (+2.1), B(5) – 5.3 (–0.2), B(6) – 4.4 (–3.5), B(10) – 7.8 (–2.6); MS (70 eV): m/z (%): 164 (38) [M^+], 162 (100) [$M^+ - \text{H}_2$].

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DNA-Based Assembly of Gold Nanocrystals**

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The ability to generate assemblies of metallic, magnetic, or semiconducting nanocrystals, in which the relative spatial arrangement of two or more distinct nanocrystals is controlled, would allow for a systematic investigation of the physical properties of these novel structures.^[1] A number of reports have described methods for organizing nanocrystals into arrays using small molecules,^[2] nano-patterned templates,^[3] and crystallization.^[4] Recently, methods that use biopolymers to assemble nanocrystals have appeared in the literature.^[5] For example, Au nanocrystals derivatized with complementary single-stranded DNAs (ssDNAs) can be hybridized to each other to form periodic arrays.^[6] In a preliminary report, we showed that nanocrystals modified with ssDNA could be arranged into homodimeric and homotrimeric assemblies.^[7] We now report the synthesis of heterodimeric and heterotrimeric “nanocrystal molecules” in which Watson–Crick base-pairing interactions are used to control the relative spatial arrangement of Au nanocrystals that are 5 and 10 nm in diameter. A preliminary characterization of the optical properties of these assemblies is also reported.

DNA is an ideal template for the formation of nanocrystal molecules due to its ability to form well-defined secondary and tertiary structures and its similarity in size to nanocrystals. To use DNA in this capacity, nanocrystals must first be complexed with ligands that stabilize them in aqueous buffers. Secondly, ssDNA must be site-specifically modified with moieties that covalently bind to the complexed nanocrystals such that the resulting conjugates effectively hybridize to complementary ssDNA. In addition, methods for purifying the nanocrystal molecules from their synthetic precursors or undesired larger assemblies are likely to be needed.

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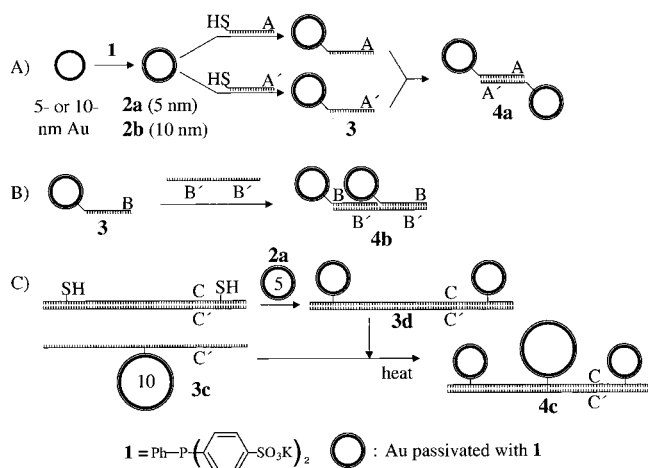
Gold nanocrystals were stabilized by complexation with dipotassium bis(*p*-sulfonatophenyl)phenylphosphane dihydrate (**1**).^[8] Gold nanocrystals with a diameter of 5 or 10 nm that were complexed with **1** ($\rightarrow$ **2a** and **2b**, respectively) did not aggregate in aqueous buffers and could be subjected to repeated cycles of NaCl precipitation. The sensitivity of **2a** and **2b** to salt concentration was size-dependent: the 5-nm nanocrystals could be manipulated in 0.3 M NaCl and 1 mM MgCl₂, but the 10-nm nanocrystals precipitated above 0.15 M NaCl and aggregated irreversibly in 1 mM MgCl₂. Both **2a** and **2b** were stable in solutions ranging in pH value from 3–9; they were also stable to heating (95 °C) for short times in aqueous buffers. Since **2a** and **2b** migrated through agarose gels and were stable to repeated cycles of separation and recovery, electrophoresis was used as the primary analytical and preparative method in these studies.

It was found that thiol groups, incorporated into ssDNA at either the 5' terminus or the C5 position of 2'-deoxyuridine nucleotides, penetrated the ligand "shells" of **2a** and **2b** and reacted directly with the surfaces of the Au nanocrystals.^[9] In a typical modification reaction, 0.9 equivalents of ssDNA were added to a 1–10 μ M solution of nanocrystal **2a** or **2b**.^[10] Although 5- and 10-nm Au nanocrystals derivatized with less than one equivalent of short ssDNA had similar mobilities to **2a** and **2b**, nanocrystals modified with longer ssDNA (>60 nucleotides) or double-stranded DNA (dsDNA) (>40 base pairs) migrated distinctly slower than **2a** and **2b** through 3% agarose gels.^[11]

Three strategies were examined for the generation of heterodimeric and heterotrimeric nanocrystal molecules (Scheme 1). The first involved the use of two complementary

approach was used to synthesize trimers with no nicks. Two complementary oligonucleotides were synthesized with either one or two thiols extending from the C-5 position of deoxyuridine nucleotides. These oligonucleotides position three nanocrystals in a collinear arrangement along one face of the double helix, with at least ten base pairs of dsDNA on each side of the modified nucleotides (Scheme 1C). For a typical assembly reaction involving either of the first two methods, conjugate **3** was directly treated with the complementary ssDNA, which was either attached to other nanocrystals (Scheme 1A) or added as a template free in solution (Scheme 1B). These mixtures were incubated from one to four days at room temperature. For the assembly synthesized according to Scheme 1C, the ssDNA with two thiols was hybridized to non-thiolated complementary ssDNA prior to the addition of Au nanocrystals to avoid the formation of a 1:1 chelate between the flexible, thiolated ssDNA and a single Au nanocrystal. Product **3d** was purified by electrophoresis and then incubated with **3c** to yield trimer **4c**. All of the desired products were purified from the crude reaction mixtures by preparative electrophoresis on 3–4% agarose gels. The Au nanocrystals were visible to the naked eye on agarose gels as red bands. The products were isolated by electrophoresis of the desired bands into glassfiber microfilters backed with dialysis membranes.

Dimer and trimer nanocrystal molecules generated by all three assembly strategies were characterized by transmission electron microscopy (TEM). A TEM image of a twice-purified 5-nm homodimer (Scheme 1B) shows that more than 90% of the Au nanocrystals present in the image are found in dimeric structures (Figure 1). To examine the level of control



Scheme 1. Synthesis strategies A–C for nanocrystal assembly (see the text for details). The labels A', B', and C' denote oligonucleotide sequences complementary to sequences A, B, and C, respectively.

5'-ssDNA/nanocrystal conjugates to form double-stranded nanocrystal molecules (Scheme 1A). Dimers and trimers synthesized by this method had zero and one single-stranded breaks (or "nicks"), respectively. In the second approach, the 5'-ssDNA/nanocrystal conjugates were assembled onto an unmodified template strand (Scheme 1B), resulting in dimers and trimers with one and two nicks, respectively. The third

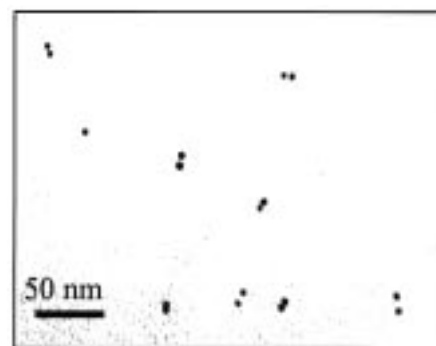


Figure 1. A typical TEM image of a 5-nm homodimer formed by the strategy in Scheme 1B; more than 90% of the Au in this image is found in dimeric structures.

over the spacing between nanocrystals in dimeric assemblies, TEM images of three sets of 10-nm homodimers (Scheme 1A) constructed with dsDNA sequences ranging from 18–38 base pairs were evaluated statistically. One hundred examples of each dimer were measured; the distances between the nanocrystal edges averaged 1.2 nm (with distances of 0–6 nm observed), 1.5 nm (with distances of 0–6 nm observed), and 5.8 nm (with distances of 0–15 nm observed) for the sequences with 18, 28, and 38 base pairs, respectively. The lengths of the dsDNAs between the nanocrystals for the sequences with 18, 28, and 38 base pairs were calculated to be 6.1, 9.5, and 12.9 nm, respectively. The high

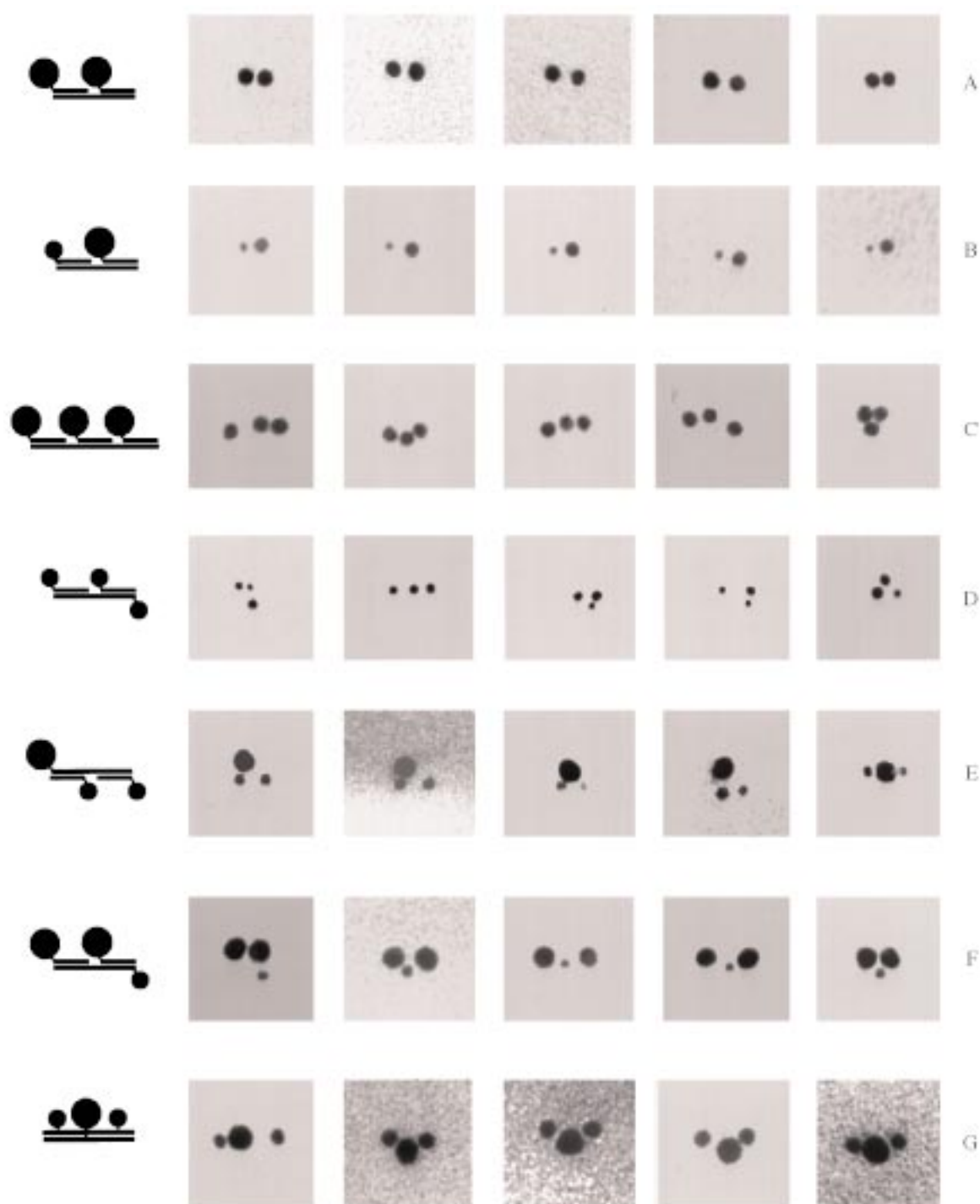


Figure 2. Schematic illustrations and representative TEM images for specific nanocrystal molecules. See Scheme 1 for the synthesis strategies. A) 10-nm homodimer, synthesis strategy B; B) 10-/5-nm heterodimer, strategy B; C) 10-nm homotrimer, strategy B; D) 5-nm homotrimer, strategy A; E) 10-/5-/5-nm heterotrimer, strategy A; F) 5-/10-/10-nm heterotrimer, strategy A; G) 5-/10-/5-nm heterotrimer, strategy C.

variability of the experimental values for the nanocrystal spacings is primarily due to the flexibility of the 1-nm-long 5' linker arms. Bending of the dsDNA, the affinity of one Au nanocrystal for another,^[12] and the process of depositing the nanocrystal molecules onto grids for TEM imaging may also affect the spacings.

Representative images of homodimers, heterodimers, homotrimers, and heterotrimers synthesized according to Schemes 1 A and 1 B, which produce nicked structures, are

shown in Figures 2 A–F. The TEM images of the trimers show spatial arrangements of the particles ranging from collinear to triangular (Figures 2 C–F), again indicating flexibility. The set of images of a heterotrimer synthesized by Scheme 1 C (Figure 2 G) shows a much more consistent arrangement of particles than do the images in Figures 2 C–F. This supports the hypothesis that nicks contribute significantly to the flexibility of DNA–nanocrystal assemblies prepared according to Schemes 1 A or 1 B.

The optical properties of Au nanocrystals and their assemblies depend on the size, shape, and/or spacings of the nanocrystals. When DNA is used to prepare extended aggregates of Au nanocrystals, pronounced changes in the optical spectra are observed that result from the collective electronic interactions of the nanocrystals.^[6] Generalized Mie theory for a Au–nanocrystal pair predicts that the plasmon band should red shift slightly, decrease in intensity, and broaden as the distance between two nanocrystals becomes smaller than the sum of their radii. The electronic interactions between Au nanocrystals separated by distances greater than the sum of their radii, however, are only expected to produce a slight broadening of the spectrum and no noticeable red shift.^[13] After normalization of the UV/Vis spectrum of the 10-nm monomeric DNA-modified gold species **3** and the spectrum of 10-nm homodimer **4a**,^[14] a decrease in the absorbance at 520 nm and some broadening of the absorption to longer wavelengths for the dimer was observed (Figure 3).

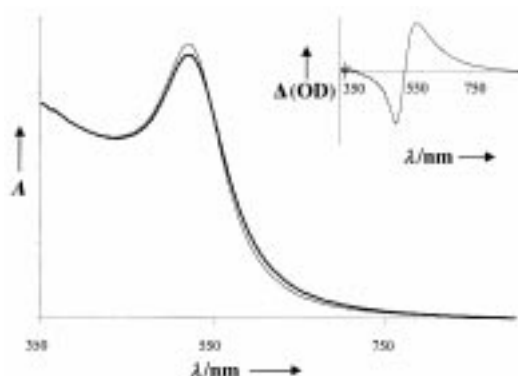


Figure 3. UV/vis spectra for 10-nm Au–ssDNA conjugate **3** (thin line) and 10-nm homodimer **4a** (bold line) assembled using 18-base-pair dsDNA. Inset: subtraction of the monomer spectrum from the dimer spectrum after normalization of the two spectra at 450 and 900 nm.

Subtraction of the spectra showed a maximum increase in absorbance at 582 nm for the dimer (inset of Figure 3). Since the effect of assembly observed in the optical spectrum of homodimer **4a** is small, the nanocrystal pairs are potentially spaced more than 5 nm apart in solution.

In this report, DNA was used to prepare specific, designed, nonperiodic arrangements of nanocrystals. The resulting assemblies are not structurally rigid, even in the case where the DNA has no nicks in it. Thus, we conclude that the present combination of linker molecules and duplex DNA is not sufficiently rigid to dictate a precise spatial arrangement of nanocrystals. In some cases, the ability to code sequences of nanocrystals without structural rigidity may be desirable. For instance, catalytic and photoactive systems may require simple colocalization of a number of nanocrystals. However, many more applications will require precise control of spatial separation, and it is quite likely that this can be achieved using oligonucleotide templates constructed from antiparallel double-crossover components.^[15]

Experimental Section

General procedures: Gold nanocrystals with a diameter of 5 and 10 nm were purchased from Ted Pella (Redding, CA). Salt **1** was purchased from Strem Chemicals (Newburyport, MA). Agarose was purchased from Gibco (Gaithersburg, MD). All biological buffers were from Sigma Chemical (St. Louis, MO); water was purified using a Millipore system. Microconcentrators were purchased from Amicon (Beverly, MA). Desalting by Fast Protein Liquid Chromatography (FPLC) was performed on a Superdex 75HR 10/30 column (3 mm 2,2-bis(hydroxymethyl-2,2',2''-nitrotritoltris(ethanol) (Bis-Tris), 1 mM ethylenediaminetetraacetic acid (EDTA), pH 6.5).

Gold complexation: For a typical reaction, an aqueous solution (100 mL) of 5-nm or 10-nm Au nanocrystals was stirred with a large excess of **1** (10–20 mg) at room temperature for more than 10 h. The nanocrystals were precipitated by adding solid NaCl to the reaction mixture until the color changed from burgundy red to blue. The sample was centrifuged (500 × *g*) to pellet the nanocrystals, and the supernatant was removed. The nanocrystals were redissolved in an aqueous solution of **1** (25 mg in 100 mL of water). Methanol was added to this solution until the color again changed from red to blue. Centrifugation and decanting were repeated, followed by dissolution of the nanocrystals in the aqueous solution of **1**.

Oligonucleotide Synthesis: The DNA was synthesized on an Applied Biosystems 391A PCRmate. All reagents used in automated synthesis were purchased from Glen Research (Sterling, VA), including the 5'-thiol modifier C6 used in the synthesis of the 5'-thiolated DNA (Scheme 1 A, B) and the amino modifier C6dT used to synthesize the trimeric system with internal thiols (Scheme 1 C). The DNA was synthesized with the 5'-trityl protecting group (trityl = CPh₃) intact and purified by HPLC using a reverse-phase C18 column (218TP54, Vydac Technologies) and a 1% per minute gradient of acetonitrile in 50 mM triethylammonium acetate (TEAA, pH 7.0) over 30 min. Once purified, the non-thiolated DNA was detritylated by treatment with 80% AcOH in water (v/v) for 30 min. The 5'-thiolated DNA was detritylated by mixing 20 nmol of oligonucleotide in 100 mM TEAA (pH 6.5) with 1 M AgNO₃ (4 μL) for 30 min at room temperature. Then 1 M 1,4-dithiothreitol (DTT, 8 μL) was added, which led to the formation of a yellow precipitate. The reaction mixture was centrifuged, the supernatant loaded onto a NAP-5 column, and the DNA eluted with 3 mM Bis-Tris and 1 mM EDTA (pH 6.5). Finally, the thiol-modified oligonucleotides were desalted by FPLC prior to use. The thiol concentration of a desalted solution was determined by titration with 5,5'-dithiobis(2-nitrobenzoic acid).

DNA Sequences: Figure 1: 5'-XCAGTCAGGCAGTCAGTCA-3', 5'-TGACTGACTGCCTGACTGTTGACTGACTGCCTGACTG-3';^[7] Figures 2 A, B: 5'-XATGGCAACTATACGCGCTAGAGTCGTTT-3' (oligo 1), 5'-XCTAATCCGCACAGCCTATCGACCATGCT-3', 5'-AGCATGGTCGATAGGAAACGACTCTAGCGC-3';^[16] Figure 2 C: (oligo 1), 5'-AAACGACTCTAGCGCAAACGACTCTAGCGCAAACGACTCTAGCGC-3'; Figures 2 D–F: 5'-XGCGCTAGAGTCGTTT-3', 5'-XAAACGACTCTAGCGCTAAACGACTCTAGCGC, where X = 5'-thiol; Figure 2 G: 5'-GGCATGGTCYACGTCACGCTAGTCAGTCATCTTGCACTAAGTCCTTGAGAACGTCACGCTAGTCAGTCAYACTCTAGCGC-3', 5'-GCGCTAGAGTATCATCTAGTACGCGTGACGTTCTCTCAAGGACYTAGTGCAAGATGACTGACTAGCGTGACGTAGACCATG-CC-3', where Y = amino modifier dT. Complementary dimers: 5'-XTGACTGACTGCCTGACTG-3', 5'-XCAGTCAGGCAGTCAGTCA-3'; 5'-XAGCATGGTCGATAGGCTGTGCGATTAG-3', 5'-XCTAATCCGCACAGCCTATCGGACCATGCT-3'; 5'-XACGTCACGCTAGTCAGTCATCTTGCACTATGTCCTTGA-3', 5'-XCTAAGGACATAGTCAAGATGACTGACTAGCGTGACGT-3', where X = 5'-thiol.

Nanocrystal molecule assembly (Schemes 1 A, B): Thiolated ssDNA (0.9 equiv) was added to a 100 mM aqueous NaCl solution of 1–10 μM **2a** or **2b** and incubated at room temperature for more than one hour to yield the oligonucleotide–nanocrystal conjugate **3**. Conjugate **3** was mixed with an equimolar amount of a complementary oligonucleotide strand, which was either attached to nanocrystals or unmodified. This mixture was incubated at room temperature for one to four days.

Nanocrystal molecule assembly (Scheme 1 C): Two complementary 80-mer strands were synthesized as shown in Scheme 1 C. Oligonucleotide C has two amino modifier dTs, whereas C' has one. An oligonucleotide identical in sequence to C' but containing no modified bases was also synthesized.

The purified amine-containing oligonucleotides (500 nmol) were suspended in aqueous 100 mM NaH_2PO_4 (250 μL , pH 7.5). A 20 mM solution (50 μL) of 3-(2-pyridylthio)propionic acid *N*-hydroxysuccinimide ester (Aldrich) in DMSO was added, and the reaction mixture was incubated at room temperature for 4 h.^[17] The reaction mixture was desalted by FPLC, purified by reverse-phase HPLC using a 0.5% per minute gradient of acetonitrile in 50 mM TEAA (pH 7) over 30 min, lyophilized, and resuspended in 100 mM NaH_2PO_4 (pH 7.5). The modified oligonucleotides were deprotected by the addition of 1M DTT (20 μL) for 30 minutes, after which they were desalted by FPLC. Deprotected oligonucleotide C' was mixed directly with 1–10 μM 10-nm Au in 0.1M aqueous NaCl solution, whereas oligonucleotide C was first hybridized to unmodified C' for 30 min followed by addition of 5-nm Au (10 equiv) in 0.1M aqueous NaCl solution. The conjugates of strand C' with a single 10-nm Au nanocrystal and of the duplexed strand C with two 5-nm Au particles were purified on 3% agarose gels and collected. Microcon 100s were used to exchange the purified conjugates into aqueous 0.1M TEAA (pH 6.5, 0.05M NaCl). Equimolar amounts of the two conjugates were mixed, heated to 95 °C for 5 min, and allowed to cool slowly to room temperature. The trimer products, which were obtained in yields of approximately 30% based on **3c**, were purified on 4% agarose gels.

Nanocrystal molecule purification: Agarose gels (3–4%) were used to analyze and isolate the desired structures. Tris-borate EDTA (1X, TBE) was used as the running buffer. Glycerol loading buffer (6X) was used to load the samples onto the gels.^[18] After electrophoresis, samples were isolated by slicing the gel in front of the desired band and inserting glassfiber microfilters (Whatman GF/C) that were backed with dialysis membranes (Spectra/Por, MWCO: 10000). The bands were incorporated into the paper by electrophoresis. The filter/membrane pieces were then placed with the filter facing down into 0.5-mL Eppendorf tubes which had been pierced at the bottom with 26-gauge needles. These Eppendorf tubes were fitted into 1.5-mL Eppendorf tubes and centrifuged for one minute (14000 $\times$ g) to isolate the desired products. Syringe tip filters (Millipore, 0.45 μm) were used to remove residual glass fibers from the resulting solutions. Typically, isolated yields were 30–40% (dimers) and 20–30% (trimers) based on the nanocrystal starting materials.

TEM procedures: A Jeol-100CX instrument was used to image the samples at 80 kV. Copper grids (400 mesh) coated with a layer of Formvar were subjected to an argon plasma (30 s at 50 mTorr), after which solutions of the nanocrystal molecules (0.1–0.01 μM) were deposited for one minute. Excess solution was removed by wicking, and the grids were dried in air. The diameters of the 5-nm nanocrystals shown in Figure 2D–G are not uniform due to contrast differences that result from variation in the thickness of the Formvar used in imaging the samples.

UV/Vis studies: UV/Vis spectra were measured on an HP8453 UV-Vis spectrophotometer using 40- or 100- μL masked quartz cuvettes. The monomer and dimer bands were collected from the same gel lanes in 1X TBE buffer. The dimer bands were typically purified a second time. Spectra were taken of monomer and dimer samples that had been diluted with 1X until the absorbances at 520 nm for the two samples were similar but not necessarily identical. The spectra for the dimer and monomer were normalized at 450 and 900 nm before comparison or subtraction.

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Coordination Chemistry of Lanthanides at “High” pH: Synthesis and Structure of the Pentadecanuclear Complex of Europium(III) with Tyrosine**

Ruiyao Wang, Zhiping Zheng,* Tianzhu Jin, and Richard J. Staples

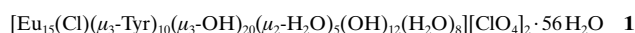
Dedicated to Professor M. Frederick Hawthorne

The design and synthesis of high-nuclearity metal complexes is arguably one of the most stimulating research frontiers in contemporary chemistry.^[1] The preparation of such complexes enables their nanoscopic dimensions to be combined with the rich redox and magnetic properties of the constituent metals, and leads to novel materials with promising electronic, magnetic, optical, and catalytic properties. A commonly employed strategy for the synthesis of polynuclear metal complexes is to utilize appropriate chelating ligands to control the hydrolysis of metal ions;^[2] the auxiliary ligands prevent extensive hydrolysis from occurring—a process that would otherwise lead to the formation of intractable metal hydroxides and/or oxides—and as a result, finite-sized poly-metal complexes can be crystallized and structurally characterized. Several classes of ligands have been utilized in this capacity, among which carboxylates,^[3] polyketonates,^[4] and alkoxides^[5] are prominent. Spectacular clusters such as polyoxovanadates,^[6] ferric wheels,^[3b] and manganese clusters^[7] have been reported and proven to exhibit rather interesting magnetic properties.^[5, 7, 8]

Despite all the progress that has been made in controlling excessive hydrolysis during the formation of metal clusters, a suitable and general class of supporting ligands is yet to be identified for the preparation of lanthanide-based clusters.^[5] Lanthanide clusters are expected to exhibit more promising magnetic properties as a consequence of the unique spectroscopic and electronic properties mainly associated with their 4fⁿ electronic configurations.^[9] With the intent of assembling novel lanthanide clusters and ultimately fabricating promising lanthanide-based magnetic materials,^[9] we have recently initiated a program to study the coordination chemistry of lanthanides with amino acids, with the hope that amino acids may serve as a novel class of supporting ligands. Although the coordination chemistry of lanthanides with amino acids has been investigated to some extent,^[10] these studies have been exclusively carried out at low pH values, typically under pH 4.

This is understandable because lanthanide ions are so prone to hydrolysis that hydroxides and/or oxides would be the final products at higher pH values. In all the reported structures of such complexes, the ligands, not surprisingly, coordinate to the metal centers through only the oxygen atom(s) of the carboxylate moiety. The amino group remains protonated and serves merely as a “spectator” fragment, which affects the structure of the complex only by imposing electrostatic and/or steric effects.^[10] At “high” pH values, however, multiple binding of a lanthanide ion by an amino acid (α -amino carboxylate, to be more exact) may be achieved with both the amino and the carboxylate groups participating in simultaneous coordination. We envision that such chelating effects may stabilize the metal complex by counteracting the undesirable hydrolysis and consequently, high-nuclearity lanthanide clusters would form.

To test the validity of this approach we have synthesized a series of lanthanide(III)–tyrosine complexes by mixing $\text{Ln}(\text{ClO}_4)_3$ ($\text{Ln} = \text{La}, \text{Pr}, \text{Nd}, \text{Sm}, \text{Eu}, \text{Gd}, \text{Dy}$) and tyrosine in an aqueous solution and subsequently adjusting its pH value to about 6. Single crystal X-ray analysis^[11] establishes an appealing pentadecanuclear structure for all these complexes and to the best of our knowledge, they are the largest members of a fascinating family of homonuclear lanthanide clusters that have hitherto been reported.^[12] Since these molecules are similar in composition, except for the different constituent lanthanide ions, and are isostructural in the solid state, we shall use the europium(III)–tyrosine complex **1** to discuss the novel features and significance of this research.



As shown in Figure 1, the complex contains 15 Eu^{III} ions and 10 tyrosine ligands in addition to the hydroxo and aquo ligands associated with hydrolysis. The metal ions are assembled into a layered structure, and each of the three parallel layers contains five Eu^{III} ions, which occupy the vertices of a nearly perfect pentagon. The two outer layers are crystallographically equivalent with an average Eu–Eu distance of 6.331 Å, while the middle layer, with an average

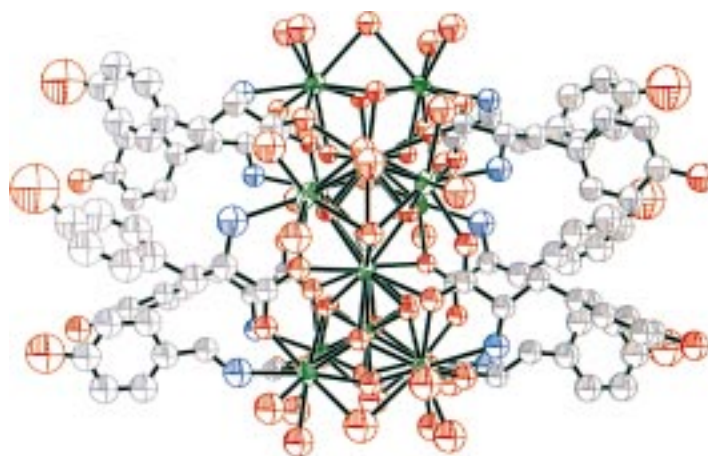


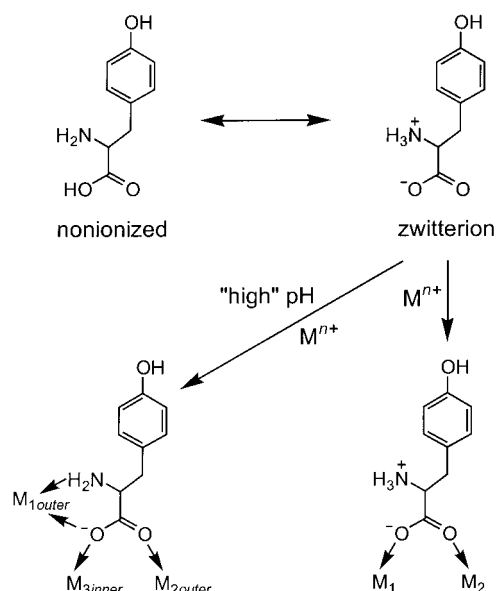
Figure 1. A perspective view of **1**: carbon: gray, chlorine: black, europium: green, nitrogen: cyan, oxygen: red. Lattice water molecules and perchlorate ions are omitted for clarity.

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Eu–Eu distance of 3.896 Å, is unique and “tucked in”. The ten tyrosinate ligands can be divided into two equivalent groups, each of which extend their 4-hydroxybenzyl side chains in the direction perpendicular to the crystallographically imposed C_2 axis. The side chains do not participate in, nor do they appreciably interfere with, the coordination. The coordination modes of the tyrosinate ligands with Eu^{III} ions in these molecules are significant: Each of the ten tyrosinate groups acts as a tetradentate ligand by utilizing both its amino and carboxylate groups (Scheme 1). The ligand can be described



Scheme 1. Two different coordination modes of a tyrosine ligand with lanthanide ions.

as a $\mu_3\text{:}\eta^1\text{:}\eta^2\text{:}\eta^1$ anion, not only linking two neighboring Eu^{III} centers within the same (outer) layer, but also coordinating to a third Eu^{III} ion in the middle layer through one of the carboxylate oxygen atoms. It is apparent that such layer-crossing chelating interactions are critical to the formation of the cluster structure. In previous studies of the coordination of lanthanide ions with amino acids, the zwitterions bind the lanthanides exclusively through the oxygen atom of the carboxylate group. Such an interaction is included in Scheme 1 for comparison. The coordination sphere of the Eu^{III} centers is completed by hydroxo and aquo ligands (Figure 2). Thus, each of the ten Eu^{III} ions in the outer layers has a coordination number (CN) of 9, and the structure can be best described as monocapped square antiprismatic, while the inner-layer Eu^{III} centers too have a CN of 9, and form irregular square antiprisms monocapped by the central chloride ion (see below). From a different perspective, the core component of the cluster may be viewed as a “wheel” of five corner-sharing cubanes, each of which consists of four Eu^{III} ions and four $\mu_3\text{-OH}$ ligands (Figure 3).

A particularly salient feature of the structure is a chloride ion being trapped within the “belly” of the barrel-shaped molecule, and serves as the bolt of the cubane wheel. The chloride ion simultaneously coordinates the five inner-layer Eu^{III} ions in a perfectly planar fashion with an average Eu–Cl distance of 3.314 Å. It is unlikely that an empty “barrel” is

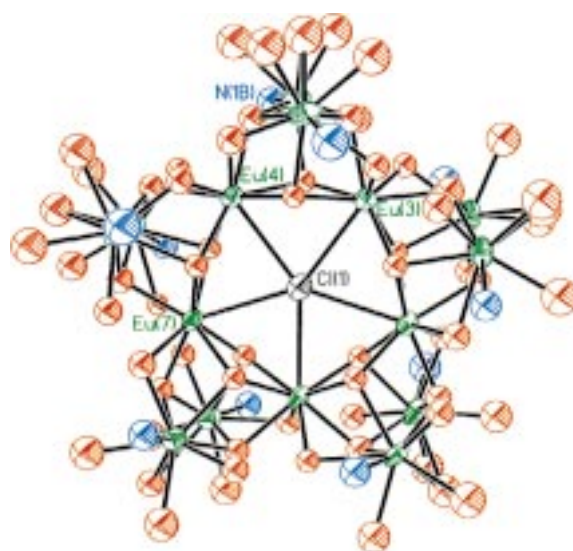


Figure 2. A perspective view of **1** showing only the Eu^{III} -coordination sphere.

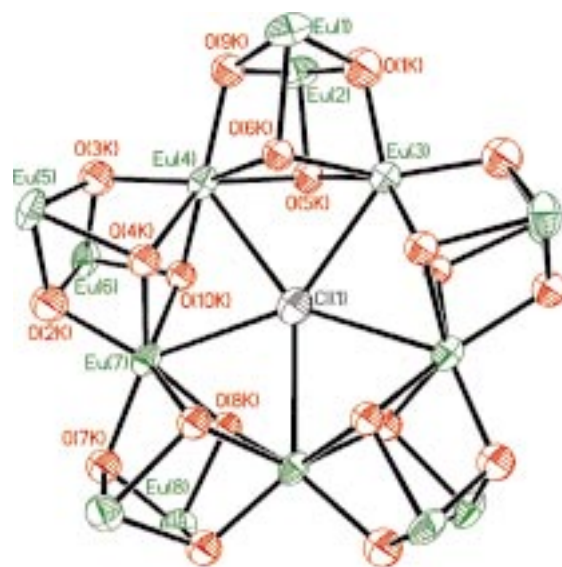


Figure 3. The “cubane-wheel” structure of **1**.

preformed for the encapsulation of the chloride ion, instead, the formation of the pentadecanuclear complex can be best described as a chloride-induced self-assembly of the lanthanide ions with the help of the tyrosine ligands. In other words, the chloride ion serves as a template in the formation of this novel cluster, and hence furnishes an exciting addition to the rare examples of anion-templated synthesis.^[13]

In summary, we have demonstrated that amino acids are an effective class of ancillary ligands in the synthesis of lanthanide clusters. The reaction described herein stands in stark contrast to previous coordination chemistry of lanthanide ions with amino acids, wherein a low pH value is, at least is commonly perceived so, mandatory in order to avoid the formation of insoluble hydrolysis by-products. It is, however, the unconventionally “high” pH condition that promotes the formation of the large molecular clusters reported here, and hence a new paradigm in lanthanide coordination chemistry. The proof-of-concept demonstrations reported herein suggest

that this synthesis concept is potentially expandable toward other metal systems. Mixed-metal clusters containing both lanthanides and d block transition elements such as copper, iron, and manganese, for example, may be prepared.^[14] The synthetic opportunities abound when the rich varieties of amino acids and transition metals including lanthanide elements are considered. Furthermore, these pentadecanuclear clusters possess unprecedented layered structures that are complex yet architecturally pleasing. The highly ordered arrangement of the metal centers in these nano-sized clusters is expected to yield useful magnetic properties.^[5, 7, 8] We believe that the novel structural patterns (and any accompanying physical/chemical properties), coupled with the ability to construct a huge variety of lanthanide clusters will undoubtedly enhance our understanding of lanthanide-based molecular magnetic materials.

Experimental Section

Tyrosine (0.181 g, 1.0 mmol) was added as a solid to an aqueous solution of $\text{Eu}(\text{ClO}_4)_3$ prepared by dissolution of Eu_2O_3 (1.408 g, 4.0 mmol) in 70% perchloric acid. To this clear solution was added one drop of 1.0 M aqueous HCl and the mixture was stirred at 80 °C for 5 min. The pH value of the above solution was then carefully adjusted by slow addition of 0.5 M aqueous NaOH until the precipitate thus formed remained. The mixture was then filtered and the filtrate was allowed to stand at room temperature. Parallelepiped of the lanthanide complexes appeared in about one week. The complex was synthesized in a typical yield of 60% (based on tyrosine). Elemental analysis calcd: Eu 37.41; found: 37.36; ESI-MS: m/z (%): 1936.1 (30) $[(M^+ + \text{H}) - 5\text{H}_2\text{O} - 2\text{ClO}_4^-]^{3+}$, 1474.7 (100) $[(M^+ + 2\text{H}) - 2\text{ClO}_4^-]^{4+}$, 1150.9 (40) $[(M^+ + 3\text{H}) - 8\text{H}_2\text{O} - 2\text{ClO}_4^-]^{5+}$.

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diffractometer at 213 K with $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). Monoclinic, space group C_2 , $a = 32.259(3)$, $b = 20.926(2)$, $c = 22.037(2) \text{ \AA}$, $\beta = 130.956(4)^\circ$, $V = 11234(2) \text{ \AA}^3$, $Z = 2$, $\rho_{\text{calcd}} = 1.801 \text{ Mg m}^{-3}$, $\mu(\text{MoK}\alpha) = 4.254 \text{ mm}^{-1}$, $F(000) = 5956$. ω - 2θ scans, 29805 reflections measured ($2\theta_{\text{max}} = 50^\circ$), of which 13780 were independent and all are observed [$I = 2\sigma(I)$], 528 refined parameters, $R = 0.0812$, $wR_2 = 0.2096$, residual electron density max./min. $2.229/-1.294 \text{ e \AA}^{-3}$, max./min. transmission 0.9583/0.7456. Data reduction was performed with the SAINT software, which corrects for Lorentz and polarization factors as well as decay. Absorption corrections were applied with SADABS supplied by G. Sheldrick. The structure was solved by the direct method with the SHELXS-97 program and refined by least squares method on F^2 , SHELXL-97, incorporated in SHELXTL-PC version 5.10. Only the metal atoms were refined anisotropically. Hydrogens were calculated by geometrical methods for the ligand and refined as a riding model. The solvated water molecules were found by using the program XWAT, supplied within SHELXTL. No hydrogen atoms were modeled for the hydroxides or water molecules in the cluster or solvated. Further details on the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de) on quoting the depository number CSD-410601.

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A Versatile Route to Porous Solids: Organic–Inorganic Hybrid Materials Assembled through Hydrogen Bonds**

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One of the many promising applications of supramolecular chemistry is in the design of crystalline host–guest materials, which have been pursued through two principal routes. First, coordination polymers^[1] have been shown to form porous materials whose host frameworks can withstand the removal/addition of guest molecules^[2] or exhibit high shape specificity in clathration.^[3] Second, organic networks have been assembled through directional intermolecular forces^[4] to form porous frameworks, which can be applied to homogeneous and heterogeneous^[5, 6] separations. Remarkably, some three-dimensional (3D) organic frameworks assembled through hydrogen bonds are rugged enough for reversible guest

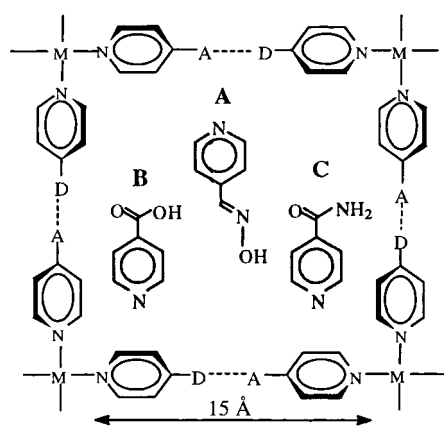
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[11] Crystal structure determination of **1**: A colorless crystal (ca. $0.15 \times 0.15 \times 0.10 \text{ mm}$) was analyzed with a Bruker SMART CCD-based

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inclusion.^[6] While it is unlikely that molecular host–guest complexes will be as robust as naturally occurring zeolites, these systems have the advantage of “tunability” where the nature and size of the pore may be altered.^[7] We are interested in a crystal engineering strategy based on a combination of the above approaches:^[8] while we are inclined to use hydrogen bonds as crystal engineering tools^[9] (they can *flex* to accommodate entering and departing guest molecules), we also recognize the advantage of using metal centers (square-planar or octahedral geometries are not easily accessible in organic chemistry). The aim is to assemble metal complexes through hydrogen bonds to form porous materials for inclusion/separation.

The assembly of the materials described herein relies on the ability of Pt^{II} and Ni^{II} ions to coordinate to four pyridine-based ligands in a square-planar arrangement. The ligands are substituted with oxime (A), carboxylic acid (B), and carboxamide (C) moieties (Scheme 1); such functional groups can



Scheme 1. A = Hydrogen-bond acceptor, D = hydrogen-bond donor, M = metal ion.

overcome competition from solvent molecules and counterions to assemble linear silver(I) complexes through complementary hydrogen bonds.^[10] Successful assembly of square-planar complexes through head-to-head hydrogen-bond interactions between the ligands affords the potential for large cavities, 15 Å × 15 Å, that are suitable for inclusion of guest molecules (Scheme 1; cavity sizes are consistently estimated allowing for van der Waals radii). As ever, the possibility for interpenetration exists,^[11] but even in that case, such large voids should only be partially filled (although examples to the contrary have been reported^[12]). Herein, we describe four structures which demonstrate the proclivity of platinum(II) and nickel(II) complexes to form crystalline host–guest materials.

In compound **1**,^[13] the metal complexes link through four unique N–H⋯O amide–amide hydrogen bonds to form cationic sheets (Figure 1); the hydrogen-bond motif results in



the formation of two unique holes in each parallel sheet. The smaller, $R_4^4(16)$ hole^[14] is blocked by nickel(II) ions in the sheets above and below, but the larger, $R_2^2(28)$ holes only partially overlap, forming channels for the perchlorate

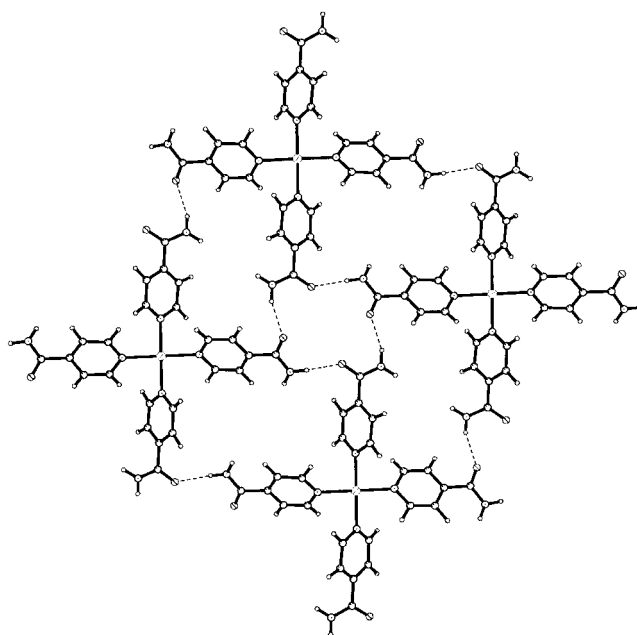
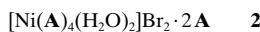


Figure 1. An infinite cationic layer in **1**, in which two unique holes $R_4^4(16)$ and $R_2^2(28)$ are formed.

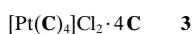
counterions.^[15] Additional hydrogen bonds between coordinated water molecules and amide oxygen atoms connect the sheets above and below to generate the three-dimensional network. As the amide–amide hydrogen bonds display catemer-type interactions (instead of head-to-head), the large holes predicted for these assemblies are not realized, and instead small channels containing counterions are present.

The versatility of the strategy is demonstrated in compound **2**, which utilizes a different type of hydrogen-bonding moiety (oxime instead of amide). The oxime hydroxy groups link



through complementary O–H⋯O hydrogen bonds to form sheets with large, $R_4^4(64)$ hourglass-shaped holes (22 Å long, 15 Å wide at the ends, 7 Å wide at the waist). The sheets are cross-linked by hydrogen bonds between the axially coordinated water molecules and the bromide counterions, forming a 3D network, where the large holes are aligned into channels. Twofold interpenetration of the 3D networks blocks the center of the large hole, leaving two smaller channels at each end. In this case, a host–guest complex is formed, and the guest molecules, 4-aldoximepyridine, are contained inside the channels (Figure 2), held in the lattice by hydrogen bonds to the bromide ion and the ligated oxime moieties.^[16] Thus, the structure of **2** demonstrates that an octahedral system can generate a 3D network with holes large enough to hold small organic molecules.

A similar square-planar system can also create a host–guest complex, as evidenced by compound **3**. In this case, *trans* ligands are linked linearly by head-to-head [$R_2^2(8)$]



amide–amide hydrogen bonds; the resulting chains are arranged parallel to each other. The linear assemblies are

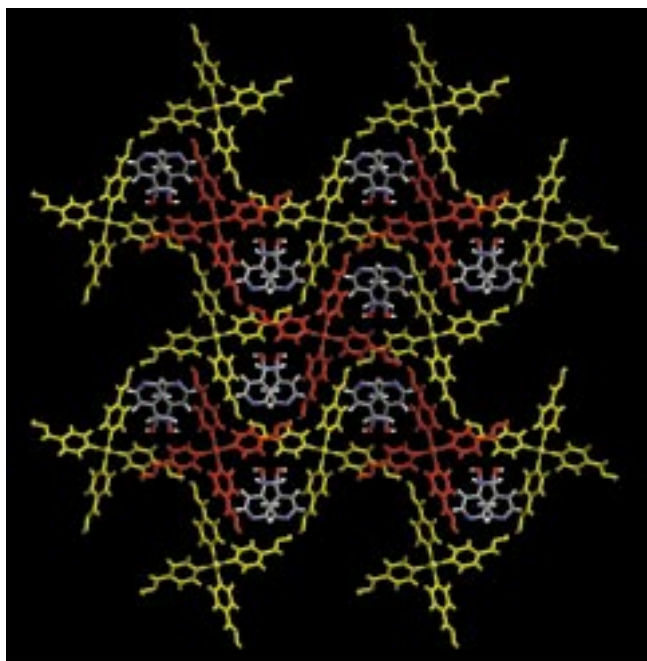
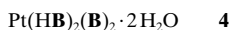


Figure 2. The structure of **2** looking down channels containing guest 4-aldoximepyridine molecules, where interpenetrating hydrogen-bonded nets are depicted in yellow and red, respectively.

interconnected perpendicular to the propagation of the chains through two inequivalent N–H⋯Cl interactions, creating a three-dimensional network which contains large square channels (Figure 3 left). The guest isonicotinamide molecules occupy these channels (Figure 3 right), and are anchored to the lattice by hydrogen bonds. Compound **3** demonstrates that even if the structure manifests head-to-head hydrogen bonds in chains (1D), rather than the sheets (2D) proposed in Scheme 1, other hydrogen-bonding interactions can create a 3D framework, and the smaller channels are still large enough to hold small organic molecules.

While the amide and oxime ligands remain protonated, thus necessitating the presence of counteranions, carboxylic acid

moieties can become deprotonated, and the lattices can be constructed without counterions. Thus compound **4**, unlike



1–3, is a neutral metal complex which forms two crystallographically unique square nets, assembled through charge-assisted carboxylate–carboxylic acid hydrogen bonds [$R_4^4(56)$] (Figure 4). The distance between the platinum(II) ions in each

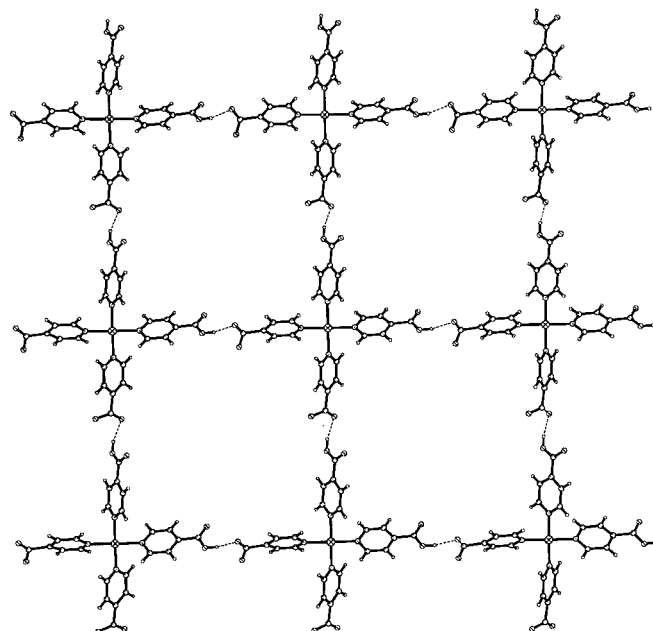


Figure 4. One of the 2D hydrogen-bonded nets in **4**, showing the large square cavities generated by hydrogen-bonded metal–complex cations.

sheet is $>15 \text{ \AA}$, leading to large square cavities. The crystal structure of **4** provides a rare example of inclined interpenetration of (4,4) nets in three mutually perpendicular planes.^[11] Each square cavity of one of the unique nets is

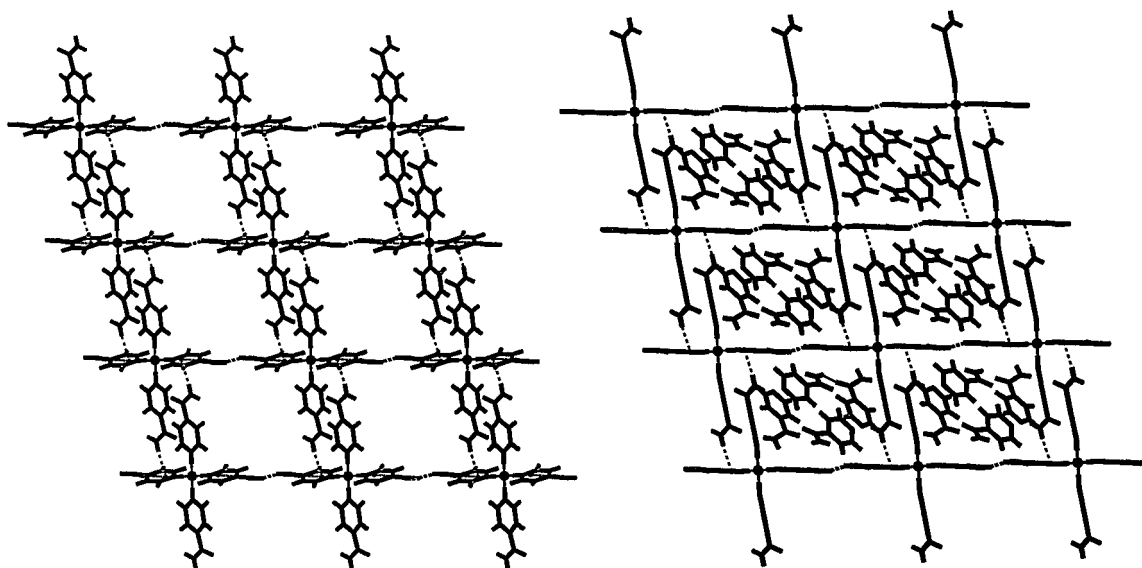


Figure 3. The structure of **3** without (left) and with (right) guest isonicotinamide molecules. In the structure with guest molecules, the ligand pyridine rings are replaced with a line for clarity.

interwoven by three other nets, while those of the other unique net are interwoven by five nets. Despite this interpenetration, channels are present which host disordered water molecules (Figure 5). The complex is thermally stable, with a melting point of $>300^{\circ}\text{C}$, indicating that the structure could remain intact for reversible addition of water or other guests in the channels.

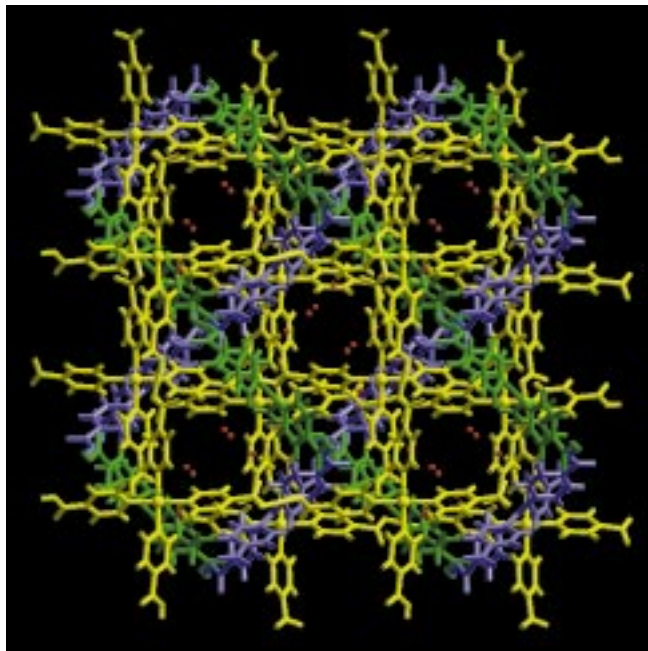


Figure 5. The structure of **4**, viewed down channels resulting from interpenetrating hydrogen bonded nets. The three mutually perpendicular nets are shown in purple, yellow, and green. The red spheres are guest water molecules.

Experimental Section

1: An aqueous solution (20 mL) of isonicotinamide (0.30 g, 2.5 mmol) was slowly added to an aqueous solution (10 mL) of $\text{Ni}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (0.18 g, 0.49 mmol). The solution was allowed to evaporate at room temperature. Irregular blue crystals formed in a few weeks (m.p. $>300^{\circ}\text{C}$).

2: A solution of 4-aldoximepyridine (0.20 g, 1.64 mmol) in ethanol was slowly added to an aqueous solution of $\text{NiBr}_2 \cdot x\text{H}_2\text{O}$ (0.14 g). The flask was covered and left to stand in ambient conditions. Slow evaporation of solvent produced blue, platelike crystals (m.p. $>300^{\circ}\text{C}$).

3: An aqueous solution of isonicotinamide (0.08 g, 0.66 mmol) was added to an aqueous solution of $[\text{NH}_4]_2[\text{PtCl}_4]$ (0.05 g, 0.13 mmol), and allowed to evaporate at room temperature. After about one week, a yellow powder was filtered off and washed with ethanol. The ethanolic filtrate and the mother liquor were mixed and allowed to evaporate, yielding irregular colorless crystals (m.p. $>300^{\circ}\text{C}$).

4: A 1:2 ethanol:water solution (30 mL) of isonicotinic acid (0.13 g, 0.94 mmol) was added to an aqueous solution (15 mL) of $\text{K}_2[\text{PtCl}_4]$ (0.07 g, 0.17 mmol) and allowed to evaporate, yielding conical colorless crystals (m.p. $>300^{\circ}\text{C}$). Typically, the yields were over 80% except in the case of **4**, which was below 40%.

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-108604–CCDC-108607 (**1–4**). 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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Photoionization and Electron Transfer of Biphenyl within the Channels of Al-ZSM-5 Zeolites

Isabelle Gener, Guy Buntinx, and Claude Brémard*

There is considerable interest in the use of crystalline aluminosilicate porous materials such as zeolites to study and try to control the photophysical and photochemical properties of the occluded molecules.^[1] The ability of zeolites to stabilize radical cations and trap electrons is now firmly established.^[2, 3] In contrast, the reverse situation in which zeolites act as electron donors is far less documented.^[4, 5] The ZSM-5 zeolites with straight (0.54×0.56 nm) and zigzag (0.51×0.55 nm) channels have been reported previously to generate radical cations spontaneously and stabilize them in the void space.^[6] While the mechanism of oxidation remains unclear today, it appears that the presence of aluminum in the zeolite is a requirement for persistent radical cations. Herein we present our most striking results concerning the photolysis of biphenyl ($C_{12}H_{10}$, BP) occluded at low coverage in nonacidic ZSM-5 zeolites. The emphasis of the paper is mainly set on the photoionization of biphenyl and electron transfer. The zeolite–radical cation–electron interactions have been tuned by varying the silicon:aluminum ratio, by using charge-balancing cations, and without any additional molecules.

Weighted amounts of bisublimed BP (0.5–1 BP per unit cell) were mixed with freshly dehydrated $M_n[Al_nSi_{96-n}O_{192}]$ ($M=Na^+$, K^+ , Cs^+ ; $n=0, 3, 6$) zeolites under an inert atmosphere in a silica cell. The FT-Raman and diffuse reflectance UV/Vis absorption spectra of the mechanical powder mixtures were recorded as a function of time. All the spectroscopic data recorded after the required equilibration period of one month at a gentle temperature ($50^\circ C$) are typical of occluded BP at low coverage within the void space. The aluminum content, the extraframework cations M^+ , and the cosorbed gas (argon or helium) do not induce dramatic

effects upon the molecular conformation of ground state BP ($BP(S_0)$) as evident from similar vibrational spectroscopic characteristics to that reported previously for $BP(S_0)$ in fluid solution and attributed to a twisted structure.^[7] The ground-state absorption electronic spectra of BP occluded at low coverage in ZSM-5 are typical of isolated chromophores.^[7]

Molecular mechanics calculations (MM), Monte Carlo simulations (MC), and molecular dynamics calculations (MD) provide evidence of the expected location, the structure as well as the diffusion of $BP(S_0)$ within the void space of the $Na_4[Al_4Si_{92}O_{192}]$ zeolites.^[8] The $BP(S_0)$ molecules were found to be located preferentially in the straight channels in the vicinity of the intersection with zigzag channels (Figure 1).



Figure 1. Cross-sectional view of biphenyl (BP, S_0) occluded in the straight channel of $Na_4Si_{92}Al_4O_{192}$ zeolite perpendicular to the b axis. The yellow Si, blue Al, and red O cylinders represent the ZSM-5 framework, the pink balls represent the extra-framework Na^+ cations, the shaded and white cylinders represent the C and H atoms of BP, respectively.

The expected site of $BP(S_0)$ in ZSM-5 is in reasonable agreement with preliminary X-ray diffraction data performed at high coverage.^[9]

It should be noted that the nearest $C \cdots Na$ and $C \cdots Al$ distances are found to be 4.66 and 7.1 Å, respectively. From MD simulations data the main role of the zeolite framework appears to be to reduce the mobility of BP within the void space, and the $BP(S_0)$ molecule performs only vibrational, internal rotation, and translational motions in the vicinity of the sorption site.^[8]

An excimer laser (248 nm, 15 ns, $0.12\text{--}30\text{ mJ cm}^{-2}$) was used as pump excitation within the $S_3 \leftarrow S_0$ ($\pi^* \leftarrow \pi$) transition of occluded BP in $M_n[Al_nSi_{96-n}O_{192}]$. This transition exhibits an intense electronic absorption around 250 nm. Decays in the UV/Vis spectra up to 300 μs were recorded after the laser photolysis of the $1BP/Si_{96}O_{192}$ and $1BP/Na_3[Al_3Si_{93}O_{192}]$ samples by the transient diffuse reflection technique.^[10] It should be noted that with the pump excitation power used, no transient spectra were obtained after photolysis of the bare zeolites.

The data processing^[11] of the transient spectra provides clear evidence of the pure component spectra and concentrations of the lowest triplet state $BP(T_1)$ (355 nm) and the radical cation $BP^{+\bullet}$ (380, 660 nm) within the experimental pump energy range.^[7] No spectral evidence of both the radical anion and trapped electron was found in either $1BP/Si_{96}O_{192}$ or $1BP/Na_3[Al_3Si_{93}O_{192}]$ samples under the experimental conditions used.^[7, 8, 12, 13] The short lifetime of $BP(T_1)$ is found to be shorter in $1BP/[Si_{96}O_{192}]$ (3×10^{-7} s) than in $1BP/Na_3[Al_3Si_{93}O_{192}]$ (2.4×10^{-6} s). In contrast, the lifetime of $BP^{+\bullet}$

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in 1BP/Na₃[Al₃Si₉₃O₁₉₂] (0.8 s) was found to be markedly longer than in 1BP/Si₉₆O₁₉₂ (1.3×10^{-5} s).^[12]

The transient species for the time resolved resonance Raman (TR³) experiments were generated through photolysis at 248 nm (15 ns, 1.4–14 mJ cm⁻²) and the resonance Raman (RR) scattering of the transient species were excited at 370 nm (8 ns, 21 mJ cm⁻²). The time delay between the pump and probe pulses can be adjusted from 50 ns to several ms. In these experimental conditions only two transient species were detected, which were unambiguously assigned to BP(T₁) and BP⁺ (Figure 2). The radical cation BP⁺ is detected for the samples ($n=0, 3, 6$; M = Na⁺) whereas BP(T₁) is detected in high yield for $n=0$ and only at low pump laser power.

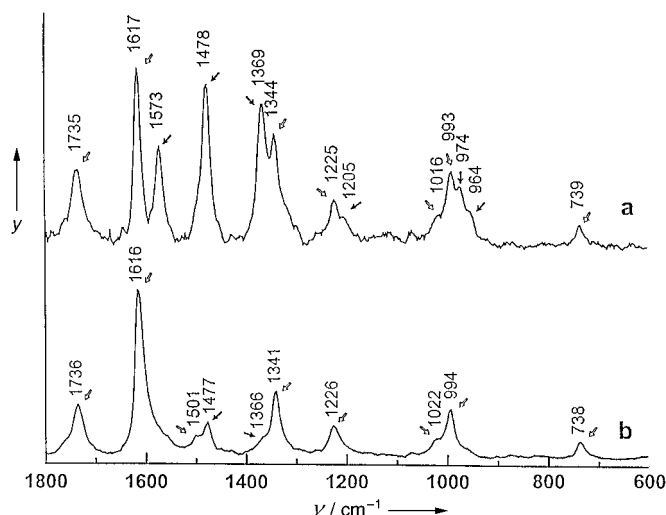


Figure 2. Time-resolved resonance Raman (TR³) spectra of occluded photoexcited biphenyl ($\Rightarrow$ BP(T₁), $\Rightarrow$ BP⁺; pump laser, 248 nm, 15 ns, 4 mJ cm⁻²; probe laser, 370 nm, 8 ns, 21 mJ cm⁻²). a) 1BP/Si₉₆O₁₉₂: spectrum obtained after 50 ns from the photoexcitation. b) 1BP/Na₃Si₉₃Al₃O₁₉₂: spectrum obtained after 50 ns from the photoexcitation. y = Raman intensity in arbitrary units.

The similarity in wavenumbers as well as in the relative intensities of the signals between the corresponding RR features of BP(T₁) and BP⁺ in solution and occluded in ZSM-5 zeolites indicates analogous molecular structures in fluid and porous media.^[7, 8, 13–16] The BP(T₁) and BP⁺ populations arise from two independent and parallel processes upon photolysis of BP(S₀) at 248 nm. The dependence of the BP(T₁) and BP⁺ yields upon pump power indicates that BP(T₁) is the major species at very low power, which suggests that BP(T₁) is produced by a monophotonic process through S₁ $\leftarrow$ S₃ internal conversion, followed by T₁ $\leftarrow$ S₁ intersystem crossing.^[7, 13] The large BP $\cdots$ BP intermolecular distance and the slow BP diffusion in the void space are in accurate agreement with the first-order rate of the decays and energy transfer through the lattice. The radical cation BP⁺ is probably generated through a biphotonic process.

The lifetime of BP⁺ appears to be dramatically dependent upon a narrow range of aluminum content of the ZSM-5 zeolites. A persistent green color can be seen after laser photolysis at room temperature of 1BP/M₆[Al₆Si₉₀O₁₉₂] (M = Na⁺, K⁺), namely of zeolites with higher aluminum content. The long lifetimes of BP⁺ in M₆[Al₆Si₉₀O₁₉₂] permit the use of

conventional spectroscopic techniques with better resolution and sensitivity to monitor the restoration of the BP(S₀) state after photolysis.

The data processing^[10] of the diffuse reflectance results (Figure 3) provides evidence of the generation of two species by the photolysis of 1BP/M₆[Al₆Si₉₀O₁₉₂]: BP⁺ and a trapped electron. The absorption bands around 460 nm were straightforwardly assigned to the trapped electron, while the bands at 370 and 670 nm correspond to BP⁺.^[7, 12] The lifetimes of BP⁺ and the trapped electron in 1BP/Na₆[Al₆Si₉₀O₁₉₂] (450, 480 nm) were found to be 17 and 155 min, respectively, while their lifetimes in 1BP/K₆[Al₆Si₉₀O₁₉₂] (448, 462 nm) were found to be 4 and 44 min, respectively. The UV/Vis spectra recorded two minutes after the photolysis of 1BP/Cs₆[Al₆Si₉₀O₁₉₂] exhibits only the absorption bands at 470 and 510 nm, which correspond to the trapped electron. The lifetime of BP⁺, which is assumed to be less than 2 min, appears markedly shorter than the trapped electron in 1BP/Cs₆[Al₆Si₉₀O₁₉₂] (131 min).^[12] Positive holes in the zeolite framework are

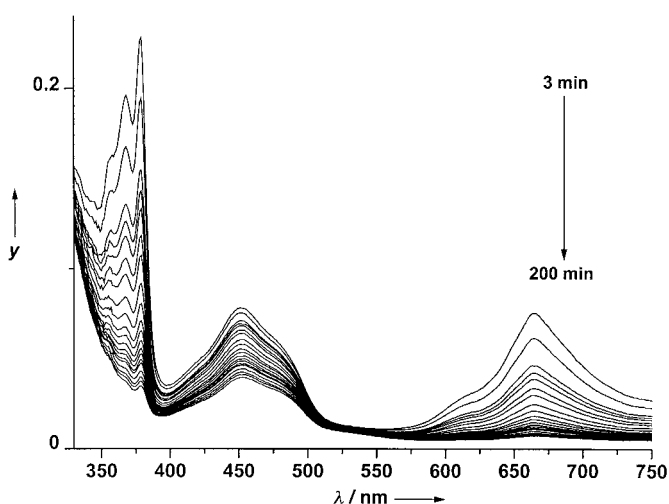


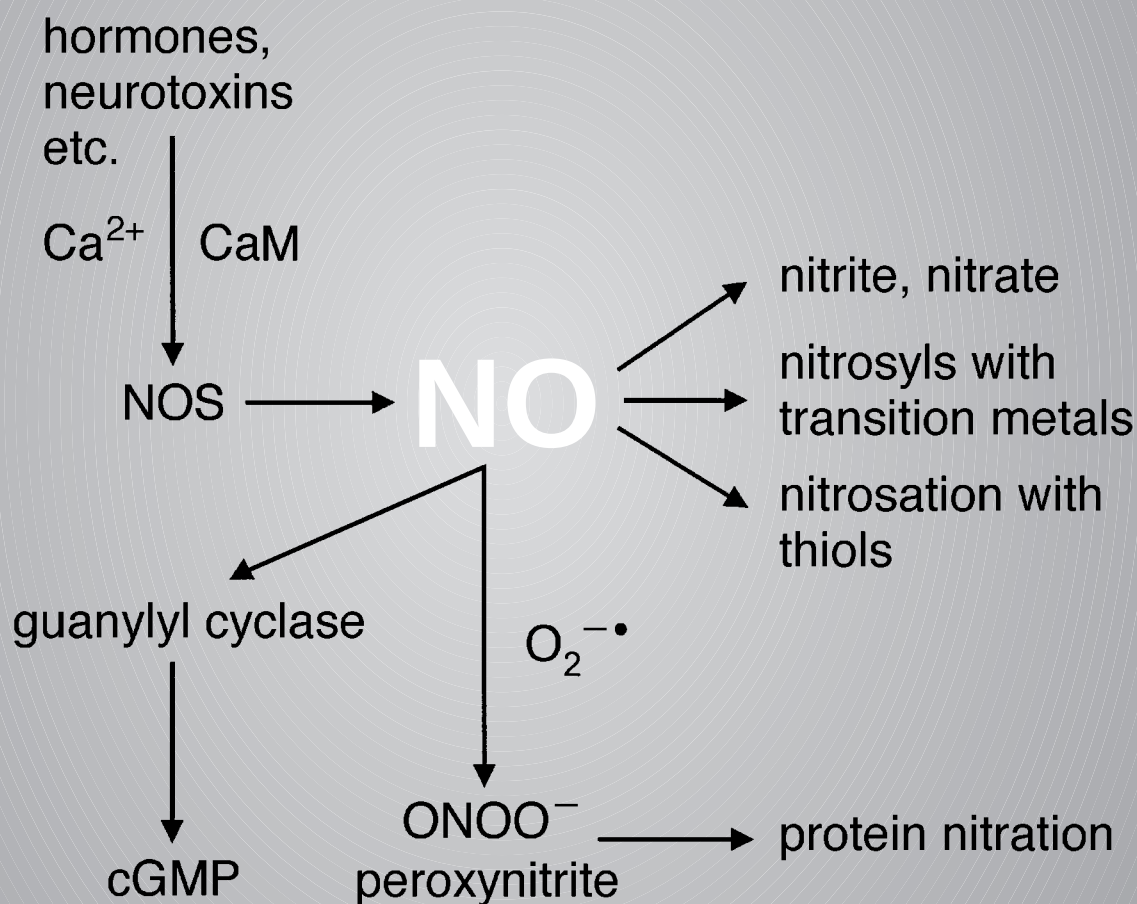
Figure 3. Diffuse reflectance UV/Vis absorption spectra of occluded photoexcited biphenyl: 1BP/Na₆Si₉₀Al₆O₁₉₂ (photolysis 248 nm, 5 s, 30 mJ cm⁻²). The spectra were recorded at room temperature from 3 to 200 min after the photolysis. y = reflectance in Kubelka-Munk units.

assumed to counterbalance the negative charge of the trapped electron. The cosorption of O₂ in the 1BP/Na₆[Al₆Si₉₀O₁₉₂] sample does not change the lifetimes of either BP⁺ or the trapped electron significantly. So, the expected formation of O₂⁻ does not occur and the electron trapping site is probably not accessible to O₂.

The EPR spectrum (77 K, Figure 4a) recorded immediately after irradiation of the 1BP/Na₆[Al₆Si₉₀O₁₉₂] sample at room temperature under argon consists of a complicated pattern containing the signals of three paramagnetic species: BP⁺, which corresponds to the seven resolved lines,^[17] a trapped electron, and a positive hole. After annealing the sample at room temperature the well resolved lines evolve to the broader remaining signals (77 K) that correspond to both the remaining trapped electron and the positive hole (Figure 4b). The seven resolved lines evolve faster for the irradiated 1BP/K₆[Al₆Si₉₀O₁₉₂] sample, whereas they are practically absent in

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Formation and fate of nitric oxide



Discovery of Some of the Biological Effects of Nitric Oxide and Its Role in Cell Signaling (Nobel Lecture)**

Ferid Murad*

The role of nitric oxide in cellular signaling in the past 22 years has become one of the most rapidly growing areas in biology with more than 20000 publications to date. Nitric oxide is a gas and free radical with an unshared electron that can regulate an ever-growing list of biological processes. In many instances nitric oxide mediates its biological effects by activating guanylyl cyclase and increasing cyclic GMP synthesis from GTP. However, the list of effects of nitric oxide that are independent of cyclic GMP is also growing at a rapid rate. For example, nitric oxide can interact with transition metals such as iron, thiol groups, other free radicals, oxygen, superoxide anion, unsaturated fatty acids, and other molecules. Some of these reactions result in the oxidation

of nitric oxide to nitrite and nitrate to terminate its effect, while other reactions can lead to altered protein structure, function, and/or catalytic capacity. These diverse effects of nitric oxide that are either cyclic GMP dependent or independent can alter and regulate important physiological and biochemical events in cell regulation and function. Nitric oxide can function as an intracellular messenger, an autacoid, a paracrine substance, a neurotransmitter, or a hormone that can be carried to distant sites for effects. Thus, it is a unique simple molecule with an array of signaling functions. However, as with any messenger molecule, there can be too little or too much of the substance, and pathological events result. Some of the methods to regulate either nitric oxide formation, metabo-

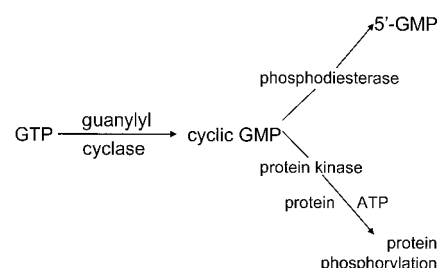
lism, or function have been in clinical use for more than a century, as with the use of organic nitrates and nitroglycerin in angina pectoris that was initiated in the 1870s. Current and future research with nitric oxide and cyclic GMP will undoubtedly expand the clinicians' therapeutic armamentarium to manage a number of important diseases by perturbing nitric oxide and cyclic GMP formation and metabolism. Such promise and expectations have obviously fueled the interests in these signaling molecules for a growing list of potential therapeutic applications.

Keywords: enzymes • nitric oxide • Nobel lecture • nucleotides • signal transduction

Introduction

As a combined degree student in the laboratories of Earl Sutherland and Theodore Rall shortly after the discovery of cyclic AMP, I was taught by them to enjoy science and I quickly became convinced of the important role of second messengers in hormonal signaling systems. After additional clinical training, I went to the National Institutes of Health (NIH) in 1967 to work with Martha Vaughan, and cyclic GMP began to emerge as another potential second messenger.

Sutherland's laboratory and Aurbach's laboratory independently described the enzyme guanylyl cyclase that catalyzed the formation of cyclic GMP from GTP.^[1, 2] Also, a new isoform of cyclic nucleotide phosphodiesterase that preferred to hydrolyze and inactivate cyclic GMP^[3] and a subsequent novel cyclic GMP-dependent protein kinase, presumably its molecular target,^[4] were described (Scheme 1).



Scheme 1. Enzymes involved in cyclic GMP synthesis, metabolism, and function.

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The cyclic AMP area was becoming very popular and crowded due to new and simpler assay systems and more and more hormones mediating their effects through this intracellular messenger. Thus, cyclic GMP became more appealing to me as I planned to launch an independent academic career when I moved to the University of Virginia in 1970. In our earliest studies, we did numerous descriptive experiments where we added one agent or another to tissues or cells to correlate cyclic GMP accumulation with the function of the preparation. While these early studies seemed necessary at this time and were productive, we gained little insight into the true regulation of cyclic GMP synthesis and its functions. We wanted to address two simple, and obviously, naïve questions: 1) how do hormones, neurotransmitters, and various ligands regulate guanylyl cyclase activity and cyclic GMP synthesis (i.e., what are the molecular coupling events between hormone binding to its appropriate receptor and guanylyl cyclase activation)? and 2) what are some biological functions resulting from increased cyclic GMP levels?

Possible Isoforms of Guanylyl Cyclase

After two to three years of what some of my trainees called “dumping experiments”, where we added various hormones and agents to preparations and measured cyclic GMP accumulation, we decided that there must be a better fundamental approach to our questions. About 1973 Hiroshi Kimura, a fellow with me, and I turned our attention to an examination of guanylyl cyclase in cell-free systems. We quickly learned that there was enzyme activity in both high-speed supernatant and particulate fractions of most tissue homogenates. Furthermore, the activities were quite different (Table 1).^[5–7] While ATP inhibited both activities, the soluble activity was more sensitive to ATP inhibition. Calcium ion could either activate or inhibit activity dependent upon its concentration, but the soluble and particulate activities had different sensitivities to these effects. The most striking difference was that the soluble activity gave linear kinetics (double reciprocal plots) with regard to the substrate GTP, while the particulate activity gave curvilinear double reciprocal plots indicating cooperativity with respect to

Table 1. Guanylyl cyclase in cytosolic and particulate fractions of homogenates with different properties.

	Soluble	Particulate
Ca ²⁺	stimulates	inhibits
ATP, IC ₅₀	0.4 mM	> 1 mM
GTP, <i>h</i> ^[a]	1.0	1.74
detergent	stimulates 50–100 %	stimulates > 300 %

[a] *h* = Hill coefficient.

GTP, with presumably multiple GTP binding sites. The Hill coefficient of the soluble enzyme was 1.0, while the particulate activity had a Hill coefficient of about 1.7.^[5–7]

From our subsequent work with the characterization and purification of the enzyme and the cloning of the cDNAs for guanylyl cyclase we were able to demonstrate definitively that several isoforms and separate gene products were present in tissues.^[8–11] Similar studies have since been done by other laboratories, most notably in the laboratory of David Garbers.^[12, 13] Today we know that numerous soluble and particulate isoforms can exist. This topic is beyond the scope of this review, and interested readers are referred to other reviews.^[8–13]

Effects of Azide, Hydroxylamine, and Nitrite

Our initial reaction to these early data was that we were dealing with two different isoforms of guanylyl cyclase, one in the cytosolic compartment and one in the membranous compartment or organelles. However, we could not exclude that such differences in the activities were due to artifacts and spurious results with crude high-speed soluble and particulate preparations. We thought that contaminating phosphatases, nucleotidases, and perhaps cyclic nucleotide phosphodiesterases might also explain our observations. At the time we had no desire or plans to purify the protein(s). A simpler approach was to add azide, pyrophosphate, hydroxylamine, phosphodiesterase inhibitors, fluoride, etc. individually or in combination to preserve the substrate or product and recharacterize the crude soluble and particulate guanylyl cyclase. Quite surprisingly, we found that azide, hydroxylamine, and nitrite activated many, but not all preparations of guanylyl cy-



Ferid Murad was born in 1936 in Whiting, Indiana. He received his BA degree from DePauw University (Greencastle, Indiana) in 1958 and his MD and PhD degrees from Western Reserve University (Cleveland, Ohio) in 1965. After two years of clinical training at Massachusetts General Hospital and three years at the National Institutes of Health, he became an Associate Professor in the Departments of Medicine and Pharmacology at the University of Virginia in 1970, where he initiated his studies with cyclic GMP and nitric oxide. In 1981 he was appointed Professor of the Departments of Medicine and Pharmacology at Stanford University and Chief of Medicine of the Palo Alto Veterans Medical Center. In 1988 he became a Vice President of Abbott Laboratories. He joined the University of Texas-Houston as Professor and Chair of the Department of Integrative Biology and Pharmacology in 1997. He has received many awards including the Ciba Award in 1988, the Lasker Award in Basic Medical Research in 1996, and the Nobel Prize in Physiology or Medicine in 1998. He is a member of many societies including the National Academy of Sciences and the Institute of Medicine.

clase.^[14–17] This was an exciting serendipitous observation for us, because any hormones or ligands that increased cyclic GMP accumulation in intact cells failed to increase cyclic GMP synthesis in cell-free preparations. We, therefore, thought that hormone–receptor coupling to guanylyl cyclase activation would be a complicated molecular event that was disrupted in cell-free preparations.

In order to establish the molecular events between hormone binding and guanylyl cyclase activation, it was necessary to have a hormonally responsive cell-free system and ultimately purified and reconstituted components of the signaling cascade. We thought that an understanding of the activation of guanylyl cyclase with azide, hydroxylamine, and nitrite could assist us in unraveling the complex signaling cascade, perhaps analogous to the stimulatory effects of fluoride that assisted with the characterization of the adenylyl cyclase system. This intuition was correct in ultimately leading us to the mechanism of hormonal activation of guanylyl cyclase as summarized below. Thus, we made a major commitment to understand the mechanisms of guanylyl cyclase activation by these agents.

Discovery of Proteins Required for Azide Activation and Inhibition

The activation by azide was tissue specific and had a brief time lag of several minutes before the time course became linear.^[14–17] Furthermore, the activation was absent in an anaerobic environment requiring air or oxygen and could be enhanced with the addition of thiols.^[14–17] These results suggested to us that azide might be converted into another compound that served as the activating species. We then did a simple and classical biochemical experiment where we mixed crude soluble cell extracts that were activated by azide with crude soluble extracts that were not activated.

In our first experiment (Table 2) we mixed crude supernatant fractions of rat liver that could be activated with azide

Table 2. Effects of sodium azide (NaN_3) on soluble guanylyl cyclase from rat liver, heart, and cerebral cortex (reprinted from references [14, 15] with permission).

Enzyme	Cyclic GMP formed ^[a]		+ NaN_3 /– NaN_3
	– NaN_3	+ NaN_3	
liver	38.8	595.4	15.3
heart	23.0	23.2	1.0
cerebral cortex	46.0	42.0	0.9
liver + heart	27.3	23.1	0.8
liver + cerebral cortex	23.0	899.0	39.1

[a] Given in pmol per mg protein per min.

with fractions of rat brain and rat heart, tissues without azide effects on guanylyl cyclase.^[14–17] Mixing extracts of liver with cerebral cortex resulted in an increased azide effect, while mixing liver extracts with heart extracts resulted in a loss of the azide effect. Our interpretation of these data was that azide activation required the presence of other factors present in liver extracts that when added to cerebral cortex permitted

azide to activate the enzyme from both tissue extracts. Furthermore, heart extracts possessed a factor or several factors that blocked azide activation in the heart extracts and in liver extracts in the mixing experiments. Subsequent experiments proved these hypotheses to be true. The apparent activating factor(s) in liver and the inhibitor(s) in heart were nondialyzable and heat labile. Thus, we began to purify and characterize these activators and inhibitors.^[14–21]

We thought that if we thoroughly understood the mechanism of azide activation of guanylyl cyclase, perhaps some day we could reconstitute the appropriate components and factors to observe hormonal activation of guanylyl cyclase in cell-free preparations, a major goal of the laboratory. This was a major research commitment that, while risky, turned out to be the correct approach and that led us to the discovery of the first biological effects of nitric oxide. Our University of Virginia colleagues and others thought we were crazy to waste our efforts in pursuing the effects of azide, a known metabolic poison that was often added to stored buffers and columns to inhibit bacterial growth.

Characterization of the Azide Activators and Inhibitors

Hiroshi Kimura, Chandra Mittal, and I purified rat liver extracts and found that the macromolecular factor required for azide activation was catalase.^[14–21] Other heme-containing proteins such as horseradish peroxidase and some of the cytochromes,^[18–21] however, could substitute for the catalase requirement. The inhibitory factors in heart extracts were also purified and were found to be hemoglobin and myoglobin.^[14–21] It rapidly became apparent that heme-containing proteins either permitted azide to activate guanylyl cyclase or blocked its activation. These stimulatory and inhibitory effects of various heme-containing proteins were important findings in that they led us to some much older literature describing the interactions of azide with catalase to generate nitric oxide.

Effects of Azide and Nitrovasodilators on Guanylyl Cyclase Activation, Cyclic GMP Accumulation in Intact Cells, and Smooth Muscle Relaxation

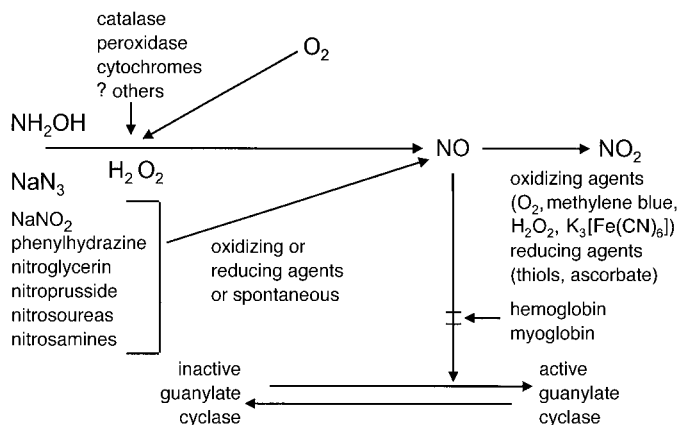
Azide, hydroxylamine, and nitrite not only activated guanylyl cyclase in cell-free extracts, but also increased cyclic GMP accumulation in numerous intact tissues and cell types including brain, liver, and some cell culture models.^[14–21] One of the new fellows in my laboratory, Shoji Katsuki, was working with bovine tracheal smooth muscle preparations that we developed to examine the role of cyclic AMP and cyclic GMP in smooth muscle motility. We developed a rather homogenous smooth muscle preparation (about 90 % smooth muscle) with a sufficient amount of tissue that permitted us to monitor motility in an organ bath after adding various agents. After quick freeze-clamping of tissue segments in liquid nitrogen, we could also measure cyclic nucleotide levels or

evaluate adenylyl or guanylyl cyclase activities.^[22–25] It was obvious to us that we should also add these new guanylyl cyclase activators to bovine tracheal smooth muscle segments and evaluate motility and cyclic GMP accumulation. We found that these agents, as with other tissues, increased cyclic GMP levels as expected and simultaneously caused relaxation in precontracted muscles.^[22–25] Similar results were observed when we examined clean gastrointestinal smooth muscle segments.^[22–25] We intentionally avoided vascular segments due to the heterogeneity of the preparations with endothelial cells, blood cells, fibroblasts, etc. We felt that we could not determine in what cell types the cyclic GMP accumulation would occur in due to the marked cellular heterogeneity. In my previous studies with rat fat pads in Martha Vaughan's lab we had great difficulty correlating the effects of some known lipolytic agents with cyclic AMP accumulation due to the cellular heterogeneity of our preparations. The studies only become meaningful and helpful when we examined cyclic AMP accumulation in homogeneous isolated fat cells.^[26–28]

After finding that these agents relaxed tracheal and gastrointestinal smooth muscle and that the increase in cyclic GMP was coincident with or preceded relaxation, we began to examine other smooth muscle relaxants such as nitroglycerin, nitroprusside, hydrazines, etc. (Scheme 2).^[19–25] All of these

Discovery of the Biological Effects of Nitric Oxide

The effects of the growing list of nitrovasodilators with nitro or nitroso functionalities and the effects of heme-containing macromolecules as factors for either activation or inhibition of these agents suggested to us that perhaps the active intermediate or proximal activator of guanylyl cyclase could be nitric oxide or one of its oxidized or reduced products. This was indeed the case.^[20–25, 30] The formation of nitric oxide from various nitrovasodilator precursor molecules or “prodrugs” could be influenced by a variety of conditions as noted in Scheme 2. Furthermore, the activity of nitric oxide could also be diminished by a variety of oxidants such as methylene blue, $K_3[Fe(CN)_6]$, excess oxygen, etc. as well as sinks to trap nitric oxide such as hemoglobin and myoglobin (Scheme 2). Delightfully, our first chemical generator to produce nitric oxide and ventilate the gas into guanylyl cyclase incubations was a success (Figure 1).^[22–25, 30] The experiment was conducted by



Scheme 2. Effects of some nitrovasodilators on cyclic GMP synthesis (reprinted with permission from references [19–21]).

agents increased cyclic GMP levels in several smooth muscle preparations as well as other tissues, caused smooth muscle relaxation, and activated guanylyl cyclase in most cell-free tissue extracts. We began to call these new guanylyl cyclase activators “nitrovasodilators”, a simplified “lab jargon” that permitted us to communicate more readily. While these new nitrovasodilators did not require catalase to activate guanylyl cyclase preparations, these effects were inhibited by hemoglobin and myoglobin. The effects of altered redox conditions, thiols, and heme-containing proteins with this growing list of nitrovasodilators were to become very important for us later in determining the effects of endothelial-derived relaxant factor (EDRF) on cyclic GMP accumulation in vascular preparations subsequent to Furchgott's discovery of EDRF in 1980.^[29]

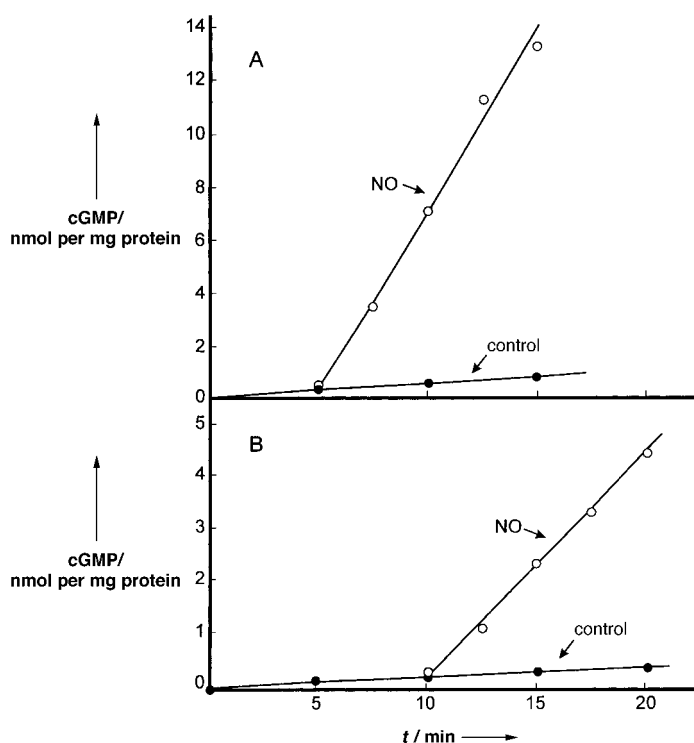


Figure 1. The first demonstration of the biological effect of nitric oxide to activate guanylyl cyclase preparations from rat lung (A) and bovine tracheal smooth muscle (B) (reprinted with permission from reference [22]).

Shuji Katsuki and William Arnold, post-doctoral fellows in my laboratory in 1976, the night before Katsuki was to complete his fellowship and return with his family to Japan. I have often thought that had Katsuki completed his fellowship earlier, our discovery of the first biological effects of nitric oxide could have been delayed considerably. Nitric oxide, unlike some other activators and nitrovasodilators, increased the activity of most guanylyl cyclase preparations and increased cyclic GMP levels in virtually all tissues tested with few exceptions (Table 3). Furthermore, the stimulatory effects of nitric oxide and various nitrovasodilators on cyclic GMP

Table 3. Effect of nitric oxide (NO) on the activity of guanylyl cyclase from various tissues.

Tissue	Frac- tion ^[a]	Cyclic GMP formed ^[b]		+ NO/ – NO
		– NO	+ NO	
rat liver	S	22.1	674.2	30.5
	P	12.4	37.3	3.9
bovine lung	S	111.7	3625.5	32.5
bovine tracheal smooth muscle	S	8.9	297.1	33.4
	P	27.0	35.3	1.3
rat heart	S	10.5	242.8	23.1
rat kidney	S	51.6	975.9	18.9
rat cerebral cortex	S	55.7	1122.6	20.2
	P	14.2	209.1	14.7
rat cerebellum	S	23.7	784.4	33.1
	P	20.6	201.2	9.8
rat skeletal muscle	S	6.1	84.0	13.8
rat spleen	S	73.5	381.7	5.2
rat small intestinal muscle	S	42.9	250.6	5.8
rat adrenal	S	37.5	394.2	10.5
rat epididymal fat	S	9.4	108.5	11.5
rat liver	D	296.2	1406.3	4.7
rat cerebral cortex	D	57.2	174.3	3.0
bovine lung	G	61.4	1672.1	27.2
rat heart	G	30.2	171.9	5.7

[a] S = soluble fraction; P = particulate fraction; D = after chromatography on DEAE-cellulose; G = after chromatography on Sephadex G-100. [b] Given in pmol per mg protein per min.

synthesis were not additive, suggesting that they all shared a similar mechanism of activation of guanylyl cyclase.^[30]

Since this was the first and only example of a free radical activating an enzyme, we committed ourselves to purifying the isoforms of guanylyl cyclase to homogeneity in order to reexamine the effects of nitric oxide. We could not exclude the possibility that nitric oxide was being converted into yet some other activating species or that it was not inhibiting an inhibitor of the enzyme in our crude preparations. The inhibition of a guanylyl cyclase inhibitor could appear in our assays as a possible activation due to disinhibition.

After purification of the soluble guanylyl cyclase to homogeneity, nitric oxide activation was still observed.^[21, 31] In fact, the apparent K_{act} for nitric oxide continued to decrease as we purified the enzyme. Purified soluble guanylyl cyclase has a K_{act} or EC50 for nitric oxide in the order of 1 to 10 nM, depending upon the conditions of the assay and the presence of other materials, such as thiols, proteins, sugars, lipids, etc., that can form complexes with nitric oxide and alter its effective concentration to activate guanylyl cyclase.^[31] These later agents can act as traps or scavengers to form nitro or nitroso adducts and complexes to increase the K_{act} and EC50 values. In some cases these complexes can themselves also become nitrovasodilators or nitric oxide prodrugs to rerelease nitric oxide under favorable conditions.

Is Nitric Oxide a Natural Endogenous Guanylyl Cyclase Activator?

We proposed in 1978 that nitric oxide formation from some endogenous precursor could perhaps explain hormone, autacoid, and neurotransmitter effects on cyclic GMP synthesis in intact cells and tissue.^[1921] We thought that perhaps appro-

priate hormone treatment could influence the redox state of cells, the formation of nitric oxide from an endogenous precursor or the metabolism of nitric oxide. We also thought that nitric oxide could be an intracellular second messenger to mediate the effects of hormones. Thus, it could perhaps join the short but growing list of second messenger molecules that included cyclic AMP, cyclic GMP, Ca^{2+} , eicosanoids, lipids, proteins, etc. Again our colleagues thought we were crazy to believe that a reactive free radical could activate an enzyme and function as a second messenger. After all, nitric oxide was a known pollutant from the combustion of numerous substances that inactivated or destroyed various macromolecules and depleted the ozone layer. It surely could not be a second messenger signaling molecule, we were told by the skeptics.

In order to prove this hypothesis it would require a major technical advance. If, indeed, nitric oxide was an endogenous second messenger and regulator of guanylyl cyclase, one would expect that tissue levels would be about 1 to 10 nM (levels near the K_{act} or EC50 values) in order to be physiologically relevant and permit the fluctuations in nitric oxide concentrations to control enzyme activity. If the concentrations in tissue were much higher, one would expect the enzyme to always be activated and the physiological significance of nitric oxide as a regulator would be less convincing and meaningful. Unfortunately, the assays for nitric oxide and its oxidation products (nitrite and nitrate) were crude colorimetric and spectrophotometric assays that were several orders of magnitude less sensitive than needed (about millimolar to micromolar). Thus, our hypothesis remained untestable for several years until we and others developed more specific and sensitive assays for the free radical.

Discovery of EDRF

In 1980 Furchgott presented a seminar in our Pharmacology Department while I was at the University of Virginia. He was excited about his recent discovery of endothelial-derived relaxing factor (EDRF) and the ability of endothelial cells to generate and release a labile substance that produced relaxation of the underlying smooth muscle in vascular segments.^[29] The effects of EDRF shared many of the features and properties of nitrovasodilators, and I suggested to him that perhaps increased cyclic GMP levels could explain his observations with light-induced and endothelium-induced relaxation. We even planned a collaboration to test this hypothesis which never materialized because of our subsequent move to Stanford University in 1981.

At the time, Robert Rapoport was one of my new fellows working with tracheal and vascular smooth muscle preparations and impatiently waiting for the collaboration to begin. After a brief visit to our new laboratory by the late Michael Peach in 1982 and his queries about our interests in EDRF, we then proceeded to demonstrate independently that EDRF did, indeed, increase cyclic GMP synthesis in the smooth muscle compartment of rat aorta segments (Figure 2).^[32–38] We observed similar effects of EDRF on cyclic GMP formation with a variety of endothelium-dependent vasodilators such as

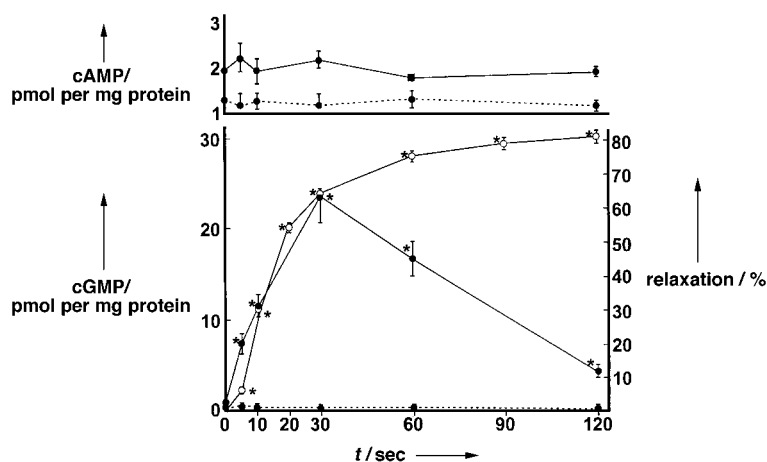


Figure 2. Effects of acetylcholine on cyclic GMP and cyclic AMP accumulation (●) and relaxation (○) of rat aorta segments with (—) and without (---) endothelium (reprinted with permission from references [32, 33]).

acetylcholine, A23187, thrombin, ATP, bradykinin, etc.^[32–38] We continued the work to show that EDRF formation as well as nitrovasodilators also increased cyclic GMP dependent protein kinase activity and altered protein phosphorylation of many endogenous smooth muscle proteins including the dephosphorylation of myosin light chain.^[32–38]

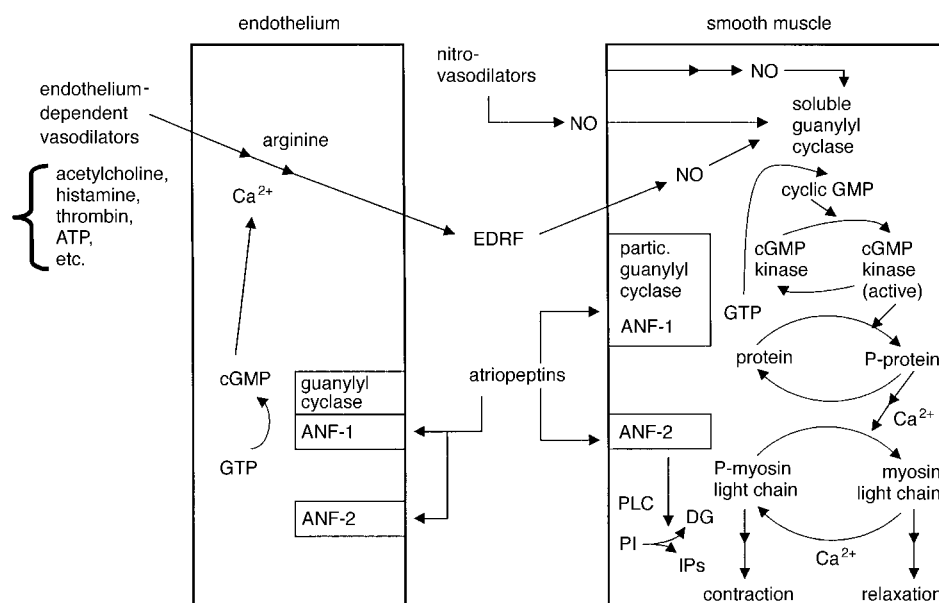
Cyclic GMP accumulation with nitrovasodilators or endothelium-dependent vasodilators produced similar effects on cyclic GMP accumulation, cyclic GMP dependent protein kinase activation and altered $^{32}\text{PO}_4$ incorporation in the same family of proteins isolated with two-dimensional polyacrylamide gel electrophoresis.^[32–38] Subsequently, we and others found that cyclic GMP decreased phosphoinositide metabolism and formation of inositol phosphates, including inositol trisphosphate, by decreasing phospholipase C activity.^[39] These and other effects of cyclic GMP would be expected to decrease cytosolic free calcium and the activity of myosin light chain kinase, a calcium/calmodulin-dependent enzyme. The effect of cyclic GMP elevating agents to decrease cytosolic free calcium levels in smooth muscle and other tissues has been subsequently shown by many laboratories.

Because of the biochemical similarities of the effects of nitrovasodilators and EDRF-producing agents, we came to view EDRF as the “endogenous nitrate or endogenous nitrovasodilator”.^[40] About six months after this review^[40] was published, Furchgott and Ignarro independently proposed that EDRF is nitric oxide at a meeting at the Mayo Clinic in the summer of 1986. I previously and continually have argued with them and others

that EDRF is probably a family of nitric oxide adducts or complexes as well as nitric oxide. This controversy remains unresolved even today in that many nitro and nitroso complexes can meet the definition of EDRF as originally defined by Furchgott.^[29] These complexes are readily formed between free nitric oxide in cells and the interstitial space with various cellular constituents such as thiols, lipids, proteins, etc. Presumably, many of these substances can readily dissociate their nitric oxide in the correct environment to account for the free nitric oxide found in some vascular preparations. Unfortunately, this pressing issue may not be resolved in the near future due to the low concentration of EDRF expected. As noted above, nitric oxide at concentrations of 1 to 10 mM are sufficient to activate guanylyl cyclase and permit the signaling event. Clearly endothelial cells and many other cells produce nitric oxide, and the activator of the

smooth muscle guanylyl cyclase is nitric oxide. The question outstanding is: what are the intervening or intermediate molecules presumably complexed with nitric oxide that Furchgott has called EDRF?

We and a number of laboratories showed that the effects of EDRF could be blocked with methylene blue, hemoglobin, and other inhibitors of nitrovasodilator activation of guanylyl cyclase (Scheme 2). The effects of nitric oxide or EDRF can also be mimicked by atriopeptins which selectively activate particulate guanylyl cyclase (Scheme 3).^[41, 42] In fact, we showed that atriopeptin receptors were heterogeneous with two receptor subclasses (ANF-R1 and ANF-R2).^[43, 44] One of the atriopeptin receptors (ANF-R1) is the extracellular domain of the transmembrane particulate guanylyl cyclase.^[45] This work has been elegantly confirmed by the cDNA cloning studies from Garbers' laboratory.^[46]



Scheme 3. Effects of endothelium-dependent vasodilators, nitrovasodilators, and atriopeptins in rat aorta segments. PLC = phospholipase C, PI = phosphoinositides, DG = diacylglycerol, IP3 = inositol phosphates (reprinted with permission from references [40, 50]).

Effects of L-Arginine on Guanylyl Cyclase Activation and Nitrite and Nitrate Formation

Degucci's laboratory was also one of the earlier few laboratories interested in guanylyl cyclase. In 1982, Degucci's laboratory reported that brain and/or neuroblastoma extracts possessed an endogenous substance that activated crude or partially purified preparations of soluble guanylyl cyclase.^[47] Furthermore, this substance was identified as L-arginine. Activation by L-arginine was blocked by hemoglobin and methylhydroxylamine, which blocked the effects of nitrovasodilators. We confirmed their observations with crude or partially purified preparations of guanylyl cyclase, but found that L-arginine failed to block our highly purified guanylyl cyclase preparations.^[68] These observations were the first to report arginine activation of guanylyl cyclase with effects similar to those of nitric oxide and nitrovasodilators. Unfortunately, neither they nor we pursued these arginine effects at the time, which could have led to the earlier discovery of nitric oxide synthase.

Subsequently Hibbs' laboratory in 1987 found that the cytotoxic effects of macrophages on tumor cells in culture correlated with the accumulation of nitrite and nitrate in the conditioned media.^[48] The cytotoxic effects and accumulation of nitrite and nitrate were increased with L-arginine and blocked with L-arginine analogues such as *N*-methyl-L-arginine, an agent that later proved to be an effective competitive inhibitor of nitric oxide synthase.

The findings of Degucci and Hibbs were important, timely, and critical observations that subsequently permitted our laboratory and many other laboratories to move forward rapidly in understanding the role of nitric oxide in cellular signaling.^[49–52] Unfortunately, their laboratories have not received sufficient recognition for these important observations.

Characterization of Nitric Oxide Synthase and Its Isoforms

Within several years many laboratories including our own began to characterize, purify, and clone this novel synthetic pathway that converted L-arginine into nitric oxide and citrulline. Initially the most active and productive laboratories included those of Marletta and Stuehr, Palmer and Moncada, Bredt and Snyder, Stuehr and Nathans, and ourselves (see references [9–11] and references therein).

The first nitric oxide synthase (NOS) isoform to be purified was the neuronal or brain NOS or NOS-1, also called Type 1 NOS or constitutive NOS. The first investigators to purify and characterize NOS-1 were Bredt and Snyder^[53] and our own laboratory.^[54–57] This was followed shortly thereafter by NOS-2 or inducible NOS, also called Type II NOS, by Stuehr and Nathans,^[58] and then NOS-3 or endothelial NOS, also called Type III NOS, in our lab.^[59–62] Monoclonal and polyclonal antibodies to the purified isoforms and synthetic peptide fragments were produced as were the cDNA clones for these three gene products (see references [9–11] and references

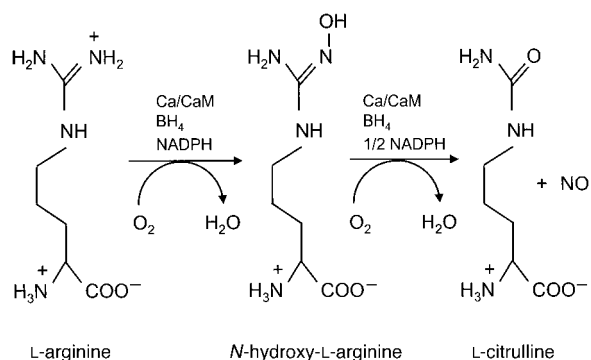
therein). The chromosomal location for each isoform has been identified as have the numerous cosubstrates, cofactors, and prosthetic groups (O_2 , NADPH, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), tetrahydrobiopterin, and heme; see reference [11] and references therein). All three isoforms are regulated by calmodulin, but only NOS-1 and NOS-3 have apparent and obvious regulation with altered cytosolic free calcium concentrations. NOS-2 contains calmodulin as a tightly bound component or subunit after translation and assembly such that dependency upon cytosolic free calcium levels is not demonstrable. The catalytically active isoforms exist as homodimers with a heme prosthetic group serving to facilitate dimer formation. The carboxy terminal domain has considerable homology between the isoforms, and is homologous to cytochrome P450. The amino terminal domain has less homology and may serve to bind to regulatory proteins and/or chaperones for its subcellular compartmentation. The homology of the three isoforms is only about 50 to 60%, while the homology of a given isoform between species can be as great as 85 to 92%. Some of the properties of the NOS isoforms are summarized in Table 4.

Table 4. Isoforms of nitric oxide synthase (NOS).^[a]

NOS-1 (155 kDa)	neuronal, brain, Type I-NOS; central and peripheral neurons, NANC neurons, islets, endometrium, skeletal muscle, etc.
NOS-2 (125 kDa)	inducible, Type II-NOS; macrophage, liver, smooth muscle, endothelium, heart, etc.; effects of LPS, cytokines and glucocorticoids
NOS-3 (135 kDa)	endothelial, Type III-NOS; endothelium, brain, heart, etc.; acylation, phosphorylation

[a] LPS = lipopolysaccharide; NANC = nonadrenergic-noncholinergic.

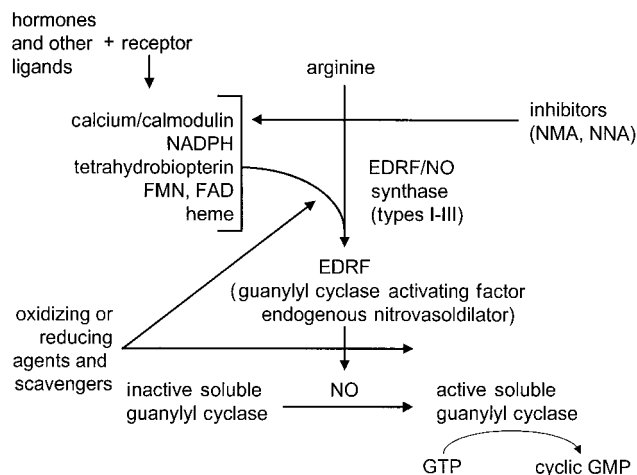
NOS converts L-arginine into L-hydroxyarginine and subsequently into nitric oxide and citrulline as summarized in Scheme 4. The transfer of electrons for the oxidation of the guanidino nitrogen atom of L-arginine and the precise role of each cofactor remains unresolved. This continues as an active area of investigation in many laboratories. Recent X-ray diffraction studies with crystals of some NOS isoform fragments are currently underway in several laboratories. These studies should shed some light upon the precise roles of the cofactors and prosthetic groups. These studies may also



Scheme 4. The nitric oxide synthetic pathway. BH_4 = tetrahydrobiopterin; Ca/CaM = calcium/calmodulin.

permit the development of highly selective and effective inhibitors of each of the NOS isoforms in order to determine other important biological roles of nitric oxide and develop new and important therapeutic agents (see below).

Presumably the *in situ* activity of NOS can be regulated by the availability of substrate, cofactors, and prosthetic groups. However, to date most of the regulation of NOS-1 and NOS-3 has focused on the alterations of cytosolic free calcium caused by a myriad of hormones, autacoids, neurotransmitters, etc. (Scheme 5).



Scheme 5. Hormonal regulators of nitric oxide and cyclic GMP formation. NMA = *N*-methyl-L-arginine, NNA = *N*-nitro-L-arginine.

NOS-2 does not appear to be present under normal conditions in most cells. However, upon exposure to endotoxin (LPS) interferon γ , IL 1, TNF α , and other proinflammatory cytokines, the levels of mRNA, protein, and catalytic activity begin to increase within one hour and reach maximal levels in 6 to 18 hours. Various antiinflammatory cytokines and glucocorticoids can decrease the induction of NOS-2. It is thought that some of the pharmacological and biological effects of these factors may be explained by increased or decreased nitric oxide formation. For example, the hypotension observed in septic shock as well as the ensuing "multiple organ failure syndrome" (MOF) may be due to excessive nitric oxide formation from NOS-2 induction.

Translational and Posttranslational Modifications of NOS

All of the isoforms can probably be phosphorylated by a variety of protein kinases including cyclic AMP dependent protein kinase, cyclic GMP dependent protein kinase, protein kinase C, and Ca^{2+} /calmodulin-dependent protein kinase.^[63] With phosphorylation, the activity of the enzyme can be increased or decreased. However, the physiological relevance of phosphorylation to regulate enzyme activity or enzyme translocation from one cellular compartment to another is unknown (see references [9–11] and references therein). In

some cell-free systems with purified NOS-1, as many as 6 to 7 moles of phosphate can be incorporated into one monomer of protein.^[63] Studies with intact cells and tissues with permeable and specific protein kinase inhibitors are required to determine the physiological relevance of NOS phosphorylation with regard to catalytic activity, enzyme compartmentation, enzyme regulation, and participation in various biological processes. Unfortunately, selective and permeable protein kinase inhibitors are not widely available.

NOS-3 is also myristoylated and palmitoylated, and acylation probably participates in the location of this isoform in the plasma membrane and/or other organelles.^[61, 64–66] Recently, Michels' laboratory has described the association of NOS-3 with the caveolae of the plasma membrane.^[65, 66] These structures also contain many other proteins participating in cell signaling events. Mechanisms to regulate and chaperon NOS-3 from the Golgi to the caveolae and the recycling of NOS-3 to the cytosol and perhaps back to the plasma membrane are areas of active investigation. These processes could perhaps serve as important sites for regulation and drug targeting.

The three gene products of NOS described to date, possible alternate mRNA splicing due to multiple promoters (i.e., NOS-1),^[67] and various posttranslational modifications should permit a multitude of NOS isoforms and activities for regulation. Undoubtedly this will be a complex problem to resolve with regard to NOS function, regulation, and selective inhibition (see below). Some cells possess only one isoform while other cells have several isoforms with different subcellular locations and perhaps different functions. The precise location of pools of nitric oxide in cells and their proximity to guanylyl cyclase, thiols, fatty acids, heme-containing scavengers or sinks, other free radicals such as superoxide anions, etc. should have marked and profound effects on the biological and/or cytotoxic properties of nitric oxide. Thus, numerous questions regarding nitric oxide formation, location, and function remain to be addressed.

Inhibitors of NOS

Numerous NOS inhibitors have been described. Novel arginine-based and guanidine-based antagonists are areas of active investigation in both academic and industrial laboratories (see references [9–11] and references therein). Most inhibitors to date are competitive antagonists and may be partly selective for one or another isoform. Commercially available compounds may show as much as 100- to 200-fold selectivity for one or another isoform. Unfortunately, to date, there are no highly specific inhibitors of one or another isoform. Such agents could prove invaluable to sort out the NOS isoforms in various cell types and the biological events that they regulate. Furthermore, investigations in this area could result in highly selective and perhaps specific and efficacious therapeutic agents without numerous side effects since one or more isoforms of NOS is found in virtually all cells with few exceptions. Currently, some NOS inhibitors are

in clinical trials for patients in septic shock. Other clinical studies are under discussion and consideration.

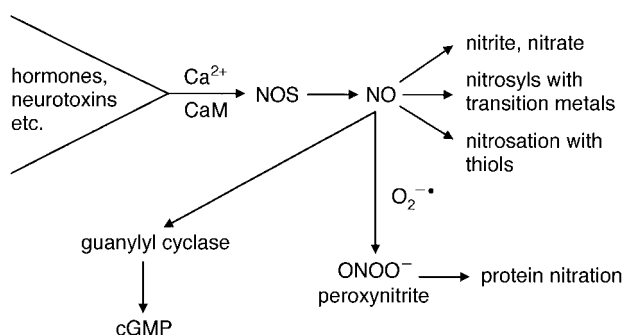
Unfortunately, the earliest clinical studies with NOS inhibitors were not sufficiently selective as expected from previous *in vitro* studies with the inhibition of NOS isoforms. Thus, the initial enthusiasm for the clinical utility of NOS inhibitors is beginning to meet with some concerns and skepticism as unwanted side effects become apparent, some of which were predictable from earlier basic biological studies. Perhaps this could serve as a lesson regarding the selection of nonspecific agents for clinical development prematurely. None the less, many believe that these molecular targets will prove to be valuable approaches to some diseases with excess nitric oxide production. However, more selective agents are necessary due to the broad distribution of the many NOS isoforms and their important and diverse roles in biological regulation.

Nitric Oxide Donors or Prodrugs in Clinical Medicine

A number of NO prodrugs have been used successfully since the use of nitroglycerin and other organic nitrates for angina pectoris more than a century ago. Nitroglycerin was discovered in 1847, and it and other organic nitrates were first used clinically in the 1870s. Ironically Alfred Nobel, who discovered how to safely formulate dynamite from nitroglycerin, was prescribed nitroglycerin for his angina pectoris late in life, but he refused to take it because of the known vascular headaches of his factory workers. Other therapeutics of the “nitrovasodilator class” include nitroprusside, organic and inorganic nitrites and nitrates, nitrosamines, hydrazines, nitrosoureas, etc. Many academic and industrial laboratories are actively synthesizing additional “NO donors” that release NO at predictable rates in specific tissues or environments or can be attached to various grafts and supports for vascular surgical procedures (see reference [11] and references therein). The controlled delivery of nitric oxide with a prodrug or device at a specific target site or vascular bed without the tolerance that is developed to nitroglycerin would be a most significant contribution to therapy of several vascular disorders and other diseases. There is currently considerable promise that such agents and delivery methods can be achieved in that the work is becoming more of a medicinal chemistry and/or engineering effort with obvious targets. An important clinical indication is reperfusion injury after surgery or instrumentation for vascular patency. There are obviously many other indications that can be listed such as vascular remodeling, tissue transplantation, cancer vascularization, etc. with the known effects of nitric oxide on platelet aggregation, vascular smooth muscle proliferation, atherogenesis, angiogenesis, cytotoxicity, etc.

One of the most rapidly advancing areas of clinical application is the inhalation of low concentrations of the NO gas (see reference [11] and references therein). Interestingly, nitric oxide at low concentrations is quite stable and minimally reactive. Its reactivity and toxicity is a second-order

reaction dependent upon the concentration of nitric oxide and its interaction with other free radicals and reactive oxygen species such as superoxide. At higher concentrations NO can interact with many transition metals, heme-containing proteins, and thiol groups, oxidize functionalities on polynucleotides (RNA and DNA) and proteins, and form strand breaks in polynucleotides (Scheme 6). There is a rapid and almost diffusion-limited interaction of nitric oxide with superoxide to form the very reactive peroxynitrite. This is becoming a very interesting area of investigation that undoubtedly relates to the toxic properties of these free radicals.



Scheme 6. Formation and fate of nitric oxide.

When low concentrations of nitric oxide are administered to premature infants with low birth weights by nasal catheters, nitric oxide has marked beneficial effects on infant mortality. The pulmonary hypertension is improved as is the pulmonary and systemic oxygenation and systemic hypertension resulting from the right-to-left shunting through the patent ductus arteriosus. There may also be beneficial effects in infants and children with congenital heart defects with right-to-left shunting and hypoxia. The beneficial effects in adult patients with various pulmonary disorders including pulmonary hypertension and/or acute respiratory distress syndrome (ARDS) are less convincing and more controversial. The controversy and variability of the response to inhaled nitric oxide

Table 5. Targets of nitric oxide and/or cyclic GMP.

guanylyl cyclase
cyclic nucleotide protein kinases
cyclic nucleotide phosphodiesterases
cyclooxygenase (COX II)
heme proteins, iron centers, and thiol groups
DNA modifications
regulation of <i>N</i> -methyl D-aspartate(NMDA)-activated glutamate receptors
phospholipase C

in adults undoubtedly relates to the etiology of their disease and the presence or absence of some “reversible components” of their pathology. Some of the molecular targets and processes regulated by NO and/ or cyclic GMP are summarized in Tables 5 and 6. These targets can obviously suggest other therapeutic uses for NOS inhibitors and NO prodrugs.

Table 6. Some processes regulated by nitric oxide and/or cyclic GMP.

relaxation of smooth, cardiac, and skeletal muscle
retinal phototransduction
intestinal secretion and ion transport
renal tubular-glomerular feedback
endothelial permeability
smooth muscle proliferation
platelet adhesion and aggregation
insulin secretion
hormone production and secretion
neurotransmission
long-term potentiation and memory
regulation of transcription
tissue injury and inflammation
pathogen cytotoxicity
tumor cytotoxicity
calcium transport and redistribution

Summary

Since our discovery of the first biological effects of nitric oxide more than two decades ago, more than 20000 publications have appeared on this topic. There have been more than 4500 to 5000 publications per year in the recent several years, and the number of publications continues to grow exponentially. It has become almost impossible for investigators to read all the abstracts of these publications, let alone the full papers. At times it can be difficult to keep up with just the titles, authors, and laboratories and remain current. This will undoubtedly test the mentorship of many of us as we train students and fellows who need exciting and novel publications and the priorities required to find positions in our competitive research society and remain funded. However, there seems to be no obvious saturation of the talent required in this exciting and growing field. Numerous important questions remain to be addressed and answered. I have attempted to raise some of these areas and questions here. What excitement it has been for those of us who joined the field early and were able to see such major advances and progress in recent years. There still remains many nuggets available in a vast gold mine of nitric oxide biology. By no means has the field been over-mined with a paucity of gems to be found.

In recent years a number of laboratories have made significant advances in nitric oxide research. Some current areas of active research are listed in Table 7. Undoubtedly, other interesting areas will also emerge and require increasing research and attention.

Table 7. Some areas of active nitric oxide research.

transcriptional regulation
overexpression and gene therapy
gene knockouts
histochemistry and hybridization
acylation, phosphorylation, etc.
cell types with NOS isoforms and functions
cyclic GMP mediated effects
noncyclic GMP mediated effects
selective/specific NOS inhibitors
novel NO donors

I would like to thank the 82 trainees to date who have shared in this work with me. I must acknowledge the major contributions chronologically of Hiroshi Kimura, Shoji Katsuki, William Arnold, Mark Braughler, John Lewicki, Scott Waldman, Robert Rapoport, Yoshinari Kamisaki, Takayashi Kuno, Masaki Nakane, Kunio Ishii, Dale Leitman, Ulrich Förstermann, Harold Schmidt, Jennifer Pollock, and many others who participated in various aspects of this work. Their collaboration, curiosity, hard work, sense of humor, and present successes have added even more to my excitement of an academic career. I wish to also thank the John S. Dunn Foundation, the University of Texas, and numerous agencies for their past grant support and in particular the National Institute of Health.

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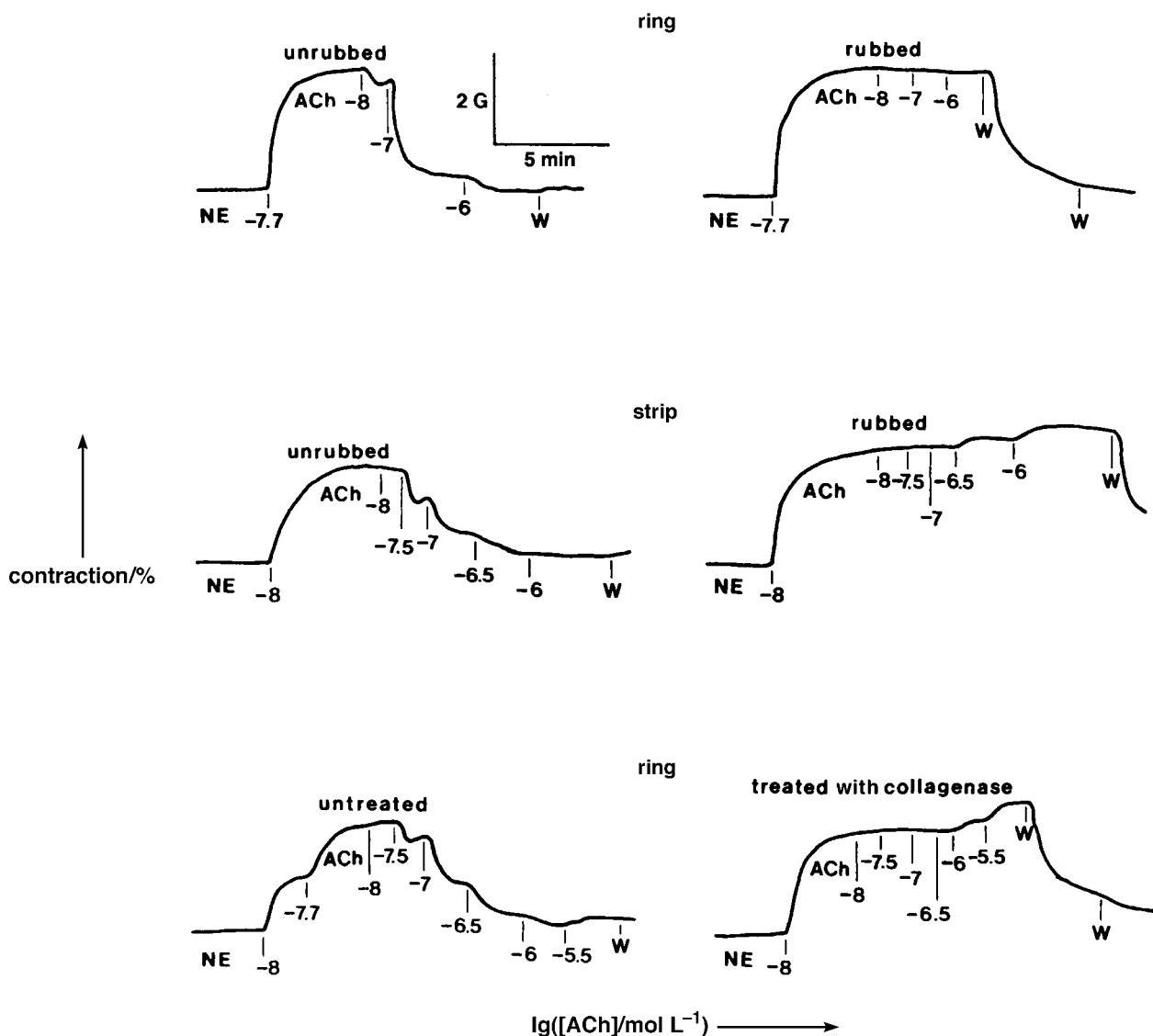
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The obligatory role of endothelial cells in the relaxation of arterial smooth muscle is clearly shown with organ-chamber experiments. Two preparations, a transverse ring and a helical strip from the same rabbit thoracic aorta, were incubated in an organ bath and contracted with noradrenaline (NE).



Addition of the vasodilator acetylcholine (ACh) results in relaxation of the smooth muscle cells (left-hand side of the picture). If the endothelial cells are removed mechanically (by rubbing) or enzymatically (by exposure to collagenase), the relaxing response is eliminated (right-hand side of the picture). After washout (W) of NE and ACh, the muscle relaxes again.

Endothelium-Derived Relaxing Factor: Discovery, Early Studies, and Identification as Nitric Oxide (Nobel Lecture)**

Robert F. Furchgott

An Accidental Finding

In a paper published 45 years ago, in which I introduced the helical strip of the rabbit aorta as a useful preparation for quantitative pharmacological studies on vasoactive drugs and drug–receptor interactions, I reported on the responses of this preparation to a number of catecholamines, sodium nitrite, histamine, and acetylcholine (ACh). One somewhat surprising finding^[1] was that ACh, well recognized as a very potent vasodilator in whole animal and perfused organ studies, elicited no relaxation but only contraction of the aortic strip, whether or not the strip was tested at rest or precontracted with a vasoconstrictor like norepinephrine. Somewhat later, we used ACh as the contracting agent when determining the relative potencies of norepinephrine, epinephrine, and isoproterenol as relaxing agents acting through β -adrenoceptors on helical strips in which α -adrenoceptors had been irreversibly blocked by pretreatment with dibenamine.^[2] The relative potencies of these catecholamines as relaxants (approximately 1:50:200) indicated a β_2 -type adrenoceptor.

In the mid 1970s, in pharmacological studies on isolated strips of guinea-pig tracheal smooth muscle, we obtained evidence that the β -adrenoceptors of that muscle, which according to dogma were pure β_2 -type, frequently had a fairly high admixture of the β_1 -type with the β_2 -type. In view of this unexpected finding, I decided that it would be well to reexamine the type of β -receptor in the smooth muscle of rabbit thoracic aorta. The plan was first to determine anew in a series of experiments the relative potencies of norepinephrine, epinephrine, and isoproterenol in producing relaxation of preparations of rabbit aorta after dibenamine pretreatment. To produce contraction after the pretreatment, we planned to use a muscarinic agonist like carbachol or ACh, as in our earlier studies. However, in the very first experiment of this planned series in May 1978, my technician did not follow

my directions correctly: early in the experiment before, blocking the α -adrenergic receptors with dibenamine, he tested carbachol for its contracting activity before rather than after (as prescribed in the directions) washout of a previous test dose of norepinephrine. The response to carbachol was not a contraction, but was a partial relaxation of the norepinephrine-induced contraction. ACh was then tested. It too produced relaxation of the aortic preparation precontracted with norepinephrine (Figure 1). This was the first time

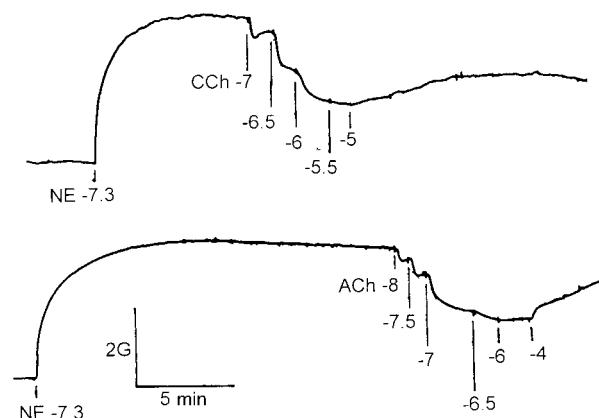


Figure 1. Polygraph tracings from the first experiment in my laboratory in which the muscarinic agonists, carbachol (CCh) and acetylcholine (ACh), unexpectedly produced relaxation of transverse rings of rabbit thoracic aorta. In this and all other experiments, unless otherwise noted, aortic preparations (rings or strips) were mounted in 20-mL all-glass organ chambers containing Krebs bicarbonate solutions aerated with O_2/CO_2 (95/5) and kept at 37°. Tracings show changes in tension under isometric conditions. (For details of experimental procedures, see reference [4].) In this and all other figures unless otherwise noted, cumulative concentrations of drugs in organ chambers are expressed as the log of molar concentrations. 2G = tension; NE = norepinephrine.

that I had ever observed relaxation of rabbit aorta in response to muscarinic agonists over the many years that I had been using this blood vessel for in vitro studies. The unexpected relaxation of rabbit aorta in vitro by muscarinic agonists was very exciting, for it was an effect that was in accord with the potent vasodilating action of these agonists in vivo.

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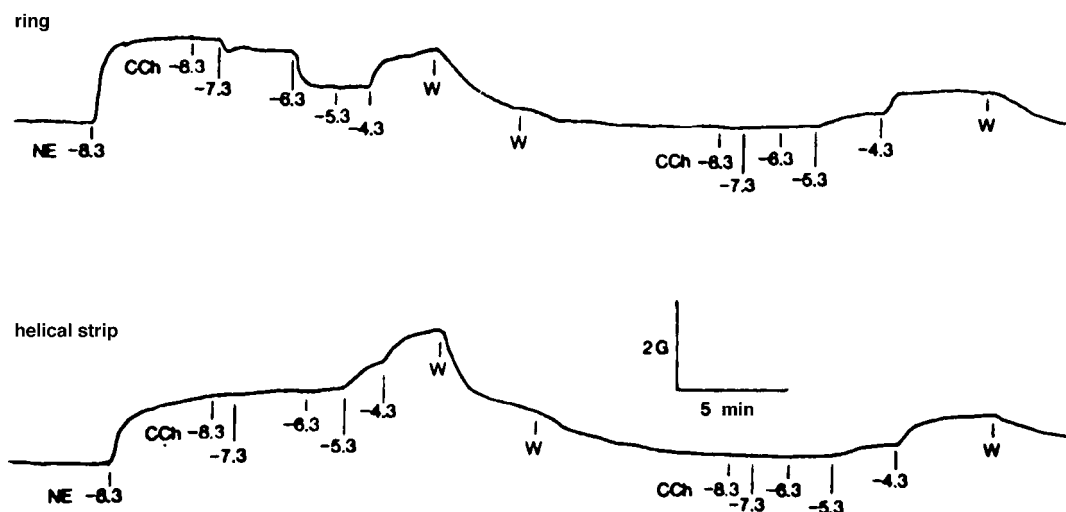


Figure 2. Comparison of responses to carbachol (CCh) of a transverse ring and a helical strip prepared from the same rabbit thoracic aorta. The ring (2.5 mm wide) was mounted on a pair of L-shaped hooks for attachment to the base of the organ chamber and to the force transducer. The strip, prepared by our standard method, had thread ties at each end for attachment. Only the ring, when precontracted with norepinephrine (NE), relaxed in response to low concentrations of CCh. It was subsequently recognized that in our standard method of preparing strips, the endothelial cells were unintentionally rubbed off the intimal surface. (There is no relaxation of the ring in response to CCh added after washout (W) of NE because such preparations exhibit practically no active contractile tension in the absence of vasoconstrictor agents.) Concentrations of drugs are expressed as the log of concentrations in g mL^{-1} . Reproduced with permission from reference [3].

Discovery and Early Studies on EDRF

Why had we failed to observe ACh-induced relaxation or aortic preparations in the past, and why did it now become manifest? A detailed account of our investigation of this matter has been published,^[3,62] so it will suffice here to outline briefly the sequence of findings. First, we were aware that the kind of preparation of rabbit thoracic aorta that we were now using was the transverse ring, rather than the helical strip which we had used in all of the earlier experiments with muscarinic agonists.

Several experiments were therefore carried out in which we compared the responses to cumulative concentrations of carbachol or ACh on helical strips and transverse rings prepared from the same aorta. Only the rings responded with

relaxation to these muscarinic agonists, while the strips responded with contraction (Figure 2).

After several weeks of work, we recognized that in any kind of preparation (ring or strip), gentle rubbing of the intimal surface, whether intentional or unintentional (as had occurred, unbeknown to us, in our standard method of preparing helical strips), eliminated the relaxing response to ACh and other muscarinic agonists. Strips as well as rings gave excellent relaxation when care was taken not to rub the intimal surface. That the loss of the relaxation response to ACh after intimal rubbing was the result of mechanical removal of the endothelial cells was clearly demonstrated with the use of a silver staining procedure to allow en face microscopy of the intimal surfaces. It was also demonstrated that complete removal of endothelial cells of the aorta by preincubation of

Robert F. Furchgott was born in Charleston, South Carolina. He received a BS degree in chemistry from the University of North Carolina in 1937 and a PhD degree in biochemistry from Northwestern University in 1940. He was at Cornell University College of Medicine from 1940–1949 and at Washington University from 1949–1956. He served as Professor



and Chairman of the Department of Pharmacology at the State University of New York Downstate Medical Center (now SUNY Health Service Center at Brooklyn) from 1956–1982, and is presently Distinguished Professor Emeritus at that institution. Since 1989 he has also been Adjunct Professor of Pharmacology at the University of Miami School of Medicine. His research interests have focussed on cardiac pharmacology, adrenergic peripheral mechanisms, theory of drug–receptor mechanisms, and vascular pharmacology and physiology. Much of his research has been carried out on isolated, living preparations of heart and blood vessels. The obligatory role of endothelial cells in the relaxation of arteries by acetylcholine and related muscarinic agonists and the demonstration that the relaxation resulted from release of a labile factor (EDRF) from the stimulated endothelial cells are among the important discoveries he has made. His outstanding contributions have been recognized by numerous prestigious awards and honors, culminating in 1998 with the Nobel Prize in Physiology or Medicine.

the intimal surface with collagenase also resulted in a complete loss of the relaxation response to ACh.

Figure 3 is a slightly modified figure from the first full paper on the obligatory role of endothelial cells in the relaxation of arterial smooth muscle by ACh.^[4]

In that paper, we also reported that in addition to the thoracic aorta, a number of other arteries from the rabbit and various arteries from other species of laboratory animals also exhibited relaxation in response to ACh only if endothelial cells were present. Cyclooxygenase inhibitors did not interfere with the endothelium-dependent relaxation. One hypothesis to explain the obligatory role of endothelial cells was that ACh acting on a muscarinic receptor of these cells stimulates them to release a non-prostanoid substance that diffuses to and activates relaxation of the subjacent smooth muscle cells. Direct evidence for this hypothesis was obtained with the so-called "sandwich" procedure in which it was shown that a transverse strip of aorta freed of endothelial cells, and therefore unable to relax on exposure to ACh, could be made to relax in response to ACh if it were remounted for recording with its endothelium-free intimal surface placed against the endothelium-containing intimal surface of a longitudinal strip of aorta (Figure 4). The ACh-induced relaxation of the transverse strip in the "sandwich" demonstrated that ACh stimulated the release of a diffusible relaxing substance (or substances) from the endothelial cells of the longitudinal strip.^[4] The relaxing substance was later referred to as EDRF (for endothelium-derived relaxing factor).^[5]

It should be noted here that although we had never observed relaxation of rabbit aortic preparations by ACh in my laboratory prior to our accidental finding in 1978, there had been occasional reports from other investigators of relaxation of isolated preparations of arteries in response to that agent. For example, Jelliffe^[6] had reported that a chain of thread-tied rings of rabbit aorta precontracted with serotonin relaxed in response to low concentrations of ACh; and Vanhoutte^[7] and Toda^[8] had found that on a number of canine arteries prepared as strips, ACh elicited significant relaxation of spontaneous or drug-induced contractions. After our findings on the obligatory role of endothelial cells in ACh-induced relaxation of arteries, it became apparent that these earlier examples of ACh-induced relaxation were dependent on the presence of endothelial cells in the preparations of the arteries being studied.

It is of some interest that an unintentional removal of endothelial cells in another vascular preparation used in my laboratory may have prevented the discovery of endothelium-dependent vasodilation at an earlier date. That preparation was the perfused rabbit central ear artery which Odd Steinland, while a graduate student in my laboratory in the early 1970s, used for investigating the inhibition by ACh and other muscarinic agonists of adrenergic neurotransmission by their action on prejunctional muscarinic inhibitory receptors. He readily demonstrated that ACh was a potent inhibitor of neurotransmission, as evidenced by its inhibition of both the release of norepinephrine and the vasoconstriction in response to nerve stimulation.^[9] To

make sure that the inhibition of the vasoconstriction was only due to inhibition of transmitter release, he tested whether infused ACh could inhibit vasoconstriction produced by infused norepinephrine, and found that it did not.^[9] About ten years later, after endothelium-dependent relaxation by ACh had been found in a large variety of arterial preparations, we were puzzled by the earlier finding that the perfused ear artery constricted with infused norepinephrine had failed to show any vasodilation in response to ACh. Dr. Steinland provided us with the answer. In his earlier work, he had not taken precautions to prevent air bubbles from entering the perfusion line and passing through the ear artery. When care was now taken to avoid the occurrence of bubbles in the perfusion stream through the artery, ACh elicited good vasodilation (relaxation) of norepinephrine-induced vasoconstriction.^[10] Apparently, in the earlier work, the bubbles had mechanically removed the endothelial cells, and we had been unaware of this action.

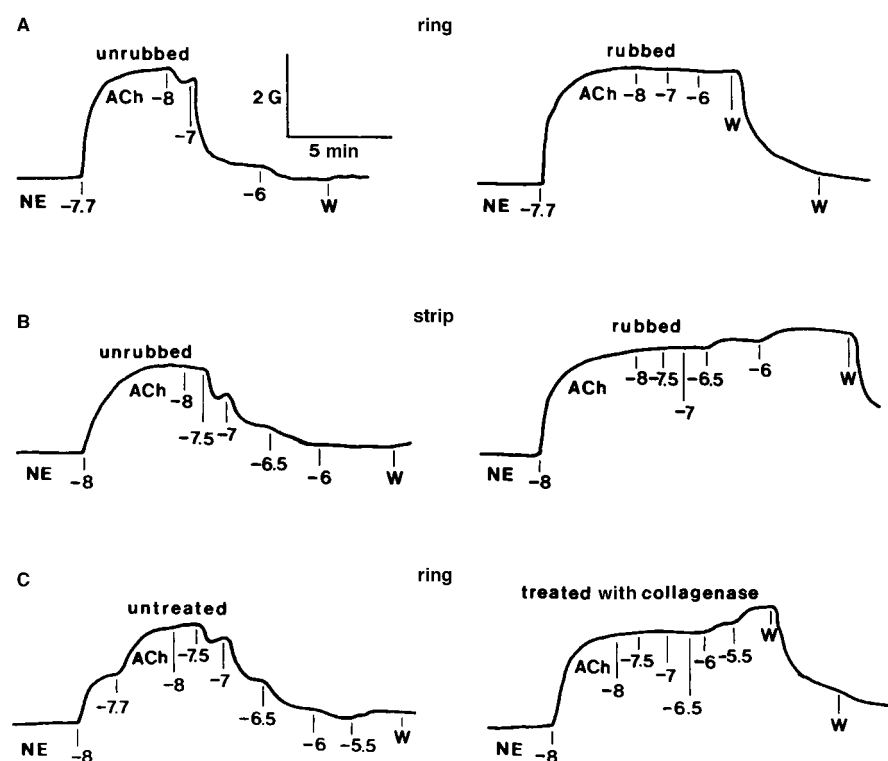


Figure 3. Recordings showing loss of relaxing response of rabbit aorta to ACh after complete removal of endothelial cells. In each case, the recording on the left was made before removal and that on the right after removal of endothelial cells. The cells were removed from the ring in A by rubbing its intimal surface with a small wooden stick, from the transverse strip in B by rubbing its intimal surface on moistened filter paper, and from the ring in C by exposing its intimal surface to collagenase. After the procedure to remove endothelial cells, each preparation was allowed to reequilibrate in its organ chamber for about 1 h before being retested. Reproduced with permission from reference [18].

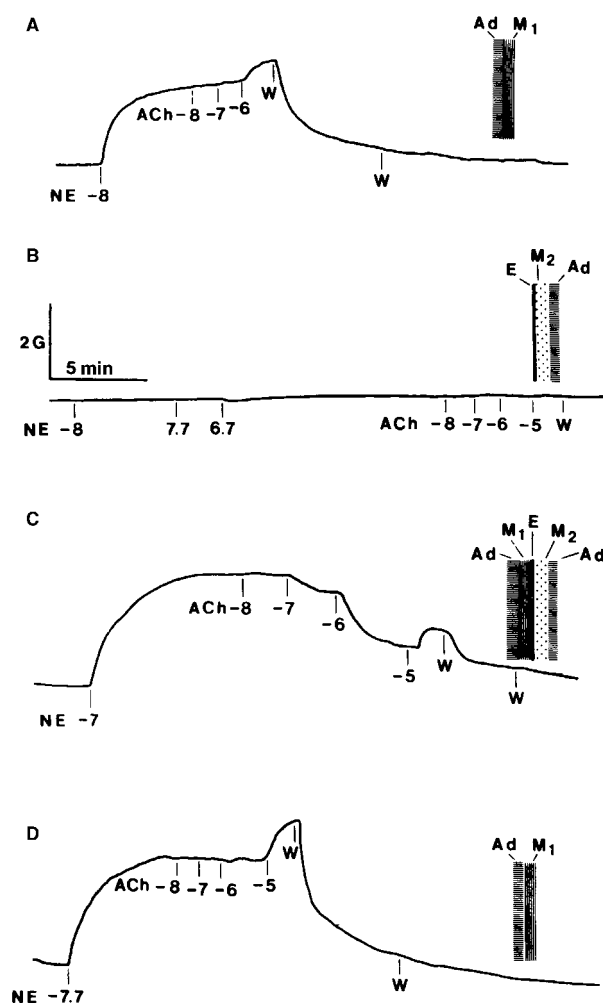


Figure 4. "Sandwich" experiment demonstrating release of a relaxing substance from endothelial cells in response to ACh. A: On a transverse strip of rabbit aorta with endothelial cells removed by rubbing (first test); ACh failed to relax the NE-induced tone. B: On a longitudinal strip from the same aorta with endothelium present (first test); NE and ACh produced only trivial changes in tension because of the orientation of the smooth muscle cells. C: On a "sandwich" made from the transverse strip without endothelium and the longitudinal strip with endothelium mounted together in clips with their entire intimal surfaces apposed, and allowed to reequilibrate (second test); ACh now produced relaxation of the NE-induced tone. D: On the transverse strip after removing the longitudinal strip with endothelium (third test); after reequilibration there was again no relaxation in response to ACh. Insets: diagrams of the vertical cross-sections of the preparations; Ad = adventitia, E = endothelial cell layer, M₁ and M₂ = medial layers of smooth muscle cells oriented vertically and horizontally, respectively. Reproduced with permission from reference [18].

Early Studies on Agents Inducing and Inhibiting Endothelium-Dependent Relaxation

Within a few years after the discovery that ACh-induced relaxation of isolated arteries was endothelium-dependent, a number of other endothelium-dependent relaxing agents were identified in organ-chamber experiments using rings or strips of blood vessels from a variety of species, including man. Among these were the calcium ionophore A23187, ATP and ADP, substance P, bradykinin, histamine, thrombin, serotonin,

and vasopressin. (For references to first reports on endothelium-dependent relaxation by these and other agents, see references [11, 12].) The early finding that the calcium ionophore A23187 was a very potent endothelium-dependent relaxing agent on all vessels tested suggested a central role for calcium in the release, or synthesis and release, of EDRF by endothelium-dependent relaxing agents.^[13]

It was soon recognized in this early work that, depending on the agent, endothelium-dependent relaxation might be limited to certain species, or even to specific arteries from a given species. For example, whereas ACh relaxed by this mechanism practically all systemic arteries from all mammalian species tested, histamine, another potent vasodilator, produced endothelium-dependent relaxation of rat^[14] but not of rabbit arteries.^[15] Another example was bradykinin, which gave endothelium-dependent relaxation of isolated canine and human arteries but not of rabbit or cat arteries.^[5] As examples of vessel specificity, it was found that noradrenaline activated endothelium-dependent relaxation in isolated canine coronary arteries by acting on α_2 -adrenoceptors of the endothelial cells, but failed to activate this kind of relaxation in canine systemic arteries,^[16] and that serotonin activated endothelium-dependent relaxation in canine coronary arteries but not in canine systemic arteries.^[16, 17]

Among the conditions and agents which were found to inhibit endothelium-dependent relaxation of arteries in the early organ-chamber experiments were anoxia and some agents that were recognized as inhibitors in the metabolic pathway of arachidonic acid (AA) from phospholipids through lipoxygenase.^[4, 18] Because it had already been reported that in certain smooth muscles there was a positive relationship between an increase in cyclic guanosine monophosphate (cGMP) and relaxation^[19–22] and that guanylate cyclase (G-cyclase) was markedly stimulated by hydroperoxides of AA,^[23, 24] I speculated 1) that EDRF is either a short-lived hydroperoxide or free radical arising as an intermediate product in the oxidation of liberated AA by the lipoxygenase pathway, and 2) that EDRF stimulates the G-cyclase of the vascular smooth muscle, causing an increase in cGMP which then somehow activates relaxation.^[18] As it turned out, this early speculation about the nature of EDRF was wrong, but that about the role of cGMP proved to be right.

Cyclic GMP as a Mediator for EDRF and Nitrovasodilators

The speculation that EDRF released by ACh would stimulate an increase in cGMP was soon proven correct in a number of independent studies by, for example, Rapoport and Murad^[25] using rat aorta, Diamond and Chu^[26] and Furchgott et al.^[27] using rabbit aorta, Holzmann^[28] using bovine coronary artery, and Ignarro et al. using bovine pulmonary artery.^[29] All of these investigators showed that a rise in cGMP accompanied the ACh-induced relaxation of the blood vessel, consistent with the proposal that the increase in cGMP in the muscle has a causal role in the relaxation (Figure 5). Endothelium-dependent relaxation of rat aorta by A23187

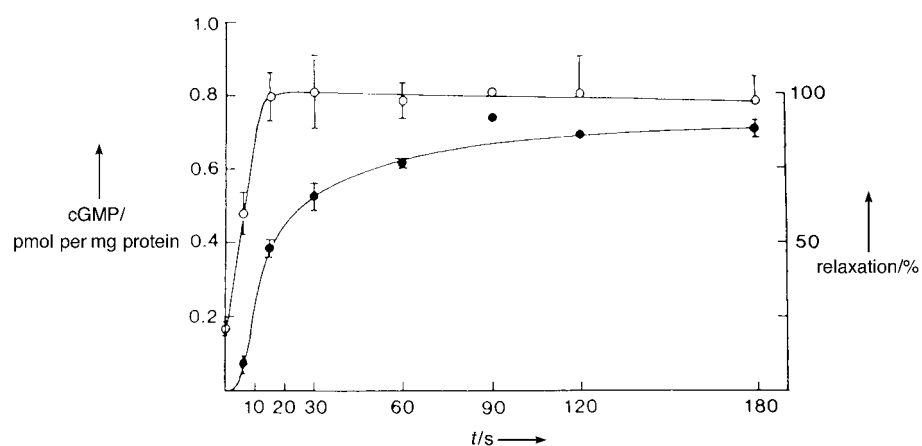


Figure 5. Time course of changes of cyclic GMP (cGMP, $\circ$) and of relaxation ($\bullet$) of endothelium-containing rings of rabbit thoracic aorta after addition of ACh. Norepinephrine ($0.1 \mu\text{M}$) was first added to produce a steady contraction. ACh ($1 \mu\text{M}$) was then added, and the tissue was instantly frozen in liquid nitrogen after the desired period of exposure to ACh. The level of cyclic AMP (not shown) did not change significantly during the endothelium-dependent relaxation. Reproduced with permission from reference [27].

and by histamine^[25] and of rabbit aorta by A23187^[27] was also accompanied by a rise in cGMP.

It is well to stress at this point that Murad and co-workers^[21, 22] had previously found that nitric oxide (NO) is a very potent stimulus of G-cyclase, and had proposed that a number of agents (e.g. glyceryl trinitrate, nitroprusside, azide), which they and others had found to increase cGMP and produce relaxation of certain non-vascular smooth muscle preparations, do so by generating NO as the proximal activator of G-cyclase. Also, Ignarro and his co-workers^[30] had found that the relaxation of a vascular preparation (bovine coronary artery) by NO as well as by nitroprusside was associated with an increase in cGMP. However, despite these early findings with NO, none of the investigators who several years later showed that endothelium-dependent relaxation of arteries in organ-chamber experiments was also correlated with an increase in cGMP^[30] hypothesized that EDRF might be the free radical NO. Certain key findings had yet to be made to foster this novel proposal.

Further Studies on EDRF, Including Those with Perfusion – Bioassay Procedures

Hemoglobin and Methylene Blue as Inhibitors

Two agents that became widely used as inhibitors of endothelium-dependent relaxation were hemoglobin (Hb) and methylene blue (MB). Hb had been found by Murad et al.^[21] to inhibit guanylate cyclase in cellular extracts, and by workers at Glasgow University^[31] to inhibit the relaxation of strips of bovine retractor penis (BRP) muscle in response either to nonadrenergic, noncholinergic (NANC) nerve stimulation or to an acid-activated extract of that tissue. Gruetter et al.^[32] had reported that methemoglobin (metHb) interfered with NO-induced increases in vascular cGMP. In our laboratory, work with Hb began in 1983 when William

Martin, who had studied the properties of extracts of the BRP muscle while a graduate student at Glasgow University,^[33, 34] came to my laboratory as a postdoctoral fellow (see below). It was soon found that Hb was an extremely potent and fast-acting inhibitor of endothelium-dependent relaxation and of the associated rise in cGMP induced by ACh, A23187, and other agents on rabbit and rat aorta.^[27, 35, 36] Myoglobin was as effective as Hb, but metHb only inhibited very weakly^[37] (Figure 6).

The degree of potentiation by Hb of contractions of arterial rings by vasoconstrictors such as phenylephrine also provided a measure of the relaxing activity of basally released EDRF.^[36] Using this measure,

the relaxing activity of basally released EDRF in the case of rings of rat aorta appeared to be considerably greater than in the case of rings of rabbit aorta. With rings from both species, addition of phosphodiesterase inhibitors (such as zaprinast and IBMX) produced relaxation associated with increases in cGMP.^[38] The responses to these inhibitors of the enzymes which hydrolyze cGMP to GMP were more pronounced in the case of rat aorta than in rabbit aorta, consistent with a higher basal release of EDRF and higher basal stimulation of G-cyclase in the former. The relaxation of endothelium-containing rings of both species produced by phosphodiesterase inhibitors could be completely blocked by Hb.

It was originally proposed that Hb inhibits relaxation and the rise in cGMP by binding EDRF as it diffuses from the endothelial cells, thus preventing it from reaching and activating the G-cyclase in the muscle cells.^[35] However, after the identification of EDRF as NO, it became apparent that the scavenging of EDRF by Hb under the usual aerobic conditions was the result of the very rapid reaction of HbO₂ with NO to form NO₃⁻ and metHb, as first reported by Doyle and Hoekstra.^[39]

Methylene blue had first been shown by Gruetter et al.^[32] to inhibit stimulation of G-cyclase by nitrovasodilators in cell-free systems and to inhibit both the relaxation and increase in cGMP elicited by these agents in vascular smooth muscle. Subsequently, MB was found to inhibit both the endothelium-dependent relaxation and accompanying increase in cGMP produced by ACh in bovine arteries^[28, 29] and by ACh and A23187 in rabbit aorta.^[27, 35] Holzman^[28] and Ignarro et al.^[29] concluded that the inhibition of relaxation was the result of the inhibition of G-cyclase by the MB. On the other hand, I and my co-workers, although agreeing that such enzyme inhibition accounted for the sustained inhibition by MB developed during prolonged exposure of the rabbit aorta, proposed that the extremely fast inhibition of endothelium-dependent relaxation immediately after addition of MB to an organ chamber (as in Figure 6) resulted from some more

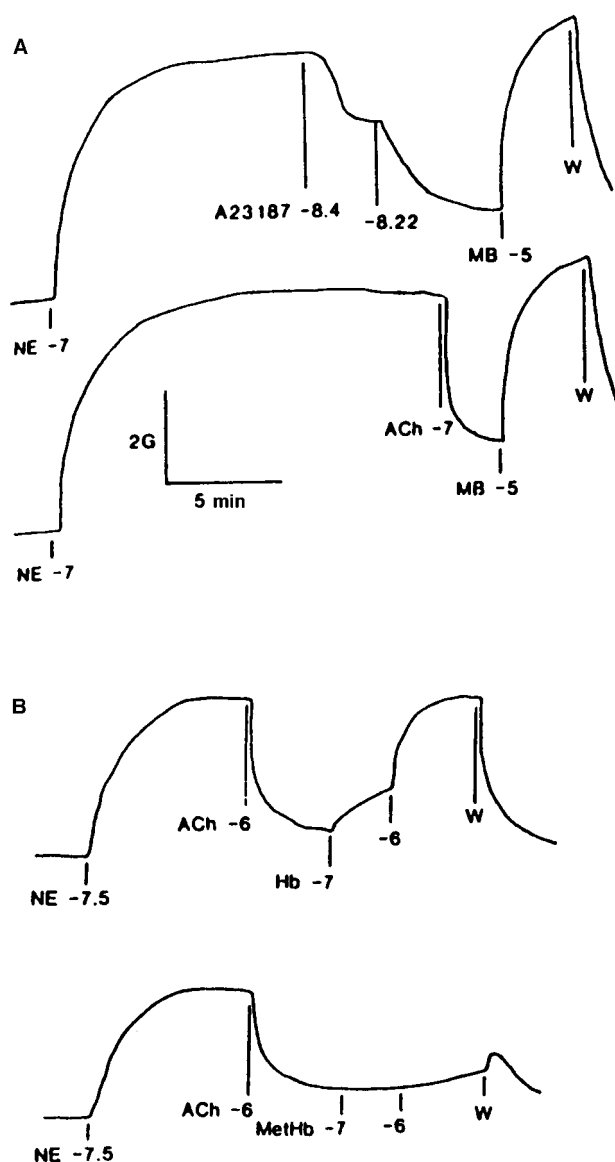


Figure 6. Examples of inhibition by methylene blue (MB) and by hemoglobin (Hb) of endothelium-dependent relaxation in rings of rabbit aorta. A: Addition of MB ($10\mu\text{M}$) rapidly and completely inhibited the ongoing relaxation produced by addition of ACh (top) and that produced by addition of the calcium ionophore A23187 (bottom). B: Addition of Hb ($1\mu\text{M}$) rapidly and almost completely inhibited the relaxation produced by addition of ACh (top); however, addition of methemoglobin (MetHb) at the same concentration inhibited ACh-induced relaxation only to a very small extent (bottom).

direct inactivation of EDRF by the MB.^[27, 35] It now appears that this more direct inactivation is due to superoxide anions, generated by reactions of the MB in the tissue.^[40]

Results with Perfusion–Bioassay Procedures

For more direct studies on the properties of EDRF than were possible in organ-chamber experiments, perfusion–bioassay procedures were developed. These procedures, although first developed in laboratories other than my own, should be considered here, because they produced results

which contributed much to the identification of EDRF. In such procedures, endothelial cells are upstream in the perfusion (or superfusion) cascade and an endothelium-denuded arterial preparation (usually a ring or one or more strips), whose contractile state is monitored, is downstream to bioassay EDRF in the perfusion fluid. In some procedures, the endothelial cells upstream were native cells on the luminal surface of a perfused artery;^[41–44] in other procedures, they were cultured cells (usually from bovine or porcine aortas) on the surface of microcarrier beads contained in a perfused column.^[45, 46]

Perfusion–bioassay procedures allowed one to determine clearly whether an inhibitor of endothelium-dependent relaxation interfered with the synthesis/release of EDRF or inactivated the EDRF after its release. In addition, by altering the transit time of the perfusate between the endothelial cells and the bioassay preparation, it was possible to study the rate of decay of EDRF. Surprisingly, estimated half-life values for EDRF ranged from as low as 4–6 s to as high as 50 s depending on the biological preparations and the experimental conditions. The likely explanation of these discrepant values came with the important finding that superoxide dismutase (SOD) markedly stabilizes EDRF released from either endothelial cells on arteries^[47] or cultured endothelial cells on microcarrier beads,^[46] clearly showing that the superoxide anion (O_2^-) very rapidly inactivates EDRF. Thus, the marked differences in half-life of EDRF reported from different laboratories could largely be attributed to differences in concentrations of O_2^- in the perfusion fluid leaving the endothelial cells under the different experimental conditions used. Subsequently, Moncada et al.^[48] were able to show with their perfusion–bioassay procedure that inhibition of endothelium-dependent relaxation by certain reducing agents (e.g. hydroquinone, phenidone, and pyrogallol) originally demonstrated in organ-chamber experiments was principally the result of inactivation of EDRF by O_2^- formed in a single-electron oxidation of the reducing agent by O_2 .

Development of perfusion–bioassay procedures also led to the important finding that increases in shear stress on endothelial cells of the perfused artery with increases in rate of flow of the perfusion fluid resulted in increases in the rate of release of EDRF.^[49, 50] Increase in flow through arteries both *in situ*^[49, 51, 52] and *in vitro* produced vasodilation that could not be blocked by inhibitors of cyclooxygenase, but was abolished by removal of the endothelium. These findings concerning increased EDRF release with increased shear stress were important for they provided evidence that EDRF has a continuous physiological role in the control of regional blood flow and regulation of blood pressure.

Findings That Led to the Identification of EDRF as NO

While Billy Martin was working in my laboratory (1983–1985), I became interested in the work that he and others had done in John Gillespie's laboratory in Glasgow in trying to identify the neurotransmitter released from NANC (non-adrenergic, non-cholinergic) nerves that elicit relaxation of

bovine retractor penis (BRP) muscle and certain other smooth muscle preparations. I learned that relaxation produced by NANC nerve stimulation and relaxation produced by a partly purified extract made by Martin from the BRP muscle (referred to as BRP inhibitory factor or BRPIF) were both inhibited by Hb^[31] and were both associated with increases in cGMP in the muscle.^[53] One important aspect of Martin's work with BRPIF extract was that it had to be acidified for a short period prior to testing in order for it to have a relaxing effect on smooth muscle preparations of retractor penis and arteries in organ baths.^[33] Once activated by acidification, the BRPIF extract gave good relaxations of the smooth muscle preparations, but the relaxations were transient, lasting no more than a few minutes.

As discussed in the paper that I presented at a symposium in Rochester, Minnesota in July 1986,^[54] I was struck by the similarities of the transient relaxations produced by the acid-activated BRPIF extract of Martin and the transient relaxations of rabbit aorta produced by acidified solutions of sodium nitrite (NaNO₂). That acidified, but not neutral, solutions of NaNO₂ produce strong transient relaxations of rabbit aorta had been found about 15 years earlier in my laboratory when a new postdoctoral fellow, assigned to obtain cumulative dose-response curves for NaNO₂, accidentally made his standard solutions of that salt with acidified diluting fluid (used in my laboratory for making stable standard solutions of catecholamines) rather than with neutral diluting fluid (Figure 7). At

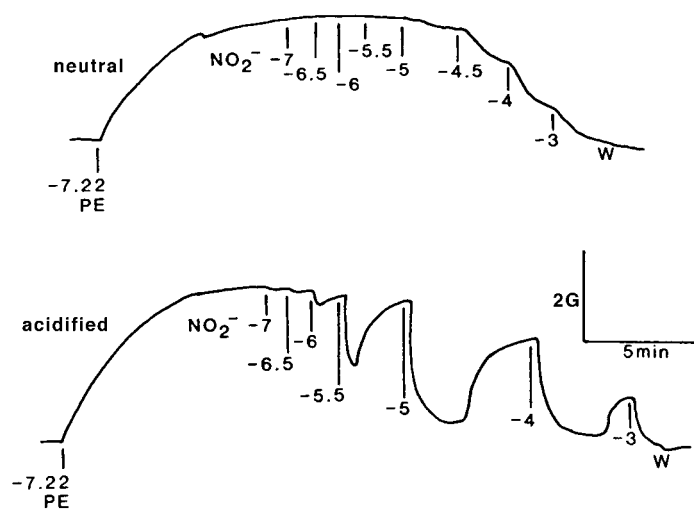


Figure 7. Comparison of the relaxing effects of cumulative additions of neutral and acidified solutions of sodium nitrite on rings of rabbit aorta. In this typical experiment, two rings from the same aorta after contraction with phenylephrine (PE) were exposed to additions of NaNO₂ diluted with neutral physiological saline and to additions of NaNO₂ diluted with the saline acidified with 11 mM HCl. The concentrations shown are the final nitrite concentrations (log of the molar concentration) in the organ chambers. Note the striking transient relaxations in the case of the acidified nitrite solutions. The rings in this experiment were denuded of endothelial cells, but results were essentially the same when endothelial cells were present. Reproduced with permission from reference [54].

that time, I became aware that the HNO₂ (pK_a=3.2) produced by acidification of NaNO₂ solutions immediately generates low concentrations of NO and NO₂ as a result of a reversible dismutation according to Equations (1) and (2).



However, when a solution of acidified nitrite is added to the oxygenated, well-buffered physiological salt solution used in our organ chambers, any NO present will be removed very rapidly—thus accounting for the transiency of the relaxation of the aortic rings receiving acidified nitrite.

In the spring of 1986, recalling the much earlier accidental finding in my laboratory with acidified sodium nitrite solutions, I began to consider the possibility that Martin's acid-activatable BRPIF was inorganic nitrite. An encouraging early finding was that the degree of transient relaxation as a function of pH of the added NaNO₂ solution in the case of rabbit aorta was similar to that found by Martin for his added extract in the case of the BRP—maximal transient relaxation at a pH of 2 or lower, and approximately half-maximal transient relaxation at a pH of 3.

By the time I had begun to use acidified nitrite solutions as a source of NO in experiments with rabbit aorta in 1986, I had also become aware of the recent findings by Gryglewski, Palmer and Moncada^[46] and by Rubanyi and Vanhoutte^[47] demonstrating that the superoxide anion (O₂⁻) rapidly inactivates EDRF and that superoxide dismutase (SOD) protects against this inactivation. Assuming that the free radical NO might rapidly react with the free radical O₂⁻, to form inactive NO₃⁻, I began to speculate on the possibility that EDRF is NO. (Later, it was pointed out by Beckman et al.^[55] that the immediate product of the reaction between NO and O₂⁻ is peroxynitrite (ONOO⁻), which is protonated to HONOO (pK_a=6.8), which decays within seconds at physiological pH.)

My speculation in the spring of 1986 that EDRF might be NO led to a large number of experiments on rabbit aorta, carried out with the assistance of D. Jothianandan and M. T. Khan, comparing the characteristics of relaxation by NO (present in acidified solutions of NaNO₂) with the characteristics of relaxation by EDRF (released by ACh). It was found that relaxation by NO, like that produced by EDRF, was strongly inhibited by Hb, strongly inhibited by MB, strongly inhibited by superoxide generators (e.g. hydroquinone, phenidone, ferrous sulfate), and potentiated by SOD when superoxide was limiting the degree of relaxation (Figure 8). It was already known that relaxation of vascular smooth muscle by NO, like that by EDRF, is accompanied by an increase in cGMNP. On finding the characteristics of NO and EDRF so similar, I had no hesitancy in proposing in the paper which I delivered at the July 1986 symposium that EDRF is NO.^[54] (Shortly after the symposium, we began using solutions of NO gas rather than acidified nitrite as a source of NO in our experiments and obtained identical results (Figure 9).) I also proposed that the acid-activatable inhibitory factor in the BRP extract of Martin and Gillespie is inorganic nitrite, and that the possibility had to be considered that the neurotransmitter of NANC nerves might also be NO.^[54]

At the same symposium at which I proposed that EDRF is NO, Ignarro, on the basis of studies in his laboratory on isolated bovine pulmonary arteries, independently made the

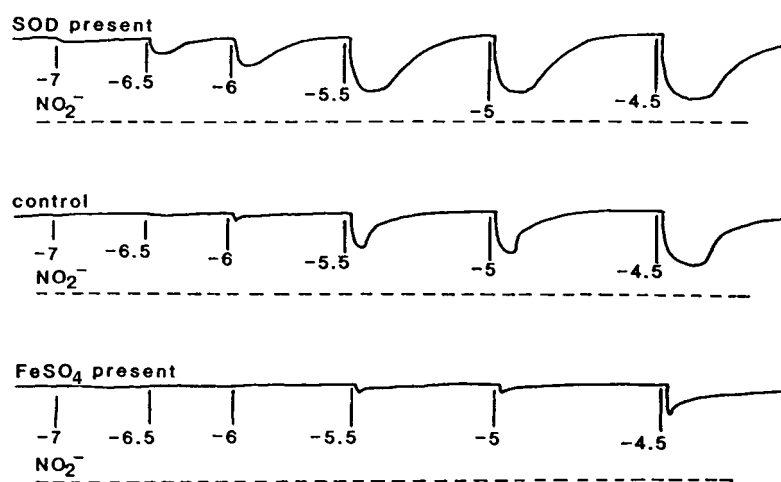


Figure 8. Transient relaxations of endothelium-free rings of rabbit aorta by nitric oxide present in acidified solutions (pH 2) of NaNO_2 , potentiation of the relaxation by SOD (25 units per ml), and inhibition by the superoxide generator FeSO_4 ($3 \times 10^{-4} \text{ M}$). Three matched rings from one aorta were contracted above basal tone (dashed line) with phenylephrine. SOD and FeSO_4 were added about 5 min before first additions of the acidified nitrite. Reproduced with permission from reference [54].

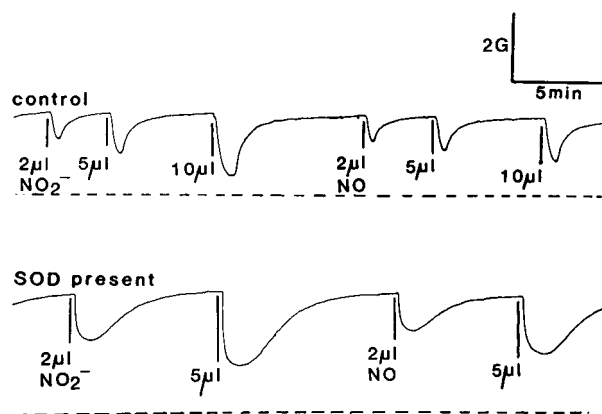


Figure 9. Similarity of the relaxing effects of a solution of acidified sodium nitrite and a solution of nitric oxide gas on rings of rabbit aorta in the absence and presence (30 units per ml) of SOD; 2.5 mM acidified nitrite (pH 2.0), $15 \mu\text{M}$ NO. (See reference [60] for a method for making solutions of NO.) Additions were to 20-mL aliquots of Krebs solution in each organ chamber, so that $2 \mu\text{L}$ of the NO solution gave an initial concentration of 1.5 nM . Both rings were precontracted with phenylephrine. The potentiation by SOD of the transient relaxations produced by the NO in the acidified nitrite and in the NO solution indicates that there was a considerable endogenous production of the superoxide anion O_2^- in the organ chambers.

same proposal. [56] (Unfortunately, the papers presented at that symposium were delayed in publication until 1988.) Soon after the 1986 symposium, three laboratories utilized perfusion–bioassay procedures to compare more accurately the biological and chemical characteristics of EDRF and NO. Ignarro and co-workers using bovine pulmonary vessels, [57] Moncada and co-workers using cultured porcine aortic endothelial cells, [58] and Khan and myself using rabbit aorta as a source of EDRF [44] all found EDRF released upstream and NO infused upstream to have similar rates of decay, similar susceptibility to inhibitors like Hb and superoxide generators, similar stabilization by SOD, etc. In addition,

Ignarro's laboratory presented spectroscopic evidence that the product of the reaction of released EDRF and Hb was the same as that of NO and Hb, and Moncada's laboratory showed that the amount of NO released by bradykinin (as determined by a chemiluminescence assay) could fully account for the relaxation of the bioassay strip by the EDRF released by the peptide. A year later, Moncada and co-workers made the major finding that the source of endothelial NO was a guanidinium nitrogen atom of L-arginine and that the enzyme responsible for its formation was an oxygenase (now called endothelial nitric oxide synthase or eNOS). [59] These findings marked the beginning of a major worldwide expansion of research on the role of NO in vascular physiology and pathophysiology.

Despite the evidence for the identity of EDRF and NO, some results obtained in various laboratories in the late 1980s did not appear to be completely consistent with that conclusion. [60]

Nevertheless, as of now, it has become generally accepted that EDRF is either NO, or some adduct that readily releases NO, or perhaps a mixture of NO and some adduct of NO. This does not preclude a contribution to endothelium-dependent relaxation in some blood vessels from the release of a non-nitric oxide endothelium-derived hyperpolarizing factor (EDHF) along with EDRF. [61]

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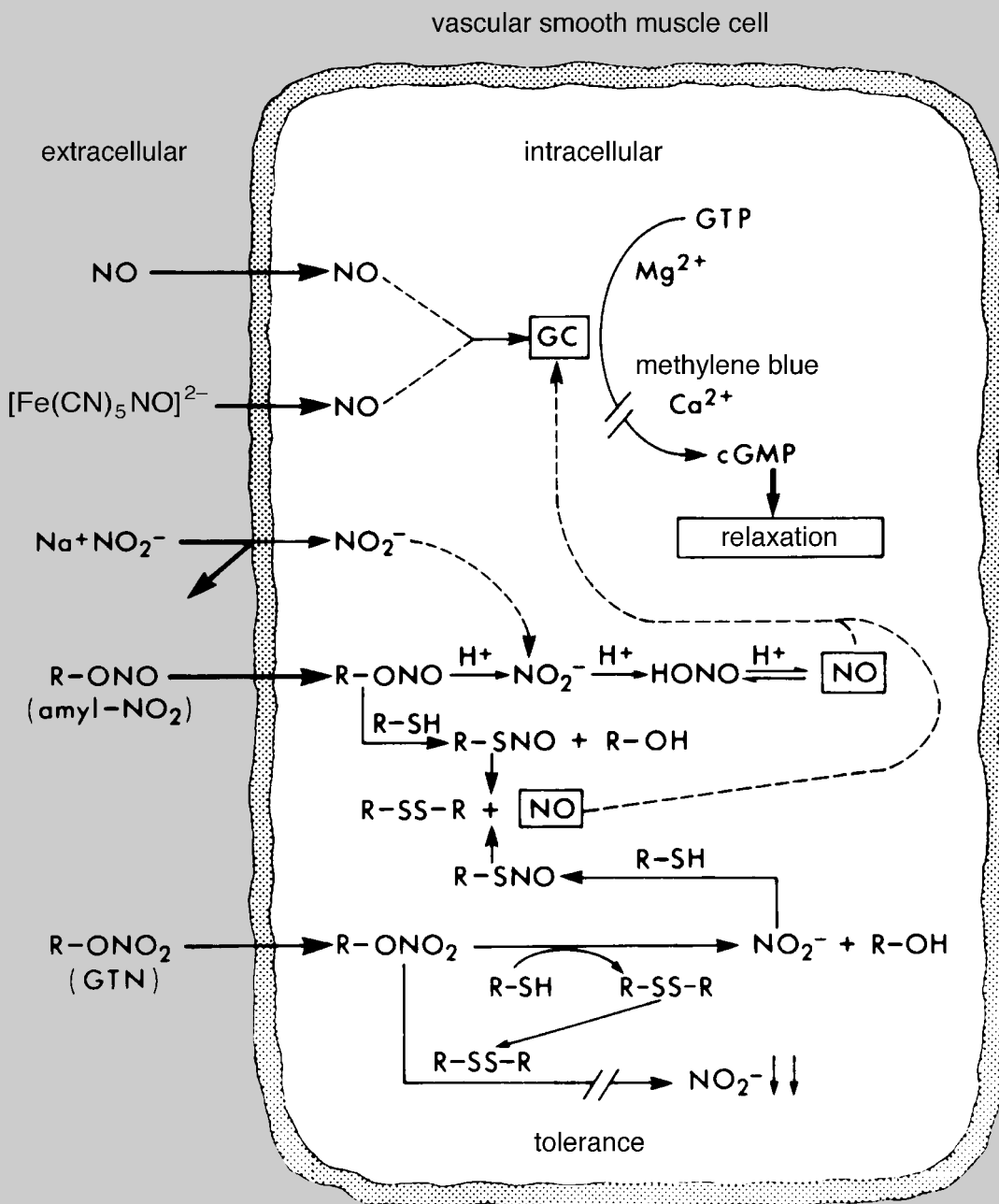
Keywords: enzymes • neurotransmitters • nitric oxide • Nobel lecture • signal transduction

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Proposed mechanism by which organic nitrate and nitrite esters, inorganic nitrite, and nitroso compounds relax vascular smooth muscle.



Nitroprusside liberates NO in solution, whereas organic nitrate and nitrite esters require chemical interaction with thiols to generate *S*-nitrosothiols, which then decompose with the liberation of NO . NO activates guanylate cyclase, and the resulting cyclic GMP causes vascular smooth muscle relaxation.

Nitric Oxide: A Unique Endogenous Signaling Molecule in Vascular Biology (Nobel Lecture)**

Louis J. Ignarro*

Introduction

My early research career in graduate school focused on trying to develop a better understanding of catecholamine mechanisms during the embryological development of the sympathetic nervous system. This research required a strong background not only in pharmacology but also in physiology and biochemistry. I recall reading some interesting papers on cyclic AMP, but I never addressed this topic in my early research. At that time, there was no such thing as cyclic GMP, and nitric oxide was considered to be no more than a pollutant in the atmosphere around us. After completing my postdoctoral training at the National Institutes of Health in Bethesda, I accepted an industrial research position to learn more about biochemical aspects of the inflammatory process in order to develop novel antiinflammatory drugs. Although my research did not yet involve cyclic AMP, I continued to read the literature on cyclic AMP and witnessed the discovery of cyclic GMP. I then decided to ascertain whether or not cyclic AMP and cyclic GMP played any modulatory roles in the inflammatory process. We found that cyclic AMP stabilized lysosomal membranes, whereas cyclic GMP promoted instability of lysosomal membranes. Moreover, cyclic AMP production was associated with the inhibition of human neutrophil function such as phagocytosis and lysosomal enzyme secretion, whereas cyclic GMP was associated with increased neutrophil function. In a different research project, it appeared that this new cyclic nucleotide, cyclic GMP, was involved in mediating the negative effects of acetylcholine in the heart, whereas cyclic AMP was already well known to elicit the opposite effects in the heart. A theme was clearly developing that cyclic AMP and cyclic GMP mediated opposing influences on cell function.

In the midst of our studies on cyclic AMP and cyclic GMP, I recall reading two interesting papers on cyclic GMP that were

published by Ferid Murad and colleagues.^[1, 2] These studies revealed that nitric oxide and nitro compounds that might release nitric oxide all activated cytosolic guanylate cyclase. Nitroglycerin was one of the nitro compounds studied. Nitric oxide and nitro compounds also stimulated cyclic GMP production in isolated tissues in vitro.^[3] These observations suggested that nitroglycerin might activate guanylate cyclase and stimulate cyclic GMP formation by mechanisms involving nitric oxide. Additional studies revealed that nitric oxide might be involved in the nonvascular smooth muscle relaxant effects of nitroglycerin and other nitro compounds.^[4] These findings prompted me to conduct some experiments to ascertain whether nitroglycerin and related organic nitrate and nitrite esters could actually release nitric oxide gas in aqueous solution. After observing the generation of nitric oxide from a series of nitro compounds that were known to be smooth muscle relaxants, we thought that nitric oxide might also be responsible for the vasorelaxant effects of nitroglycerin and that cyclic GMP might be the intracellular second messenger mediating this effect of nitric oxide. Accordingly, a series of experiments was conducted to test the hypothesis forwarded by Ferid Murad that nitroglycerin, nitroprusside, and nitroso compounds all cause relaxation of smooth muscle, including vascular smooth muscle, by liberating nitric oxide, which elicits its action by stimulating the production of cyclic GMP.

Mechanism of Release of Nitric Oxide from Nitroglycerin and Other Nitro Compounds

The most important experiment that we conducted to test the hypothesis that nitric oxide is responsible for the vasorelaxant action of nitroglycerin was to determine whether nitric oxide itself causes vascular smooth muscle relaxation. In 1979 we reported that nitric oxide gas is a potent relaxant of bovine coronary artery and activates guanylate cyclase isolated from this tissue (Figure 1).^[5] This observation confirmed what Ferid Murad had found and extended the hypothesis to include vascular smooth muscle. Therefore, nitroprusside, nitroglycerin, and related nitro compounds appeared to relax vascular smooth muscle by liberating nitric oxide, which then stimulates cyclic GMP formation and

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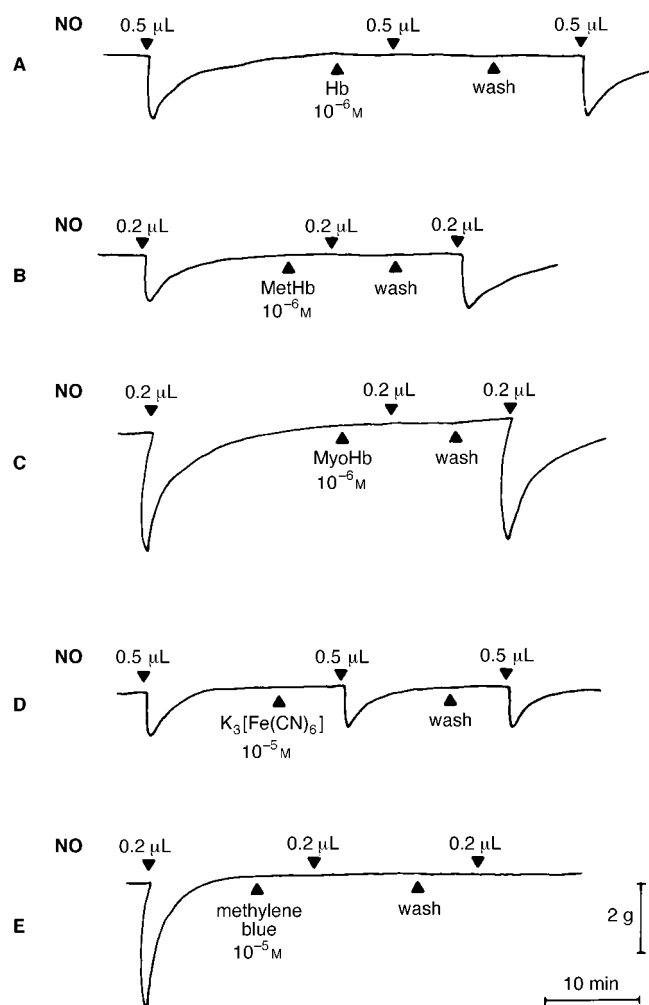


Figure 1. Relaxation of strips of bovine coronary artery by nitric oxide (NO). Strips were mounted, equilibrated, and set up for recording changes in isometric force as described previously.^[5] Strips were submaximally precontracted by exposure to 30 mM KCl. The concentrations of NO were estimated to be in the range of 0.01 to 0.05 μM . A) Hemoglobin (Hb), B) methemoglobin (MetHb), C) myoglobin (MyoHb), D) potassium ferricyanide, and E) methylene blue were added at the concentrations illustrated. Modified from reference [5] with permission.

results in vascular smooth muscle relaxation (Figure 2). The hypothesis that cyclic GMP is involved in smooth muscle relaxation was met with some controversy in the late 1970s because of the prevailing view that cyclic GMP and cyclic

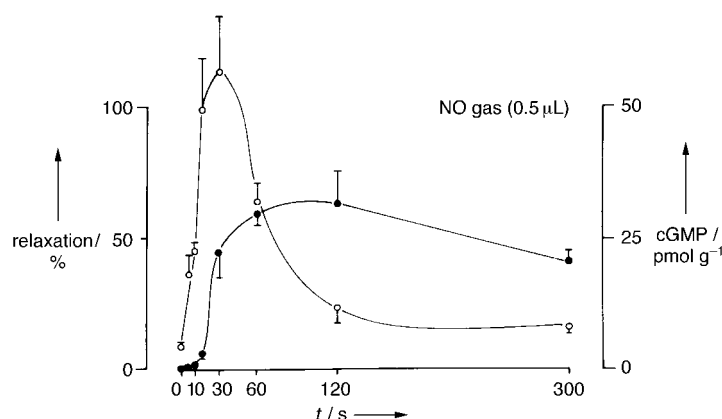
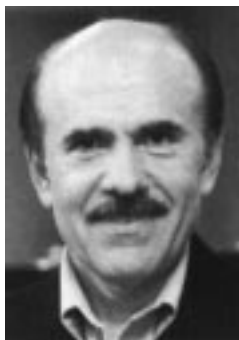


Figure 2. Time course of cyclic GMP accumulation (○) and relaxation (●) of bovine coronary artery elicited by nitric oxide gas (NO). Strips were mounted, equilibrated, and set up for recording changes in isometric force as described previously.^[5,36] Strips were submaximally precontracted by exposure to 30 mM KCl. Relaxation and cyclic GMP (cGMP) levels were measured in the same arterial strips.^[10,36] Data are expressed as the mean $\pm$ SEM using 16 arterial strips from four separate experiments. Modified from reference [36] with permission.

AMP mediate opposing biological actions. Cyclic AMP was known to mediate the smooth muscle relaxant effects of certain catecholamines and prostaglandins, and cyclic GMP was thought to be involved in mediating smooth muscle contraction. However, the work conducted in several laboratories including those of Ferid Murad and myself indicated that cyclic GMP and cyclic AMP can mediate common biological actions in smooth muscle such as relaxation.

The next step in elucidating the precise mechanism of action of nitroglycerin was to ascertain how nitric oxide was released from the parent molecule in smooth muscle tissue. The spontaneous release of nitric oxide from nitroso compounds such as nitroprusside had been understood, but nitroglycerin and other organic nitrate and nitrite esters are chemically stable compounds that would have to undergo enzymatic or chemical reactions to release nitric oxide. In early experiments, we found that thiols such as dithiothreitol enhanced the activation of guanylate cyclase by nitroprusside and *N*-nitroso compounds without affecting enzyme activation by nitric oxide itself.^[6–8] The mechanism of this enhancement was attributed to a reaction between thiol and nitroso compound to form an *S*-nitrosothiol which decomposed with the discharge of nitric oxide. A series of *S*-nitrosothiols were



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synthesized and found to be labile nitric oxide donor agents. In experiments using nitroglycerin, we found that nitroglycerin alone did not activate guanylate cyclase and that added dithiothreitol or glutathione failed to modify this lack of effect of nitroglycerin on guanylate cyclase. In the presence of cysteine, however, nitroglycerin caused a marked activation of guanylate cyclase.^[9] Sodium nitrite also failed to activate guanylate cyclase in the absence of added thiols, but unlike nitroglycerin a variety of thiols unmasked the enzyme-activating action of sodium nitrite. Additional experiments revealed that cysteine reacts with nitroglycerin, whereas sodium nitrite reacts with any of a variety of thiols to generate the corresponding *S*-nitrosothiol.^[10] The *S*-nitrosothiols decompose readily with the release of nitric oxide, which activates guanylate cyclase. These concepts are illustrated schematically in Figure 3. Examples of such *S*-nitrosothiols are *S*-nitroso-*N*-acetylphenylamine (SNAP), *S*-nitrosoglutathione (GSNO), and *S*-nitrosocysteine (CYSNO), each of which we synthesized and first examined their biological effects in mammalian systems.^[7–10] These *S*-nitrosothiols

turned out to be useful nitric oxide donor agents that are now readily employed to test the effects of nitric oxide in vitro and in vivo.

Inhibition of Platelet Aggregation by Nitric Oxide

In reviewing the literature on nitroglycerin and nitroprusside, I came across a paper showing that nitroprusside inhibited human platelet aggregation.^[11] The obvious question raised was whether nitroprusside inhibited platelet aggregation by mechanisms involving nitric oxide and cyclic GMP just as had been shown for the smooth muscle relaxant effect of nitroprusside. Using a platelet-rich plasma fraction from human volunteers, we showed that nitric oxide was a potent inhibitor of platelet aggregation induced by ADP, thrombin, collagen, and other aggregatory agents, and that the anti-platelet effect of nitric oxide was mediated by cyclic GMP.^[12] The anti-platelet action of nitroprusside appeared to involve the actions of *S*-nitrosothiols, which were shown to be potent inhibitors of platelet aggregation.^[13] Human platelets were rich in cytoplasmic guanylate cyclase, and nitric oxide activated partially purified guanylate cyclase from platelets by heme-dependent mechanisms.

Thus, nitric oxide not only caused vascular smooth muscle relaxation but also inhibited platelet aggregation, and both biological responses were mediated by cyclic GMP. It is perhaps of interest that these two biological actions of nitric oxide were discovered before or at the time of the discovery of endothelium-dependent vascular smooth muscle relaxation by Robert Furchgott in 1980.^[14] In an attempt to better understand the signal transduction mechanisms involved in the stimulation of cyclic GMP formation by nitric oxide, experiments were designed to elucidate the biochemical mechanism by which nitric oxide activates guanylate cyclase.

Mechanism of Activation of Guanylate Cyclase by Nitric Oxide

Some of the early mechanistic experiments on guanylate cyclase focused on the possible involvement of enzyme-bound thiol groups in the regulation of catalysis.^[15–18] We followed up on these studies and found that guanylate cyclase was very sensitive to oxygen, undergoing reversible inactivation with increasing oxygen tension.^[19] Sulfhydryl oxidizing agents produced identical effects which were prevented and reversed by addition of sulfhydryl reducing agents. Both basal enzymatic activity and activation of the enzyme by nitric oxide were affected similarly. Protection against enzyme inactivation was afforded by addition of either excess substrate (MgGTP) or thiol antioxidants. The thiol 2,3-dimercaprol, which possesses vicinal dithiol groups, but not dithiothreitol markedly inhibited guanylate cyclase activity, indicating that two closely juxtaposed SH groups may be located at the active site. Additional studies suggested that enzyme SH groups might bind to nitric oxide as part of the enzyme activation process. However, these studies were not definitive in terms of defining the mechanism of activation of guanylate cyclase by

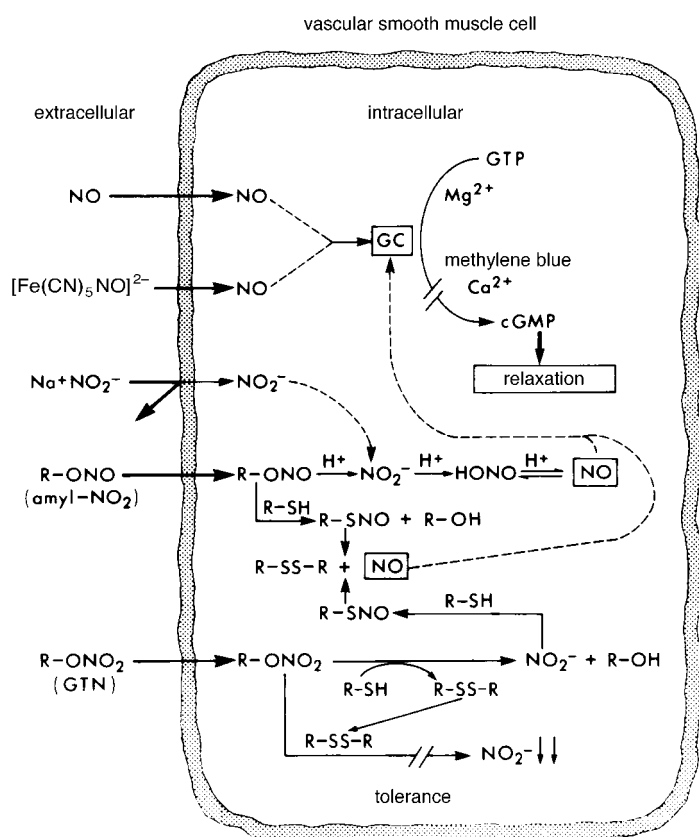


Figure 3. Schematic diagram of the proposed mechanisms by which organic nitrate and nitrite esters, inorganic nitrite, and nitroso compounds relax vascular smooth muscle. Nitroprusside liberates NO in solution, whereas organic nitrite and nitrate esters require chemical interaction with thiols to generate *S*-nitrosothiols, which then decompose with the liberation of NO. NO activates guanylate cyclase, and the resulting cyclic GMP causes vascular smooth muscle relaxation. Tolerance to nitroglycerin develops as a result of depletion of thiols and therefore production of *S*-nitrosothiols and NO. This is a schematic representation of data published by Ignarro et al.^[10] NO = nitric oxide; $[\text{Fe}(\text{CN})_5\text{NO}]^{2-}$ = nitroprusside; R-ONO = organic nitrite ester; amyl-NO₂ = amyl nitrite; R-ONO₂ = organic nitrate ester, GTN = nitroglycerin; GC = cytosolic guanylate cyclase; cGMP = cyclic GMP; R-SH = thiol; R-SNO = *S*-nitrosothiol.

nitric oxide. After reading two papers published by Craven and DeRubertis,^[20,21] who suggested that heme may be required for guanylate cyclase activation by nitric oxide, we decided to purify cytosolic guanylate cyclase and look for enzyme-bound heme. Heme iron, when in the reduced ferrous state, has a high binding affinity for nitric oxide, and this might explain why nitric oxide is such a potent activator of guanylate cyclase. Sure enough, cytosolic guanylate cyclase purified from bovine lung was found to contain one mole of heme bound per mole of enzyme dimer.^[22,23] Similar results were reported by another group.^[24]

We wanted to ascertain whether the heme bound to guanylate cyclase was indeed required for activation by nitric oxide and whether the heme played any role in the catalytic mechanism of the enzyme. Various procedures were attempted to remove the heme from guanylate cyclase without destroying the enzyme, but this approach was unsuccessful at first. Accidentally, we found that passing relatively crude cytosolic fractions containing guanylate cyclase through ion-exchange resins such as diethylaminoethyl cellulose at 9 °C instead of 2 °C (the cold room thermostat was not operating properly) caused the heme to detach from the enzyme without causing enzyme inactivation.^[23] That is, basal catalytic activity remained unaltered but guanylate cyclase was incapable of being activated by nitric oxide. Preformed nitrosyl-heme, however, markedly activated the heme-deficient enzyme. Addition of heme back to enzyme reaction mixtures completely restored activation of heme-deficient guanylate cyclase by nitric oxide. Eventually, we learned how to prepare heme-containing, heme-deficient, and heme-reconstituted forms of guanylate cyclase from rat liver and bovine lung with relative ease.^[25] The interpretation of the data was clear (Figure 4). Cytosolic guanylate cyclase was a hemoprotein, and the function of the heme prosthetic group was to bind to and react with nitric oxide to form the nitrosyl-heme complex and allow enzyme activation by nitric oxide. In addition, guanylate cyclase possessed at least two reactive SH groups at or near the catalytic site, which had to be in the

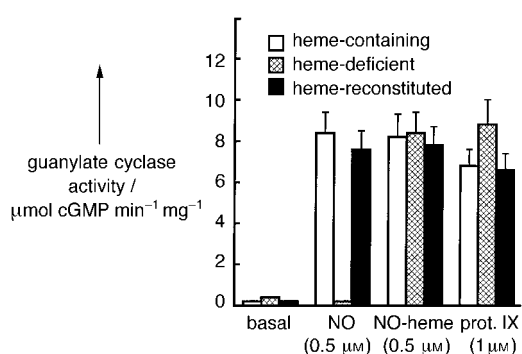


Figure 4. Heme-dependent activation of cytosolic guanylate cyclase by nitric oxide (NO). Guanylate cyclase was purified from bovine lung and assayed as described.^[22] The purified enzyme contained stoichiometric quantities of heme. Heme was removed from aliquots of native enzyme to yield heme-deficient guanylate cyclase, and the heme-deficient enzyme was reconstituted with stoichiometric quantities of heme as described.^[23,25] Enzyme reactions were conducted for 5 min under initial velocity conditions. Data represent the mean $\pm$ SEM of 12 determinations from three separate experiments. NO-heme = preformed nitrosyl-heme; prot. IX = protoporphyrin IX.

reduced state for the full expression of catalytic activity and activation by nitric oxide.^[19]

The next step was to elucidate the mechanism by which nitrosyl-heme activates guanylate cyclase. This was a difficult question to answer because the structure of guanylate cyclase was unknown. After studying the published interactions between hemoglobin or myoglobin and nitric oxide, we came up with a hypothesis to explain how nitrosyl-heme activates guanylate cyclase. We envisioned that heme (iron-protoporphyrin IX) was bound to guanylate cyclase at a site that was adjacent to the catalytic site that binds the substrate MgGTP. As in other hemoproteins, the axial ligand (bond between heme iron and protein) was thought to involve a histidine residue in guanylate cyclase, thereby yielding a five-coordinate complex (where the enzyme protein and each of the four pyrrole nitrogen atoms are ligands for the heme iron). Removal of the heme group does not influence basal catalytic activity, but abolishes enzyme activation by nitric oxide. We proposed that nitric oxide forms a covalent bond with the reduced heme iron (ferrous) to yield a five-coordinate complex rather than a six-coordinate complex. This means that the axial ligand would have to break and the resulting nitrosyl-heme complex would undergo a change in configuration while binding to the enzyme protein. This conformational change near the catalytic site could increase the affinity of guanylate cyclase for MgGTP and also increase the maximal velocity of catalysis (Figure 5). In order to test this hypothesis, protoporphyrin IX (heme without its iron) was tested to see if it could activate heme-deficient guanylate cyclase. The reasoning behind this experiment was that the axial ligand between heme iron and guanylate cyclase might create steric hindrance to the binding of MgGTP to the catalytic site, whereas protoporphyrin IX should bind to the heme binding site but create no steric hindrance because of the absence of iron, and the result should be enzyme activation by protoporphyrin IX. That is exactly what we observed. Protoporphyrin IX was a potent activator of guanylate cyclase,^[22] and it mimicked the activating effect of nitrosyl-heme in that enzyme activation by protoporphyrin IX was heme-independent, like that for preformed nitrosyl-heme.^[26] A kinetic analysis revealed that protoporphyrin IX and nitrosyl-heme activated guanylate cyclase by kinetically indistinguishable mechanisms.^[26]

These observations revealed that guanylate cyclase possessed a porphyrin binding site that was responsible for enzyme activation by nitric oxide. More elaborate studies were later conducted by others^[27–31] to verify our original hypothesis.^[32] Revelation of the crystal structure of cytosolic guanylate cyclase will undoubtedly explain the more precise mechanisms by which nitric oxide activates guanylate cyclase.

Identification of Endothelium-Derived Relaxing Factor as Nitric Oxide

Most of the research discussed above was conducted before or shortly after the discovery of endothelium-dependent vasorelaxation by Robert Furchgott in 1980.^[14] The knowledge that nitroglycerin was such a potent vasorelaxant and the

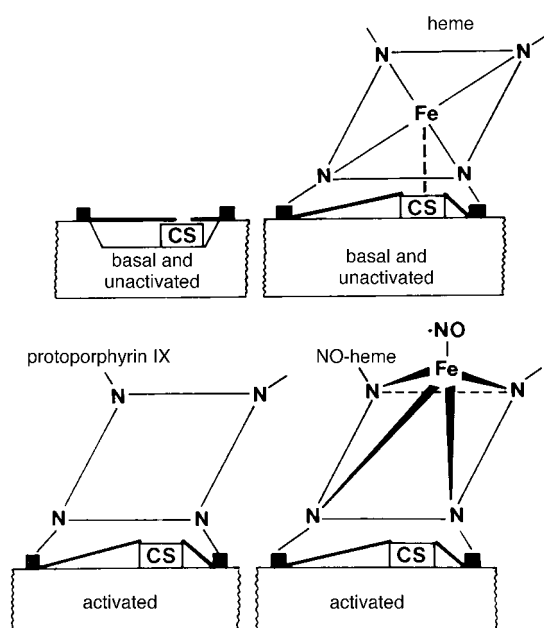


Figure 5. Schematic illustration of the mechanism of heme-dependent activation of cytosolic guanylate cyclase by nitric oxide (NO). This schematic is based on the knowledge that heme is required for the activation of guanylate cyclase by NO. In the basal or unactivated state and in the absence of the heme prosthetic group, the catalytic site (CS) in guanylate cyclase is only partially exposed so that substrate (MgGTP) availability is limited and cyclic GMP production is limited. In the presence of the heme prosthetic group (native enzyme) but in the absence of any NO (basal or unactivated state), catalytic activity is still minimal because of steric hindrance at the catalytic site. Enzyme-bound heme permits exposure of the catalytic site, but the axial ligand (bond between heme iron and enzyme protein near the CS) limits access of substrate to CS, thereby limiting cyclic GMP production. NO binds to heme iron to form the nitrosyl-heme adduct as illustrated. This NO-heme complex remains as a five-coordinate complex, thereby resulting in the breakage of the axial ligand and exposure of the CS to enzyme substrate. The activated enzyme shows up to a 400-fold increase in $V_{\max}$ and a threefold decrease in the K_m for MgGTP. Addition of protoporphyrin IX to heme-deficient guanylate cyclase mimics the effect of NO-heme because protoporphyrin IX binding allows exposure of the CS without any steric hindrance due to the absence of heme iron.

more recent findings that nitric oxide was a potent vasorelaxant and inhibitor of platelet aggregation led to the question of why mammals have receptors for such potent pharmacological compounds. This thought suggested that mammals might possess either an endogenous nitro compound that releases nitric oxide or nitric oxide itself. The early studies on endothelium-derived relaxing factor (EDRF) were conducted without any knowledge about its chemical structure. We started to study endothelium-dependent vasorelaxation in 1983 because we wanted to determine whether cyclic GMP was involved, and not because we thought that EDRF might be nitric oxide. Indeed, a separate project was also underway to find the endogenous nitric oxide. Just as we completed our experiments on EDRF and

cyclic GMP, Ferid Murad's group published a paper showing that endothelium-dependent vasorelaxation by acetylcholine and other agents was associated with cyclic GMP production in vascular smooth muscle.^[33] We observed similar results and also found that methylene blue, an inhibitor of guanylate cyclase activity, prevented both the cyclic GMP accumulating effect and vasorelaxant effect of acetylcholine.^[34] At first, we entertained the idea that acetylcholine might be stimulating the formation of a metabolite of arachidonic acid, which then somehow activated guanylate cyclase to elevate cyclic GMP levels. This view was supported by studies from this and other laboratories showing that agents that interfere with the formation of arachidonic acid and certain arachidonic acid metabolites also interfere with acetylcholine-mediated vasorelaxation.^[33–35] But I was not satisfied with this view because experiments in my laboratory failed to reveal that anything other than nitric oxide could activate guanylate cyclase. After reviewing our data again, we suddenly realized that our finding that methylene blue prevented both the increase in cyclic GMP and vasorelaxation in response to acetylcholine was similar to our earlier finding on the prevention of both cyclic GMP formation and vasorelaxation in response to nitric oxide.^[36] This indicated that the vasorelaxant action of acetylcholine was pharmacologically similar to that for nitric oxide. This concept is indicated in Figure 6, which was published in 1984.^[34] We were cautious, however, and did not suggest in writing that EDRF might be nitric oxide. Later, after other laboratories showed that EDRF was a very unstable molecule,^[37,38] we realized that the data from several laboratories were consistent with our view that EDRF and nitric oxide were closely similar. The two distinct projects in my laboratory, one focusing on EDRF/cyclic GMP and the other focusing on endogenous nitric oxide, converged, but we were not yet ready to propose that EDRF was nitric oxide.

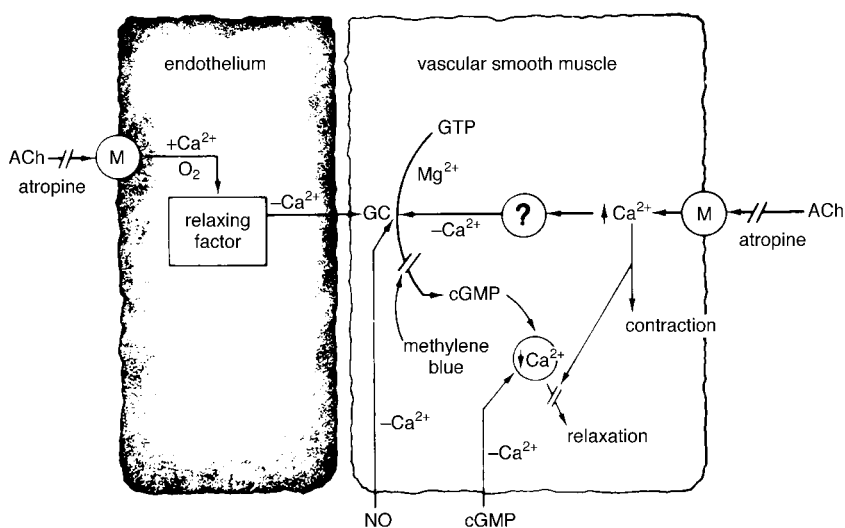


Figure 6. Schematic diagram of the similarities in the mechanisms of vascular smooth muscle relaxation elicited by acetylcholine and nitric oxide (NO). Acetylcholine and NO cause vasorelaxation by mechanisms involving cyclic GMP formation. Blockade of cyclic GMP formation by methylene blue inhibits vasorelaxation by both acetylcholine and NO, as illustrated. ACh = acetylcholine; M = muscarinic receptor; GC = cytosolic guanylate cyclase; cGMP = cyclic GMP; $-Ca^{2+}$ = calcium-independent mechanism; $+Ca^{2+}$ = calcium-dependent mechanism. Modified from reference [34] with permission.

A series of experiments was designed to test our yet unpublished hypothesis that EDRF might be nitric oxide. The first experiment was to determine whether EDRF released from artery and vein could activate guanylate cyclase and thereby account for elevated tissue cyclic GMP levels in response to acetylcholine or bradykinin, and this study was published in 1986.^[39] Isolated arterial and venous rings prepared from bovine intrapulmonary vessels were incubated in reaction mixtures containing cytosolic guanylate cyclase purified from bovine lung. Addition of acetylcholine to reaction mixtures containing arterial rings resulted in the activation of guanylate cyclase, and bradykinin elicited the same effect in reaction mixtures containing either arterial or venous rings. Activation of guanylate cyclase by acetylcholine and bradykinin was dependent on the presence of an intact endothelial layer in arterial and venous rings, and the response was blocked by methylene blue and enhanced by antioxidants. These experiments revealed that EDRF from artery and vein activates guanylate cyclase by mechanisms that can be inhibited by methylene blue and enhanced by antioxidants. Clearly, the data were pointing toward the possibility that EDRF is nitric oxide or a chemically related substance such as a labile nitroso compound. The next experiment was to determine whether the activation of guanylate cyclase by EDRF was heme-dependent, as is enzyme activation by nitric oxide. Experiments similar to those described above were repeated using guanylate cyclase that had been purified in both the heme-containing and heme-deficient forms. Sure enough, guanylate cyclase activation by EDRF, like enzyme activation by nitric oxide, was heme-dependent (Figure 7). At the end of the first experiment of

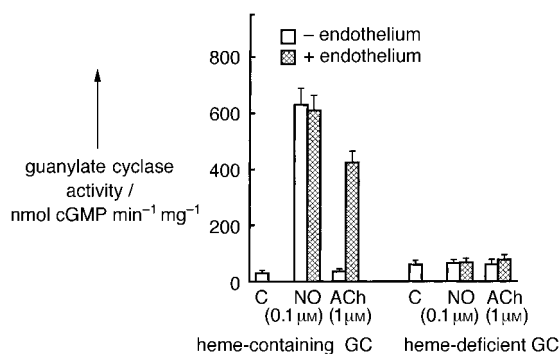


Figure 7. Endothelium-dependent and heme-dependent activation of cytosolic guanylate cyclase by endothelium-derived relaxing factor (EDRF) released from arterial rings. These experiments were based on techniques described previously.^[39] Bovine intrapulmonary arterial rings were prepared with and without intact endothelium and mounted in tissue baths for monitoring vasorelaxation. Acetylcholine (ACh) and nitric oxide (NO) were added to tissue baths to monitor relaxant responses. Acetylcholine elicited endothelium-dependent, whereas NO elicited endothelium-independent vasorelaxation. After the first round of addition to the tissue baths, the rings were rapidly detached and lowered into reaction vessels containing either heme-containing guanylate cyclase or heme-deficient guanylate cyclase, to which was added acetylcholine or NO. As illustrated, acetylcholine and NO caused heme-dependent activation of guanylate cyclase, but only NO caused endothelium-independent activation of guanylate cyclase. The conclusion was that acetylcholine causes the formation of NO from the vascular endothelium, and NO causes heme-dependent activation of guanylate cyclase. Data represent the mean $\pm$ SEM of 12 determinations from six separate experiments. C = control.

this series, we knew that we had it. EDRF must be nitric oxide. But I wanted to conduct more definitive experiments before going out on a limb and advocating that EDRF is nitric oxide. A bioassay cascade apparatus was set up in order to study the chemical and pharmacological properties of EDRF released from artery and vein, and to compare these with the properties of nitric oxide. These experiments revealed the very close similarity of EDRF and nitric oxide, and were presented at several conferences in 1986 and early 1987^[40–42] before their publication in 1987.^[43, 44]

There was one experiment in particular that convinced me beyond any shadow of a doubt that EDRF was nitric oxide. We took advantage of a previous experiment in my laboratory on guanylate cyclase, in which we demonstrated that guanylate cyclase was a hemoprotein, like hemoglobin, that reacted with nitric oxide to form the nitrosyl-heme adduct by monitoring the spectral shift in the Soret region.^[23] This classical chemical analysis provides proof of the presence of heme iron, and established guanylate cyclase as a hemoprotein. Accordingly, we conducted similar experiments with EDRF released from cultured bovine aortic endothelial cells and showed that EDRF caused a spectral shift in hemoglobin that was identical to that obtained for authentic nitric oxide (Figure 8).^[43] Suddenly, all previous data from our laboratory and several other laboratories made complete sense and were consistent with the view that EDRF was nitric oxide. EDRF is a small, labile, and lipophilic molecule that is inactivated by oxygen and oxygen-derived radicals, activates cytosolic guanylate cyclase by heme-dependent mechanisms, causes smooth muscle relaxation and inhibition of platelet aggregation and adhesion by cyclic GMP dependent mechanisms, and whose actions are blocked by hemoproteins or methylene blue but enhanced by antioxidants or oxygen radical scavengers.

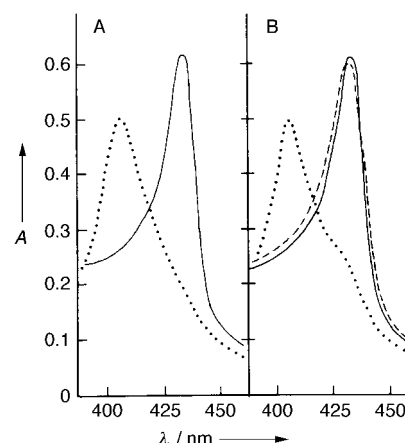


Figure 8. Identification of endothelium-derived relaxing factor (EDRF) as nitric oxide (NO) by spectrophotometric analysis of the reaction between deoxyhemoglobin and NO or EDRF. The EDRF was generated from freshly isolated bovine aortic endothelial cells stimulated by the calcium ionophore, A23187. Experiments were performed in quartz cuvettes as described previously.^[43] A) The solid curve signifies 5 μ M deoxyhemoglobin alone; the dotted curve signifies deoxyhemoglobin gassed with NO. B) The solid curve signifies 5 μ M deoxyhemoglobin alone; the dashed curve signifies deoxyhemoglobin after reaction with 10^6 endothelial cells; the dotted curve signifies deoxyhemoglobin after reaction with endothelial cells in the presence of 1 μ M A23187. Data illustrated are from one experiment representative of three separate experiments. Modified from reference [43] with permission.

At the time we were conducting some of the above experiments, other laboratories were conducting experiments that turned out to be consistent with the view that EDRF was nitric oxide. For example, hemoglobin was reported to interfere with acetylcholine-elicited, endothelium-dependent vasorelaxation,^[45, 46] superoxide anion was shown to decrease whereas superoxide dismutase increased the chemical stability of EDRF,^[57, 38] and EDRF inhibited platelet aggregation.^[47] All of these effects on or actions of EDRF were identical to those for nitric oxide. Despite this obvious analogy, no one wanted to go out on a limb and propose that EDRF was nitric oxide, except for Robert Furchgott and myself, when we independently proposed this hypothesis at the same international conference in Rochester, Minnesota in June of 1986.^[40]

The Physiological Significance of Nitric Oxide in Vascular Biology

Soon after the discovery that EDRF is nitric oxide, evidence was presented that vascular endothelial cells can produce nitric oxide and citrulline from arginine.^[48] This finding was remarkably similar to the observation in cytokine-activated macrophages that nitrite (NO_2^-) and nitrate (NO_3^-) are produced from arginine.^[49–51] Indeed, these studies revealed that a structural analogue of arginine, N^G -methylarginine, inhibited the formation of NO_2^- and NO_3^- from arginine in macrophages and also inhibited the production of nitric oxide from arginine in endothelial cells. Confirmation of these original observations and unequivocal demonstration of an enzyme that catalyzes the conversion of arginine into nitric oxide plus citrulline was made by Bredt and Snyder.^[52] The enzyme was termed nitric oxide synthase, and this study started an avalanche of research leading to the isoform characterization, purification, elucidation of catalytic mechanisms, and structure–function characterization of nitric oxide synthase.

During this period of time, incredible process was also being made on the physiological and pathophysiological properties of endogenous nitric oxide. Vascular endothelial cells synthesize nitric oxide continuously for the purpose of causing vasodilation to limit any increases in systemic blood pressure.^[62, 63] This conclusion was drawn from experiments in which N^G -methylarginine, a competitive inhibitor of nitric oxide synthase, caused a sustained increase in systemic blood pressure in animals after intravenous injection. The hypertensive response was completely reversed by intravenous injection of excess L-arginine, the substrate for nitric oxide synthase. The continuous generation of endothelium-derived nitric oxide appears to occur as a consequence of continuous blood flow or shear stress,^[64] which can trigger the calcium-dependent activation of endothelial nitric oxide synthase.^[65] Endothelium-derived nitric oxide also functions to inhibit platelet aggregation and adhesion,^[66] and to slow or prevent proliferation of the underlying vascular smooth muscle.^[67] Research continues on the elucidation of the protective role of nitric oxide in reperfusion or reoxygenation injury, restenosis, and atherosclerosis. The protective effects of nitric oxide may stem from its capacity to improve local blood flow,

inhibit thrombosis or cell adhesion, interfere with cell proliferation, and inhibit key enzymes involved in mediating cellular modification or destruction.

The neurotransmitter function of nitric oxide was first shown in the brain.^[68] These observations were based on the findings that glutamate and nitric oxide stimulate the formation of cyclic GMP in the brain, and that arginine can also elevate cyclic GMP levels under certain conditions. The function of nitric oxide as a neurotransmitter in the central nervous system remains unknown, but one possible hypothesis has been that nitric oxide modulates long-term potentiation in the control of learning and memory.^[66] The bad side of nitric oxide is that excessive production of nitric oxide in the brain stimulated by glutamate can lead to serious tissue injury in specialized regions of the brain.^[69] Moreover, excessive production of nitric oxide in close proximity to oligodendrocytes can lead to inhibition of myelin formation and perhaps multiple sclerosis.^[70] Evidence has amassed that nitric oxide is an important transmitter of the nonadrenergic-noncholinergic (NANC) neurons that innervate many smooth muscle tissues such as the airways, gastro-intestinal tract, and genito-urinary tract.^[66]

We explored the possibility that nitric oxide might be the principal neurotransmitter that mediates penile erection. The reason for pursuing this project was that before 1990 the physiological mechanism by which nerve stimulation promotes erectile function was unknown. The common belief was that excitation of the parasympathetic nervous system caused acetylcholine-mediated erectile responses. The erectile response consists of neuronally mediated relaxation of both vascular and nonvascular smooth muscle associated with the cavernous or trabecular or sinusoidal arrangement of arterioles in the corpus cavernosum. The problem that this hypothesis presented to pharmacologists and physiologists is that the cholinergic neurotransmitter, acetylcholine, cannot relax non-vascular smooth muscle and can relax vascular smooth muscle only by endothelium-dependent mechanisms. It was viewed highly unlikely that acetylcholine, a quaternary ammonium compound (highly charged), would reach the vascular endothelial cells from the neuronal synapses at the vascular smooth muscle cells. However, excitation of any nitric oxide containing NANC nerves that innervate the nonvascular and vascular smooth muscle in the corpus cavernosum would be expected to promote extensive smooth muscle relaxation and consequent penile erection. At the time of our first experiment, it was not known whether or not erectile tissue in mammals was innervated by NANC nerves, and we were not equipped to conduct such histochemical experiments. Instead, we used the classical tissue bath approach in which strips of corpus cavernosum isolated from rabbit and human penis were mounted in bath chambers, precontracted, and then electrically stimulated in order to cause smooth muscle relaxation.

Our first experiment revealed that NANC stimulation of isolated strips of rabbit corpus cavernosum resulted in smooth muscle relaxation that was blocked by inhibitors of nitric oxide synthase, hemoglobin, and methylene blue.^[71] Nitric oxide in the form of *S*-nitrosothiols also caused smooth muscle relaxation, and electrical stimulation of the tissue resulted in elevated levels of both nitrite (NO_2^-) and cyclic

GMP.^[71] The conclusion was clear. Nitric oxide is the neurotransmitter in NANC nerves that innervate the corpus cavernosum, and nerve stimulation results in nitric oxide mediated vascular and nonvascular smooth muscle relaxation. The action of the nitric oxide neurotransmitter is, in turn, mediated by cyclic GMP. Therefore, the signal transduction pathway involving nitric oxide as a neurotransmitter appears to be the same as that for endothelium-derived nitric oxide. Additional experiments with human corpus cavernosum yielded virtually identical data as the experiments using rabbit tissue.^[72, 73] Figure 9 illustrates the typical marked but transient smooth muscle relaxation responses elicited by electrical stimulation of corpus cavernosum from both rabbit and human. We were struck with the very transient nature of the responses and the fact that these electrically induced responses could be mimicked by addition of authentic nitric oxide gas. Perhaps the most convincing experiment indicating that electrical stimulation triggers erectile function by mechanisms involving nitric oxide was the finding that *N*^G-nitro-arginine, a competitive inhibitor of nitric oxide synthase, abolished the electrical responses, whereas excess enzyme substrate, *L*-arginine, overcame the inhibitory effect of *N*^G-nitroarginine (Figure 10). Our early studies also revealed that inhibition of the degradation of cyclic GMP through the use of phosphodiesterase inhibitors caused an enhancement of both electrically induced smooth muscle relaxation and nitric oxide elicited smooth muscle relaxation.^[72–74] The data for enhancement of nitric oxide elicited relaxation of corpus cavernosum by the cyclic GMP phosphodiesterase inhibitor, M&B 22,948 (zaprinast), is illustrated in Figure 11. These observations indicated clearly that relaxation of corpus cavernosum smooth muscle by NANC nerve excitation or addition of nitric oxide is mediated by cyclic GMP.

These observations shed a great deal of light on the physiological mechanism of penile erection by revealing a critical signal transduction pathway that could be modulated by drugs. For example, drugs that interfere with the formation or action of nitric oxide or cyclic GMP could also interfere with the erectile response, and might be useful for treating priapism. But much more importantly, drugs that enhance the formation or action of nitric oxide or cyclic GMP could also enhance the erectile response, and might be effective in the treatment of impotence, the most prevalent medical disorder in men. In 1998 the drug sildenafil (Viagra) was marketed for the effective treatment of impotence. Sildenafil is a relatively selective inhibitor of one of the phosphodiesterase isoforms that degrades cyclic GMP and is present in relatively large quantities

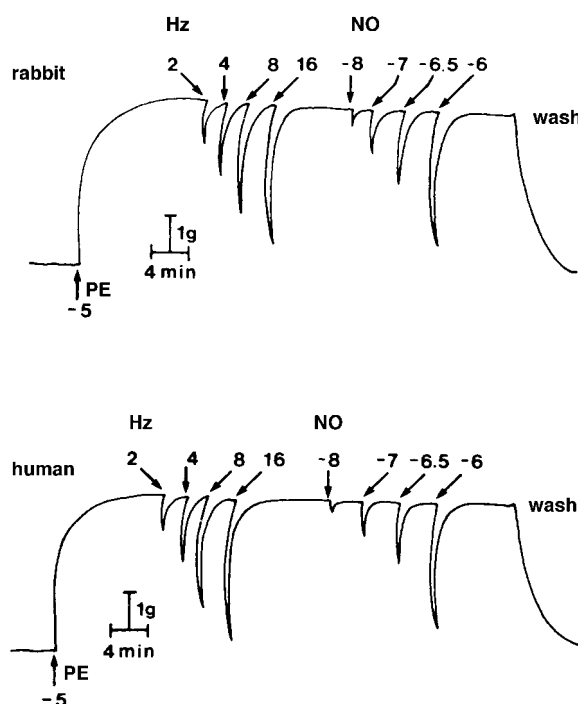


Figure 9. Relaxation of corpus cavernosum smooth muscle by nitric oxide (NO) closely resembles relaxation elicited by electrical field stimulation of NANC nerves. Strips of corpus cavernosum from rabbit and human penis were prepared and mounted in tissue baths, and isometric tension recordings obtained.^[71, 73] Smooth muscle was precontracted by addition of phenylephrine (PE) as shown. Electrical field stimulation was conducted at 2, 4, 8, and 16 Hz as shown. Concentrations of NO and PE are expressed as logs of molar concentrations. Tracings are representative of 12–18 strips from four rabbits or four humans.

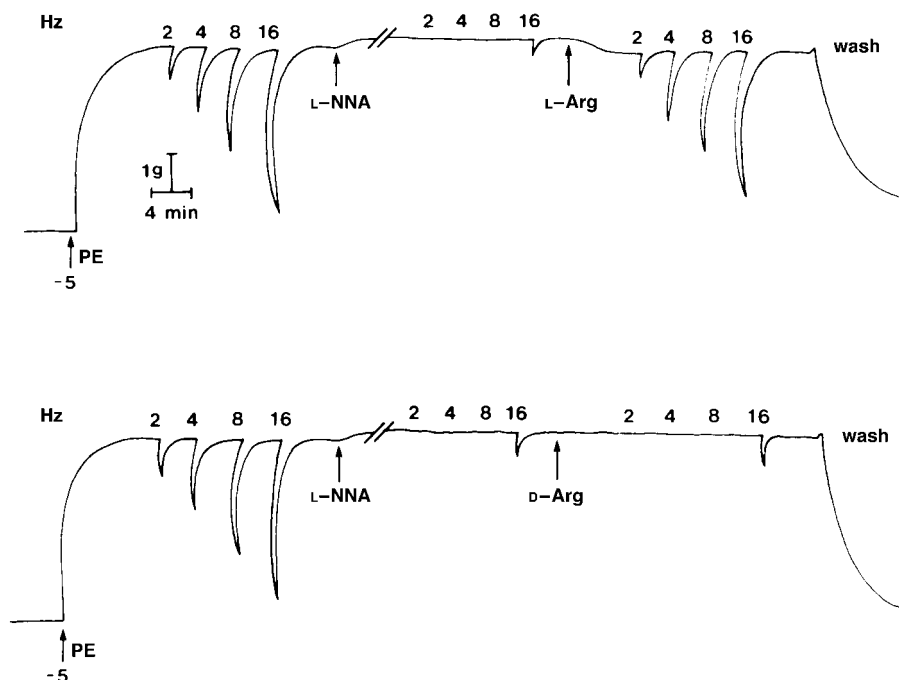


Figure 10. Relaxation of human corpus cavernosum smooth muscle by electrical field stimulation is blocked by nitric oxide synthase inhibitors. Strips of corpus cavernosum from human penis were prepared and mounted in tissue baths, and isometric tension recordings obtained.^[71, 73] Smooth muscle was precontracted by addition of phenylephrine (PE) as shown. Electrical field stimulation was conducted at 2, 4, 8, and 16 Hz as shown. *N*^G-Nitroarginine (*L*-NNA) and arginine (*L*-Arg or *D*-Arg) were tested at 30 and 300 μ M, respectively. Tracings are representative of 12–18 strips from four humans.

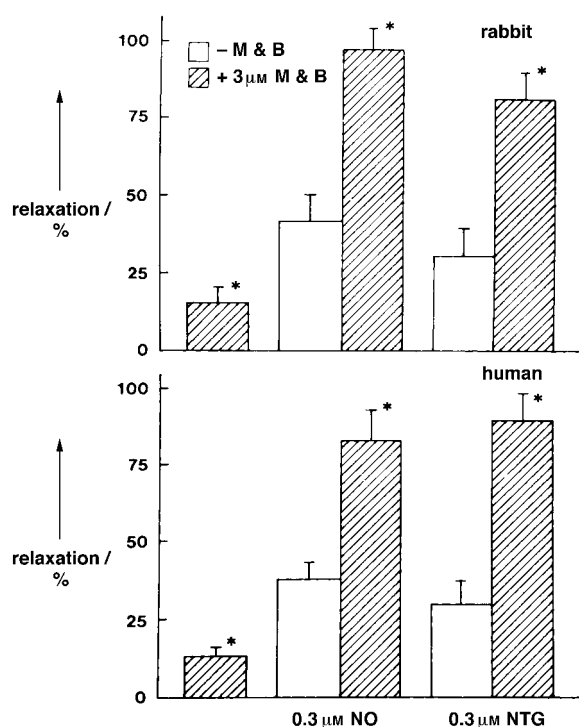


Figure 11. Enhancement of nitric oxide (NO) elicited and nitroglycerin (NTG) elicited relaxation of rabbit and human corpus cavernosum smooth muscle by M&B 22,948 (M&B). Strips of corpus cavernosum from rabbit and human penis were prepared and mounted in tissue baths, and isometric tension recordings obtained.^[71,73] Smooth muscle was precontracted by addition of 10 μ M phenylephrine. Each point represents the mean $\pm$ SEM of 9–12 strips from four rabbits or four humans. The asterisks indicate that all values obtained in the presence of M&B are significantly different ($p < 0.001$) from corresponding values obtained in the absence of M&B.

in the corpus cavernosum. Therefore, sildenafil allows intracellular cyclic GMP in corpus cavernosum smooth muscle to accumulate to high levels when ever there is a nitric oxide signal coming from the NANC nerves. Penile erection resulting from any form of excitation of the NANC nerves innervating the corpus cavernosum, whether mental or physical, would be enhanced by sildenafil, but the drug alone would not be expected to cause an erectile response in the absence of any NANC nerve excitation. Sildenafil appears to be very effective in treating various types and degrees of impotence. Our improved understanding of the physiology of erectile function will enable scientists to unravel the pathophysiology and causes of impotence, thereby further opening the doors to develop even more promising drugs for the treatment of impotence.

Future Directions for Nitric Oxide Research in Vascular Biology

The knowledge that vascular endothelium-derived nitric oxide and neuronally generated nitric oxide promote the relaxation of vascular and nonvascular smooth muscle associated with the vascular system will undoubtedly afford scientists and clinicians the opportunity to make great advances in the diagnosis, treatment, and prevention of life-threatening cardiovascular disorders such as coronary artery

disease, essential hypertension, stroke, vascular complications of diabetes, and related diseases. Already, it appears that nitric oxide plays many critical physiological roles in the regulation and protection of numerous tissue and organ functions. Defects or lesions in the nitric oxide–cyclic GMP pathway can probably account for much of the observed pathophysiology of cardiovascular disease. The development of a better understanding of basic physiological mechanisms involving the nitric oxide–cyclic GMP pathway should lead to the development of novel therapeutic strategies to reduce the morbidity and mortality attributed to cardiovascular disease.

Many individuals have been responsible for my development as a scientist and to whom I am grateful. My mentors Fred Shideman and Elwood Titus taught me the meaning of concepts like the experimental approach, the establishment and testing of the hypothesis, the critical evaluation of experiments that not only work but also do not work as expected, and the importance of thinking. In my first job after my postdoctoral training, Barbara Petrack taught me the meaning of respect and hard work in science. In my early academic career, my colleagues Bill George and Phil Kadowitz provided strong encouragement and motivation. Jim Fisher consistently motivated me by example to develop into an effective teacher of both medical and graduate students. Perhaps I owe my most profound gratitude to Gautam Chaudhuri, who provided the greatest encouragement and motivation for me to continue my work and take it to new highs. All of my graduate students, postdoctoral fellows, clinical fellows, and visiting scientists have made substantial contributions. These individuals include (in alphabetical order) John Adams, Ernesto Aeberhard, Nicole Arabolos, William Aronson, Bryan Ballot, Barbara Barry, Georgette Buga, Theresa Burke, Peggy Bush, Russell Byrns, Stella Cech, Gordon Cohen, Ronald Day, Jonathan Degnan, James Edwards, Richard Fitch, Michele Gold, Jeanette Griscavage, Rosemarie Gross, Carl Gruetter, Darlene Gruetter, Richard Harbison, Toshio Hayashi, Adrian Hobbs, Philip Horwitz, Howard Lipton, Dennis McNamara, B. Theo Mellion, Steve Napoli, Eliot Ohlstein, Richard Paddock, Waldemar Radziszewski, Norma Rogers, Robert Smith, Hugo Vargas, Liu Hua Wei, Michael Wolin, and Keith Wood.

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A sure way of obtaining accurate calculated results is shown in this picture:
It goes from the top left (HF/STO-3G) to the bottom right (FCI/ ∞).

Method \ Basis	HF	MP2	MP3	MP4	QCI	FCI
STO-3G						
6-31G						
6-31G(d)						
6-31+G(d)						
6-311+G(d)						
6-311+G(2df)						
∞						
Basis \ Method	HF	MP2	MP3	MP4	QCI	FCI

Quantum Chemical Models (Nobel Lecture)**

John A. Pople*

1. Introduction

The fundamental underpinnings of theoretical chemistry were uncovered in a relatively short period at the beginning of the present century. Rutherford's discovery of the nucleus in 1910 completed the identification of the constituent subparticles of atoms and molecules and was followed shortly thereafter by the Bohr treatment of electronic orbits in atoms, the "old quantum theory". The relation between the positive nuclear charge, atomic number, and position of an atom in the periodic table was uncovered by 1913. It proved difficult to extend Bohr's orbits to a polyatomic situation and the next advance had to await the development of the wave theory of matter and the associated quantum mechanics in the early 1920s. By 1926 Heisenberg had developed matrix mechanics and Schrödinger had proposed the basic nonrelativistic wave equation governing the motion of nuclei and electrons in molecules [Eq. (1)].

$$\mathcal{H}\Psi = E\Psi \quad (1)$$

This is a differential eigenvalue equation for the energy E and wavefunction Ψ of a particular state. $\mathcal{H}$ is the Hamiltonian operator and Ψ depends on the cartesian and spin coordinates of the component particles. The only further restrictions are the permutational symmetry requirements for Ψ (antisymmetry for fermions such as electrons and symmetry for bosons). A relativistic generalization of this equation was proposed a short time later by Dirac.

The Schrödinger equation is easily solved for the hydrogen atom and found to give results identical to the earlier treatment of Bohr. With inclusion of relativistic corrections from the Dirac equation, almost perfect agreement was found with experimental spectroscopic data. However, exact solution for any other system was not found possible, leading to a famous remark by Dirac in 1929:

"The fundamental laws necessary for the mathematical treatment of a large part of physics and the whole of

chemistry are thus completely known, and the difficulty lies only in the fact that application of these laws leads to equations that are too complex to be solved."

This was a cry both of triumph and of despair. It marked the end of the process of fundamental discovery in chemistry but left a colossal mathematical task of implementation. In retrospect, the implied finality of the claim seems excessively bold. In 1929 there had only been one preliminary approximate quantum mechanical calculation on the hydrogen molecule by Heitler and London, leading to a value of the bond energy of only about 70% of the experimental value. Nevertheless, the physicists were highly confident and most moved on to study the internal structure of the nucleus during the 1930s. In fact, their boldness was apparently justified, for no significant failure of the full Schrödinger–Dirac treatment has ever been demonstrated.

This was the challenge presented to the early quantum chemists by 1930. Given the hopelessness of exact solution, how would it be possible to develop approximate mathematical procedures that could a) assist the qualitative interpretation of chemical phenomena and b) provide predictive capability. Attempts to approach this problem by a model approach is the topic addressed here.

2. Features of Theoretical Models

A theoretical model for any complex process is an approximate but well-defined mathematical procedure of simulation. When applied to chemistry, the task is to use input information about the number and character of component particles (nuclei and electrons) to derive information and understanding of resultant molecular behavior. Five stages may be distinguished in the development and use of such a model:

1. Target

A target accuracy must be selected. A model is not likely to be of much value unless it is able to provide clear distinction between possible different modes of molecular behavior. As the model becomes quantitative, the target should be that data is reproduced and predicted within experimental accuracy. For energies, such as heats of formation or ionization potentials, a global accuracy of 1 kcal mol⁻¹ would be appropriate.

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2. Formulation

The approximate mathematical procedure must be precisely formulated. This should be general and continuous as far as possible. Thus, particular procedures for particular molecules or particular symmetries should be avoided. If this can be done, the procedure becomes a full theoretical model chemistry, which can be explored in detail as far as available resources permit.

3. Implementation

The formulated method has to be implemented in a form that permits its application in reasonable times and at reasonable cost. In recent times, this stage involves the development of efficient and easily used computer programs. It is closely comparable to the stage of building equipment in an experimental investigation.

4. Verification

The next step is to test the model against known chemical facts to determine whether the target has been achieved. If quantitative accuracy is being sought this can be done by various statistical criteria such as the root-mean-square difference between the results of the theoretical model and experimental data. In selecting such a data set, it is important to make it as broad as possible, while limiting it to experimental facts known to be of high quality. If the results of such a comparison do meet the target requirements, the model may be said to be validated.

5. Prediction

Finally, if the model has been properly validated according to some such criterion it may be applied to chemical problems to which the answer is unknown or in dispute. If the experimental data set is sufficiently broad, there is a reasonable expectation that the results will be accurate to something like the target accuracy. This stage, of course, is the one of most interest to the larger chemical community.

One further aspect of theoretical models is the introduction of empirical parameterization. Models which utilize only the fundamental constants of physics are generally termed *ab initio*; if some parameters are introduced, which are determined by fitting to some experimental data, the methods are semiempirical. Clearly, there is a wide range of possible empiricism, as will be noted in subsequent parts of this article.

3. Hartree–Fock Models

During the 1930s most work was of a qualitative nature, treating the electrons as moving in independent molecular orbitals. However, the foundations of the orbital theory of many-electron systems was laid by Hartree, Fock, and Slater. If the $2n$ electrons in a closed-shell molecule are assigned to a set of n molecular orbitals ψ_i ($i = 1, \dots, n$), the corresponding many-electron wavefunction can be written as Equation (2).

$$\Psi = (2n!)^{-1/2} \det[(\psi_1 \alpha)(\psi_1 \beta)(\psi_2 \alpha) \dots] \quad (2)$$

Here the molecular orbitals ψ_i are taken to be orthonormal and α and β are spin functions. This single-configuration wavefunction is usually described as a Slater determinant.

If the molecular orbitals ψ_i are varied to minimize the energy, calculated as the expectation value of the full Hamiltonian $\mathcal{H}$ [Eq. (3)], then the energy E is fully defined

$$E = \langle \Psi | \mathcal{H} | \Psi \rangle \quad (3)$$

and, according to that variational principle, is an upper bound for the exact Schrödinger energy from the full wave Equation (1). This procedure leads to a set of coupled differential equations for the ψ_i , as first derived by Fock. The method is known as Hartree–Fock theory, with early applications having been made (to atoms) by Hartree.

Following the break due to World War II, work on quantum chemistry resumed in a number of countries. In Cambridge, Lennard-Jones and his group (of which I became a member in 1948) reexamined the Hartree–Fock equations with a view to transforming the orbitals ψ_i into localized or equivalent orbitals, representing bonding and lone electron pairs, concepts widely used in the qualitative description of molecular structure. However, the coupled three-dimensional differential equations appeared intractable and little progress was made towards their solution.

A major advance occurred in 1951 with the publication from Chicago of the Roothaan equations.^[1] (Actually, these had been circulated in a report some time earlier.) Roothaan considered molecular orbitals that were restricted to be linear combinations of a set of prescribed three-dimensional one-electron functions χ_μ ($\mu = 1, 2, \dots, N$, $N > n$) [Eq. (4)].

$$\psi_i = \sum_{\mu=1}^N c_{\mu i} \chi_\mu \quad (4)$$

John A. Pople was born in 1925 in Burnham-on-Sea in Somerset (UK). He studied at Trinity College Cambridge and was awarded his BA in 1946 and MA in 1950. In 1951 he received his PhD in Mathematics. He stayed at Cambridge until 1958.



He then worked at the National Physical Laboratory in Teddington until 1964 and at the Carnegie-Mellon University in Pittsburgh, Pennsylvania. After about 30 years he moved to Northwestern University as a Trustees' professor. He has received many awards in his long research career: He is a Fellow of the British Royal Society and the American Physical Society and has received, for example, the Marlow Medal of the Faraday Society, the Pauling Award, the U.S. Senior Scientist Award from the Humboldt Foundation, the Davy Medal from the British Royal Society, and the Wolf Prize. He also is a member of the Editorial and Advisory Boards of *Molecular Physics*, *Chemical Physics*, and *Computers in Chemistry*. His most recent research interest is the comparison between density functional and conventional orbital theories.

Variation of the total energy [Eq. (3)] was then carried out with respect to the coefficients $c_{\mu i}$. This leads to a set of algebraic equations, which can be written in matrix form [Eq. (5)] (using real functions and atomic units throughout). The individual matrix elements are defined in Equations (6)–(11). In these and subsequent equations, we follow a useful practice of using roman suffixes for molecular orbitals ψ and

$$FC = SCE \quad (5)$$

$$F_{\mu\nu} = H_{\mu\nu} + \sum_{\lambda\sigma} P_{\lambda\sigma}[(\mu\nu|\lambda\sigma) - (\mu\lambda|\nu\sigma)/2] \quad (6)$$

$$H_{\mu\nu} = \int \chi_{\mu} \mathcal{H} \chi_{\nu} d\tau \quad (7)$$

$$S_{\mu\nu} = \int \chi_{\mu} \chi_{\nu} d\tau \quad (8)$$

$$E_{ij} = \varepsilon_i \delta_{ij} \quad \text{with } \delta_{ij} = 1 \text{ for } i=j \text{ and } 0 \text{ for } i \neq j \quad (9)$$

$$P_{\mu\nu} = 2 \sum_i^n c_{\mu i} c_{\nu i} \quad (10)$$

$$(\mu\nu|\lambda\sigma) = \int \int \chi_{\mu}(1) \chi_{\nu}(1) (1/r_{12}) \chi_{\lambda}(2) \chi_{\sigma}(2) d\tau_1 d\tau_2 \quad (11)$$

greek for the expansion functions χ . $\mathcal{H}$ is the core Hamiltonian, describing motion of a single electron moving in the bare field of the nuclei. The eigenvalues ε_i are the one-electron Fock energies, with the lowest value for n corresponding to the occupied molecular orbitals 1, 2, $\dots$ n .

These nonlinear equations provide a complete mathematical model if the prescribed functions χ_{μ} are uniquely specified by the nuclear positions. They are often referred to as self consistent field (SCF) equations. In the earliest versions of molecular orbital theory, the χ_{μ} were chosen to be the atomic orbitals of the component atoms, in which case the theory was described as LCAOSCF for “linear combination of atomic orbitals”. More generally, the set $\{\chi_{\mu}\}$ is referred to as the basis set. Normal practice is to choose basis functions that are centered at the nuclei and depend only on the atomic number (positive charge) of that nucleus.

The Roothaan-type of equations can be extended to electron configurations in which some orbitals are doubly occupied and some singly. Another extension is one in which electrons of α -spin and β -spin are assigned to different molecular orbitals ψ^{α} and ψ^{β} . This is usually referred to as a spin-unrestricted configuration. There will be two sets of coefficients $c_{\mu i}^{\alpha}$ and $c_{\mu i}^{\beta}$. The corresponding generalization of the Roothaan equations was published by the author and Nesbet in 1954.^[2] These are usually denoted as Unrestricted Hartree–Fock or UHF, and the option of double and single occupation as Restricted Open Hartree–Fock or ROHF.

The introduction of basis set expansions played a major role in the development of quantum chemistry. It changed the mathematical task from the numerical solution of coupled differential equations (following the atomic work of Hartree) to the double challenge of evaluation of the three- or six-dimensional integrals (7), (8), and (11), followed by solution of the algebraic SCF Equations (5). If analytic integration were possible, the model could become precise in the sense that good arithmetic correctness would be possible, even though the underlying approximations (use of a single configuration determinant and a finite basis) might still be unsatisfactory.

During the 1950s integral evaluation was regarded as the main barrier to progress. The best choice of basis functions for LCAOSCF theory appeared to be Slater-type atomic orbitals (STO), which have exponential radial parts by analogy to the hydrogen atom. The one- and two-electron integrals (7), (8), and (11) can then be evaluated analytically in the two-center case. However, great difficulties were encountered for the three- and four-center cases. It was common to describe this impasse as “the nightmare of the integrals”.

There were two responses to the integral difficulties. One was to make approximations for the more difficult integrals and to introduce parameters for others, with values obtained by empirical fits to experimental data. This practice became known as semiempirical. The alternative of proceeding without approximation or empirical parameterization was, at the time, necessarily limited to very small molecules and became known as the *ab initio* approach. The most widely used semiempirical methods were based on the zero-differential-overlap approximation, in which products of different atomic orbitals $\chi_{\mu}\chi_{\nu}$ are neglected in most integrals. This approximation, when applied to the π electrons of conjugated organic molecules, became known as the Pariser-Parr-Pople (PPP) theory.^[3–5] It was later generalized to the treatment of all valence electrons in the CNDO and INDO theories^[6] (1964–1966) and then pursued at a more empirical level by the group of M. J. S. Dewar. The CNDO/INDO methods were genuine chemical models in the sense that they could be used to study many molecules, vary structure to determine equilibrium geometries, and generate potential surfaces. However, they were limited by uncertainty over the consequences of the massive integral approximations and the large number of empirical parameters.

Within the *ab initio* community, a truly major development was the introduction of gaussian-type basis functions. In 1950 S. F. Boys,^[7] working in Cambridge, had demonstrated that all integrals in SCF theory could be evaluated analytically if the radial parts had the form $P(x,y,z) \exp(-r^2)$, where $P(x,y,z)$ is any polynomial in the cartesian coordinates x, y, z . Initially, this appeared to be of limited value, since single gaussian functions were poor approximations to atomic orbitals, but it was clear that prospects would improve if larger numbers of basis functions could be handled. For several years there was competition between proponents of Slater-type and gaussian-type basis sets.

The 1950s also saw the introduction of computers into quantum chemistry. By the time of the 1959 meeting there were already several groups developing *ab initio* programs, using both Slater and gaussian bases. Early codes for two-center integrals with Slater basis functions were developed in Chicago and used by Ransil in the first full LCAOSCF treatment of diatomic hydrides. At the same meeting Boys presented several prescient papers describing simple SCF calculations using gaussians. During the early 1960s other general purpose programs were developed, notably the gaussian packages POLYATOM and IBMOL, leading to a number of individual computations of molecular orbitals at the LCAO or minimal basis level.

My own research group began *ab initio* work in 1968 with the development of the GAUSSIAN program. At that time

the relative cost of *ab initio* LCAOSCF and CNDO computations on small organic molecules was over 1000. The original intention was to use full *ab initio* results to test various integral approximations that were less severe than the use of zero differential overlap. However, in the course of developing the program, Warren Hehre and I were able to generate a new integral algorithm that improved efficiency for highly contracted gaussian basis sets by more than two orders of magnitude.^[8] This was based on a method of axis rotation inside inner loops, thereby limiting the number of arithmetic operations in the innermost sections of the program. Using a procedure of least-squares fitting Slater-type basis functions by a fixed contraction of K gaussians, we were able to reproduce the results of earlier full Slater results on a series of small molecules. The choice $K=3$ proved adequate and led to the STO-3G basis and the general theoretical model HF/STO-3G. This was published in 1969^[9] and the code was made generally available as GAUSSIAN70 shortly thereafter.

Investigation of the minimal HF/STO-3G model quickly showed major failures. Comparison of some isomeric species (for example, propene and cyclopropane) showed too much stability for single bonds, relative to multiple bonds. This can be traced to the failure of the minimal basis to describe anisotropic atoms. In acetylene, for example, the carbon $2p_z$ atomic orbitals should be much tighter than $2p_x$; this effect cannot be properly simulated by the isotropic structure implied by a minimal basis with identical $2p$ functions in all three directions. This difficulty can be overcome by using two basis functions per valence atomic orbital instead of one. Such a basis is 6-31G, which has a single contracted 6-gaussian basis function for the inner shell, a set of inner 3-contracted gaussians, and a set of outer uncontracted gaussians for the valence shell of each atom. This is an example of a split-valence basis. Another similar commonly used type of basis set is double-zeta, in which there are two basis functions per atomic orbital for all atomic shells.

There are several notable failures for split-valence bases. In the first place, such bases tend to favor structures of high symmetry. For example, the ammonia molecule NH_3 is predicted to have a trigonal structure which is too close to planarity. This deficiency can reasonably be attributed to the fact that, in a planar structure, the lone pair of electrons are assigned to a nitrogen orbital that is pure p -type, which cannot mix with higher angular momentum d -type functions, whereas in a nonplanar structure the lone-pair orbital is a sp mixture, for which further stabilization by d -mixing is possible. A second deficiency in Hartree–Fock studies at the split-valence level is an exaggeration of polarity, as measured by electric dipole moments. This can also be attributed to restriction of lone-pair orbitals to pure p -type. The $3p_z$ lone-pair orbitals in HCl , for example, will probably be polarized towards hydrogen if mixing with a d_z basis is allowed, thereby reducing the predicted dipole moment.

Considerable improvement is found in Hartree–Fock models if a single set of uncontracted d -functions are added on each heavy (non-hydrogen) atom. Such a basis is 6-31G* or 6-31G(d).^[10, 11] If a single set of uncontracted p -functions is added on each hydrogen atom, the basis is denoted by 6-31G** or 6-31G(d,p). These additional basis functions are

termed polarization functions. The full model with the 6-31G* basis is then described as HF/6-31G*. Other important basis set extensions are the introduction of higher polarization functions (as in 6-31G(2df,p), which contains two sets of d -functions and a set of f -functions on heavy atoms and a single set of p -functions on hydrogen) and the use of diffuse functions, which are particularly important for anions and electronic states. The latter are denoted by a plus sign as in 6-31+G(d).

The Hartree–Fock model HF/6-31G* has proved quite effective in the description of molecular conformations. Its overall performance in this and other regards has been documented elsewhere.^[12] It is notably successful in giving differences of different isomeric forms of organic molecules, where no major changes of bond lengths are involved. Rotational potentials about single bonds were successfully explored using this level of theory.^[13] A particular example is the anomeric effect in carbohydrate chemistry, which was not properly understood until the interaction of rotational potentials about geminal C–O single bonds was investigated using HF/6-31G* theory.^[14]

4. Correlated Models

The major fault implicit in all Hartree–Fock models is neglect of electron correlation between the motions of electrons of antiparallel spin ($\alpha\beta$ correlation). In the very early days of quantum chemistry it was recognized that neglect of correlation led to severe underestimation of bond dissociation energies. This may be understood qualitatively by considering the process of complete homolytic dissociation of a bond in which one electron ends up on one center and one on the other. If the motion of the two electrons is uncorrelated, there will be a finite possibility of both electrons ending up on the same center.

Neglect of $\alpha\beta$ electron correlation is implicit in the use of a single-determinant wavefunction; improved wavefunctions necessarily involve the use of many determinants. Most practical correlation procedures start with the Hartree–Fock determinant and form linear combinations with other determinants. It is particularly convenient to form additional determinants from the unoccupied or virtual molecular orbitals, which are the higher eigenfunctions of the Fock operator. If a finite basis is used, with $2n$ electrons and N cartesian basis functions, there will be $N - n$ virtual orbitals, which may be occupied by α or β electrons.

At this point, it is convenient to change the notation somewhat and use spin orbital basis functions which are products of the cartesian basis functions and the α or β spin functions. N is now the size of this spin orbital basis (twice the number of cartesian basis functions) and n is the total number of electrons. This notation enables us to use a common notation for both spin-restricted and spin-unrestricted cases. If labels $i, j, k, \dots$ are used for occupied spin orbitals and labels $a, b, c, \dots$ for virtual spin orbitals, then single-determinant functions using Fock orbitals may be classified as unsubstituted (that is, Hartree–Fock) Ψ_0 , singly substituted Ψ_i^a , doubly substituted Ψ_{ij}^{ab} , and so forth. A general multi-

determinant wavefunction can then be written as Equation (12). The a coefficients can be determined by variation to

$$\Psi = a_0 \Psi_0 + \sum_{ia} a_i^a \Psi_i^a + \sum_{ijab} a_{ij}^{ab} \Psi_{ij}^{ab} + \dots \quad (12)$$

minimize the calculated energy. This is the method of configuration interaction (CI). If only singles are mixed in, no energy lowering follows, since the occupied orbitals are already optimized. The simplest effective form of CI allows for doubles only in Equation (12). This is usually denoted by CID. If singles are also included, the method is CISD. These configuration interaction techniques were first implemented as iterative schemes around 1970 and are still often used in practical computations. If all possible substitutions are included in the expansion (a large but finite set if a finite basis set is used), the method is described as full configuration or FCI. The FCI procedure, although desirable in principle, is usually too costly to apply except for very small systems.

Although CID and CISD are well-defined models, given a standard basis set, they suffer some serious disadvantages. These have to do with size consistency. If a method such as CID is applied to a pair of completely separated systems, the resulting energy is not the sum of the energies obtained by applying the same theory to the systems separately. If CID is applied to two separated helium atoms, for example, the wavefunction does not allow for simultaneous excitation of pairs in each atom, this being strictly a quadruple excitation. This failure of CID and CISD models is likely to lead to poor descriptions of large molecules and interacting systems.

A second general method of incorporating electron correlation is to treat its effects by perturbation theory. Suppose we define a perturbed Hamiltonian as Equation (13), where F_0 is

$$\mathcal{H}(\lambda) = F_0 + \lambda \{\mathcal{H} - F_0\} \quad (13)$$

the Fock Hamiltonian (for which the single determinants in Equation (12) are exact eigenfunctions), then Ψ_0 is the appropriate wavefunction if $\lambda = 0$, and the exact (FCI) Ψ is obtained if $\lambda = 1$. The perturbation procedure used expands the computed energy in powers of λ [Eq. (14)], cuts the series

$$E(\lambda) = E_0 + \lambda E_1 + \lambda^2 E_2 + \lambda^3 E_3 + \dots \quad (14)$$

off at some level, and then puts $\lambda = 1$. This perturbation method was first introduced by Møller and Plesset^[15] and is often denoted by MP n if terminated at order n . The MP1 energy ($E_0 + E_1$) is identical to the Hartree–Fock value. MP2 is the simplest practical perturbative procedure for electron correlation and incorporates only effects of double substitutions. At third order, MP3 also involves only double substitutions. At the fourth order level, MP4 includes a description of the (indirect) effects of singles, the leading contributions of triples, and some treatment of certain quadruple substitutions.

Møller–Plesset theory is size-consistent if the computations are carried out completely at any given order. Difficulties are that the terms become algebraically complicated at higher orders and also are increasingly costly to apply. In fact, Hartree–Fock theory (with no integral approximations)

scales as N^4 , MP2 as N^5 , MP3 as N^6 , and MP4 as N^7 . The triple contributions in the MP4 energy are the most expensive and generally limit the applicability of Møller–Plesset theory to this level. The MP2, MP3, and MP4 models were implemented by several groups in the 1970s and incorporated into the GAUSSIAN program.^[16, 17]

A third general approach to correlation theory is the use of coupled cluster methods, originally introduced into quantum chemistry by Cizek.^[18] If the configuration interaction CID wavefunction is written as Equation (15), where T_2 is an

$$\Psi = (1 + T_2) \Psi_0 \quad (15)$$

operator specifying all double substitutions, with undetermined coefficients, then the corresponding coupled-cluster function (CCD) is written as Equation (16). The CCD

$$\Psi = e^{T_2} \Psi_0 \quad (16)$$

coefficients are determined, not by the variational method, but by requiring zero projection of $(\mathcal{H} - E) \Psi$ onto Ψ_0 and all Ψ_{ij}^{ab} . This method was first implemented in 1978.^[19–21] Single substitutions are incorporated by using the operator $\exp(T_1 + T_2)$ instead of $\exp(T_1)$. This then defines a CCSD model.^[22, 23]

Unlike CISD, the CCSD method is size-consistent. The cost is of the order N^6 , as for CISD. Being nonvariational, the resulting total energy is no longer an upper bound for the exact result, but it is generally thought that the achievement of size consistency is a matter of greater importance. Another, slightly simpler, method is quadratic configuration, denoted QCISD. This is also size-consistent and can be regarded as an approximation intermediate between CISD and CCSD.

The QCISD and CCSD methods take no account of the effects of triple substitutions, known to be important by studies at the MP4 level. A useful way to take account of triples is to carry out an iterative QCISD or CCSD computation and then do a single computation of the effects of triples, using the single and double amplitudes already found. These are the QCISD(T) and CCSD(T) methods.^[24, 25] A third related method is the Brueckner-doubles method, BD(t),^[26] which alters the underlying occupied orbitals so that there is no singles mixing. All three of these methods are superior to MP4 in that, when the energy is expanded in a Møller–Plesset series, complete agreement with a FCI expansion is obtained up to fourth order and many other terms at higher order are also included.^[27] In fact, the QCISD, CCSD, and BD methods have the further advantage of being completely correct for composite two-electron systems such as a set of isolated helium atoms.

The cost of QCISD(T) or CCSD(T) scales as iterative N^6 , followed by a single computation at N^7 . They represent the most sophisticated correlation methods that are simple enough to be incorporated into general theoretical models at the present time.

5. General Energy Models

In recent years progress has been made in developing models which reproduce chemical energies to an accuracy

approaching that achieved in good experimental work. The description of model features in the two previous sections indicates that two main features are involved, basis set and level of correlation. The options available are usefully summarized in a two-dimensional model chart as shown in Figure 1. The various correlation methods are displayed horizontally in order of increasing sophistication from left to right. Basis sets are displayed vertically, becoming more flexible from top to bottom. At the far right, full configuration interaction (FCI) represents complete solution within the finite space defined by the basis. At the bottom of the table, we have (in principle but not in practice) the results of applying a complete basis set. At the bottom right, application of a complete basis set with full configuration interaction

Basis \ Method	HF	MP2	MP3	MP4	QCI	FCI
STO-3G						
6-31G						
6-31G(d)						
6-31+G(d)						
6-311+G(d)						
6-311+G(2df)						
∞						Eq. (1)

Figure 1. General model table. QCI refers to QCISD(T).

corresponds to full solution of the nonrelativistic Schrödinger equation.

Each empty box in this chart represents a well-defined size-consistent theoretical model as specified in Section 2. Clearly, we may test each level to find how far we have to proceed from the top left to the bottom right for acceptable agreement between theory and experiment. Eventually, adequate performance will be achieved, if the underlying assumptions of quantum mechanics are correct.

In practice, full models usually have to make some compromises to achieve a wide range of applicability. If the prediction of energies is most important, a common practice is to carry out a geometry optimization (to an equilibrium structure, for example) at some lower level of theory and then make a final, more expensive, computation at a higher level. A useful notation for this type of composite model is “model-1//model-2”, meaning single-point calculations using model-1 on geometrical structures determined by model-2.

To illustrate these ideas we give a partial description of the G3 model for molecular energies, which has been recently published.^[28] This is a refinement of previous energy models G1 and G2 which have been under development for more than a decade.^[29,30] The main computational steps are summarized in Figure 2.

In addition to the standard type of basis sets already described, a large basis (G3large), which permits a flexible

Basis \ Method	HF	MP2	MP4	QCI
6-31G(d)	freq	opt-1	2	3
6-31+G(d)		4	5	
6-31G(2df,p)		6	7	
G3large		8		?

Figure 2. G3 model table. QCI refers to QCISD(T), freq stands for the estimated zero-point energy, and opt-1 stands for the geometry optimization of the first calculation step.

description of the whole space with inner shells, is added. This basis is so large that only MP2 computations are reasonably possible. Geometrical structures in the G3 model are determined at the MP2/6-31G(d) level.^[31] This is followed by a sequence of single-point calculations that aim to estimate the results of a potential QCI/G3large energy, by assuming that effects of some of the improvement steps can be treated additively. The actual formula used is Equation (17). Earlier

$$\begin{aligned} ? = & 2 + (3 - 2) + (5 - 2) \\ & + (7 - 2) + (8 - 1) - (4 - 1) - (6 - 1) \end{aligned} \quad (17)$$

studies^[32] had indicated that this kind of additivity was reasonably accurate. (It should be noted that all correlation computations except the full MP2/G3large are

carried out in the “frozen core” approximation, with only interactions between valence electrons being treated.)

An important contribution to total molecular energies is the zero-point vibrational energy. This is estimated in G3 theory by using harmonic frequencies calculated at the HF/6-31G(d) level and then empirically scaled by a factor 0.8929 (HF theory being known to systematically overestimate the magnitudes of frequencies^[33]). In addition, a small correction is added for the spin-orbit splitting in isolated atoms, obtained from experimental data.^[34]

The computations as described up to this level give a reasonable account of significant energy differences, such as dissociation energies and ionization potentials. However, there is a significant systematic error, with all binding energies being slightly too low. This can be reasonably interpreted as being due mostly to the limitation in the basis sets being used. An accurate description of the wavefunction cusp at the point where electrons of opposite spin come to the same point in space requires basis sets involving high angular momentum. Another reason is that, in molecules, the symmetry is lower than in atoms and again neglect of the effects of higher angular momentum basis functions will favor atomic relative to molecular energies.

These difficulties can be partly overcome by adding a small empirical correction, depending on the number of electrons, and distinguishing between atoms and molecules. The theory

therefore becomes semiempirical, or perhaps “slightly empirical” since the parameters are small and their origin is partly understood. This higher level correction (HLC) is $-An_\beta - B(n_\alpha - n_\beta)$ for molecules and $-Cn_\beta - D(n_\alpha - n_\beta)$ for atoms (including atomic ions), where n_α and n_β are the numbers of α and β electrons, respectively, with $n_\alpha \geq n_\beta$. This completes the specification of a total G3 energy for any atom or molecule.

The parameters A , B , C , and D are determined as part of the validation process. This is carried out using a large set of 299 experimental energy differences involving molecules up to the size of benzene (42 electrons). These data include 148 heats of formation, derived from heats of atomization, 85 ionization potentials, 58 electron affinities, and 8 proton affinities. All of these experimental results are believed to be known to an accuracy of 1 kcal mol⁻¹ or better. Values of A , B , C , and D are obtained by minimization of the mean absolute deviation between theory and experiment. These are (in millihartrees) 6.386, 2.977, 6.219, and 1.185, respectively. The resulting mean deviation is 1.02 kcal mol⁻¹, close to the target accuracy. The corresponding root-mean-square deviation, which lays more emphasis on the poorer levels of agreement, is 1.45 kcal mol⁻¹. However, nearly 88% of the G3 deviations fall in the range -2.0 to $+2.0$ kcal mol⁻¹. These results are significantly better than the prior G1 and G2 models, which use a smaller database of experimental facts.

The poorest results are worthy of note. Largest absolute deviations are 4.9 kcal mol⁻¹ (C₂F₄) for heats of formation, 7.0 kcal mol⁻¹ (B₂F₄) for ionization potentials, 4.2 kcal mol⁻¹ (NH) for electron affinities, and 1.8 kcal mol⁻¹ (PH₃ and SH₂) for proton affinities.

6. Conclusions

The current status of ab initio quantum chemical models is that some success has been achieved in approaching experimental accuracy in predictive power. The target of 1 kcal mol⁻¹ is not far away for small molecules containing up to about fifty electrons. However, the G3 model has a number of remaining deficiencies that merit further attack.

1. The use of an empirical correction that depends only on the number of electrons is undesirable. One consequence is that the model becomes discontinuous in some manner. For example, if a bond is broken, the electron count of paired versus unpaired electrons has to change at some point, thereby providing a discontinuity in the potential surface. The same criticism can be applied to the use of different parameters for atoms and molecules. Some form of extrapolation is probably necessary, but it would be much better if this could be carried out in a continuous and differentiable manner.
2. The G3 model is based on MP2/G-31G(d) geometries, which are known to show considerable errors.^[31] Some of the failures of the final energies can be attributed to this; clearly a method which would reproduce known bond lengths and angles more accurately would be preferable.
3. No account is taken of relativity in the G3 model. The total energy of a molecule is known to depend significantly on

relativistic corrections, particularly for inner-shell electrons. However, considerable cancellation of errors occurs in processes such as bond dissociation. Nevertheless, some inclusion of relativistic contributions to chemical processes is clearly desirable.

4. The applicability of the G3 model to large systems is presently limited by the very expensive treatment of the triples terms, where computational cost scales as the seventh power of the size of the system. The magnitude of these terms is small, but not insignificant. A simpler treatment of three-electron effects would be desirable.

Finally, some brief comment should be made about theoretical models based on density functional theory (DFT). Such methods do not handle the two-electron interactions explicitly but rather allow for them using properties of the one-electron density. This leads to lower cost and therefore a wider range of applicability. Recent forms of DFT have also introduced a considerable amount of empirical parameterization, sometimes using the same set of experimental data. At the present time the principal limitation of DFT models is that there is no clear route for convergence of methods to the correct answer, comparable to the ab initio chart shown in Figure 1. Interaction between these two groups of theoretical chemists is a hopeful direction for future progress.

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Polymer-Supported Synthesis of Non-Oligomeric Natural Products**

Christine Watson*

Introduction

Combinatorial chemistry has become an important tool in the discovery of novel compounds for pharmaceutical research, materials science, molecular recognition, and asymmetric catalysis.^[1] Solid-phase organic synthesis enables the most efficient preparation of libraries of compounds through the use of split-and-pool techniques and rapid, automated purification procedures. Since the pioneering work of Merrifield^[2] in the 1960s, solid-phase synthesis of polypeptides^[3] and oligonucleotides^[4] has become routine, and more recently significant progress has been made in the synthesis of oligosaccharides^[5] and small organic molecules.^[6] However, further development in this area will require an increase in the scope of solid-phase chemistry and, in particular, the development of more sophisticated chemical transformations that operate reliably on the solid support.

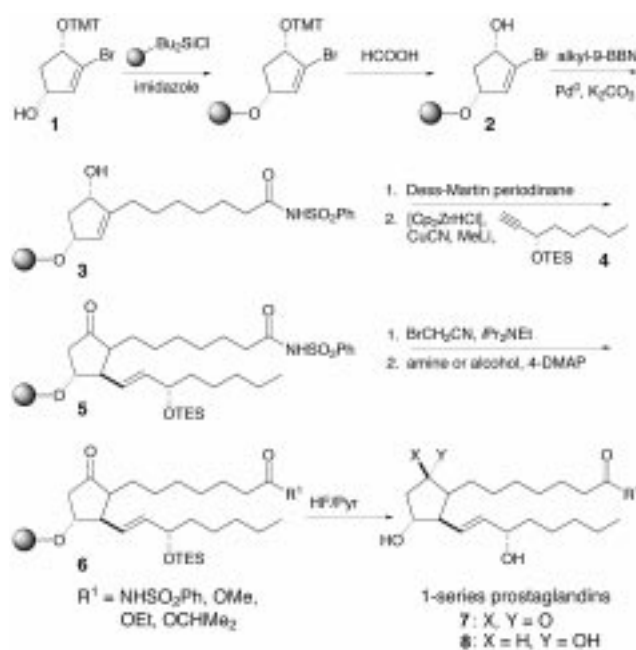
Since the early days of organic chemistry, natural products have served as an inspiration for the development of new methodology and strategies in organic synthesis. Recently, a number of ground-breaking solid-phase syntheses of non-oligomeric natural products have been reported, which demonstrate the imagination and creativity required for success in this challenging field. This article will present an outline of the synthetic approaches as well as modifications employed to extend the chemistry to the generation of libraries.

Polymer-Supported Synthesis of Prostaglandins

Prostaglandins have inspired and challenged synthetic chemists for decades. Their complex structures, incorporating an array of sensitive functionalities, coupled with their potent biological activity have stimulated many total syntheses as well as the preparation of hundreds of analogues for pharmaceutical evaluation.^[7] Even after so much research into the solution synthesis of these natural products, their preparation on a polymer support remains a significant challenge. Solid-phase reactions frequently require quite

different conditions from those in solution, in part due to differences in steric hindrance and hydrophobicity within the resin matrix. For a successful solid-phase reaction to occur, a resin must be selected that swells well in the solvent of choice and is inert to the reaction conditions. The linker must also be stable throughout the synthesis, but ultimately must allow release of the natural product from the support. Moreover, since different resin-bound intermediates cannot be separated, it is crucial that reactions are driven to completion and side reactions are minimized to avoid formation of complex mixtures at the end of a multistep synthesis.

The approach to the synthesis of the 1-series prostaglandins adopted by Ellman et al. involved attachment of the cyclopentene core (**1**, Scheme 1) to a dibutylsilyl chloride substi-



Scheme 1. Synthesis of the 1-series prostaglandins according to Ellman et al.^[8] 9-BBN = 9-borabicyclo[3.3.1]nonane, 4-DMAP = 4-dimethylaminopyridine, Pyr = pyridine, TES = triethylsilyl, TMT = trimethoxytrityl.

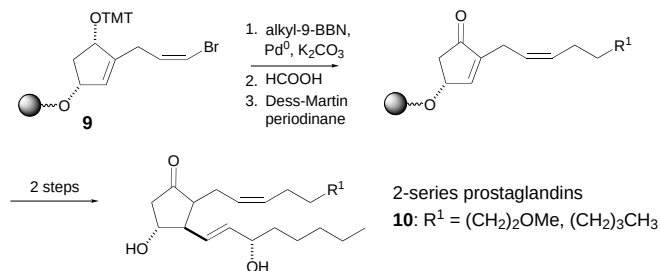
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tuted polystyrene resin.^[8] Polystyrene swells well in many commonly used organic solvents, and it was envisioned that treatment with fluoride would result in release from the support along with removal of any silyl protecting groups. The nature of the silylalkyl groups had to be carefully optimized as

a result of the high sensitivity of the final prostaglandin structure: For example, release from a diisopropylsilyl linker required harsher conditions, which resulted in elimination of the β -hydroxy ketone functionality. The choice of alcohol protecting group was also crucial as deprotection of the more stable dimethoxytrityl group resulted in some cleavage from the resin.

The first side chain was incorporated by means of a Suzuki coupling on vinyl bromide **2**, under similar conditions to those reported by Johnson and Braun for the solution-phase reaction.^[9] Oxidation of **3** to the corresponding enone was then achieved with Dess–Martin periodinane in refluxing THF. The efficiency of the synthesis might have been improved by incorporating the desired enone functionality at the start of the synthesis, as in the approach of Johnson and Braun; however, this resulted in some decomposition of the base-sensitive β -alkoxy group under the conditions of the Suzuki reaction. Subsequent addition of the cuprate derived from **4** gave ketone **5**, for which the stereochemistry of the α -chain was not reported. Elaboration of the sulfonamide was achieved by activation with bromoacetonitrile and then coupling to a series of nucleophiles to form acid derivatives **6**. Treatment with HF/pyridine resulted in the release of the prostaglandin E₁ analogues (**7**) from the resin in 49–58% yield (from **2**). Alternatively, ketone **5** could be reduced with L-selectride before further elaboration and cleavage, permitting access to prostaglandin F_{1 α} derivatives **8**. In addition, simply using the chain-extended bromide **9** (Scheme 2) as the



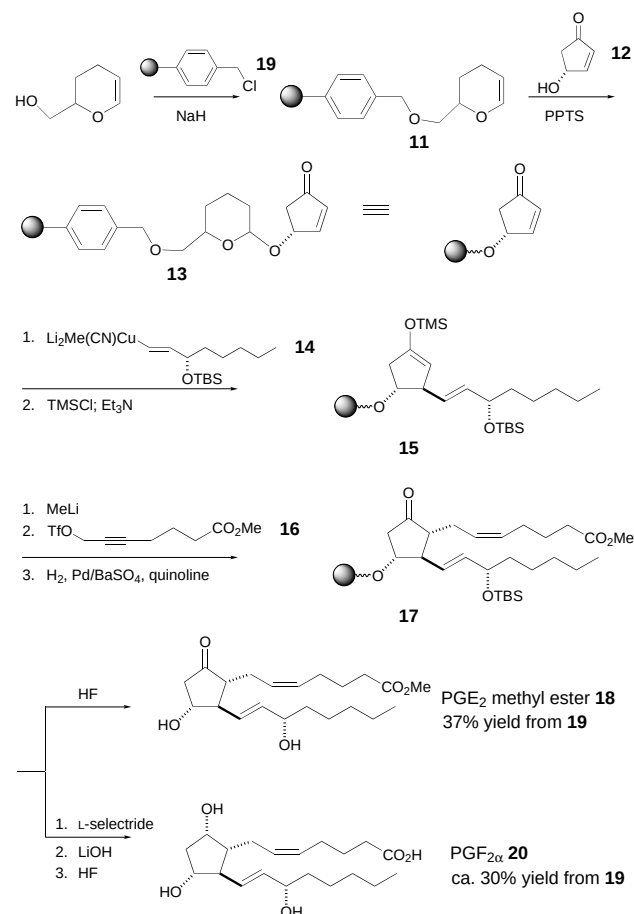
Scheme 2. Synthesis of the 2-series prostaglandins according to Ellman et al.^[8]

starting point for a similar synthetic sequence allowed ready access to the 2-series prostaglandins **10**. With modification of the separate components in the synthesis, Ellman et al. were able to prepare a number of analogous structures, thus forming a small library of prostaglandins.

This synthesis serves to illustrate some general features of solid-phase organic chemistry. It is obvious that optimized conditions for solution reactions cannot be applied directly to the solid phase, but must be reinvestigated on the resin-bound material. In addition, a large excess of reagents is often required to ensure complete conversion for solid-phase reactions. For example, five equivalents of the alkyl-9-BBN derivatives were employed in the above Suzuki coupling as compared to 1.5 equivalents of reagent in the corresponding solution transformation. Solid-phase reactions are also frequently carried out at higher temperatures and for longer times, as such reactions tend to be slower than their solution-phase counterparts.

Janda and co-workers adopted Noyori's convergent three-component strategy^[10] for the synthesis of prostaglandin E₂ (PGE₂) methyl ester.^[11] The synthesis was carried out in a liquid-phase rather than a solid-phase environment using noncross-linked chloromethylated polystyrene.^[12] This support is soluble in most commonly employed organic solvents (CH₂Cl₂, CHCl₃, EtOAc, THF), so that reactions and product characterization can be carried out using standard solution techniques. As the polymer is insoluble in methanol and water, extraction of an organic reaction mixture with water removes inorganic contaminants, and then precipitation of the support with methanol separates the desired polymer-bound materials from unbound organic by-products. As in solid-phase synthesis, different polymer-bound components cannot be separated, and consequently many of the lessons learned using a liquid-phase support are applicable to solid-phase synthesis. Liquid-phase organic synthesis can also be applied to the construction of combinatorial libraries.

The soluble polystyrene resin was functionalized to the tetrahydropyran derivative **11** (Scheme 3), which was allowed to react with cyclopentene core **12** to generate the first support-bound intermediate (**13**). In a similar manner to the solution approach of Gooding,^[13] the second component, the long-chain cuprate **14**, underwent a conjugate addition to the enone, and the resulting enolate was trapped with chlorotri-



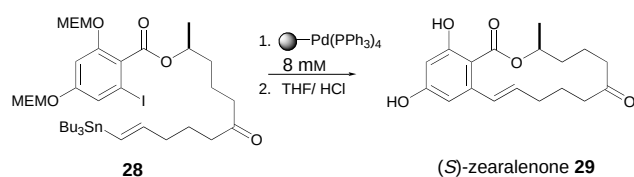
Scheme 3. Synthesis of prostaglandin E₂ methyl ester and prostaglandin F_{2 α} according to Janda et al.^[11] PPTS = pyridinium *p*-toluenesulfonate, TBS = *tert*-butyldimethylsilyl, TfO = trifluoromethanesulfonyl, TMS = trimethylsilyl.

methylsilane to give **15**. Generation of the more reactive lithium enolate followed by alkylation with triflate **16**, the third component, gave a complex alkyne which was reduced to form **17**. As in the solution synthesis, this combination of reactive enolate and reactive electrophile appeared to circumvent problems of enolate equilibration and β -elimination frequently observed in this system. Since the polymer support was soluble in the benzene/cyclohexane solvent mixture, the heterogeneous partial hydrogenation of the intermediate alkyne could be carried out under standard solution conditions and monitored by NMR analysis, minimizing over-reduction. Treatment with HF resulted in the release of **18**, the methyl ester of PGE₂, in 37% yield from **19**. As in the Ellman et al. synthesis, reduction of **17** with L-selectride before cleavage provided access to PGF_{2 α} (**20**).

One of the advantages of the three-component strategy is the opportunity it provides for diversity. Thus the use of a range of functionalized cyclopentene cores, cuprates, and triflates could be envisioned to provide access to a library of prostaglandin analogues. Comparison of the liquid-phase and solution-phase approaches reveals that solution-phase reaction conditions could be applied with only minor modifications, as anticipated given the “solutionlike” environment of the soluble polystyrene resin.

Cyclorelease Strategies in Solid-Phase Synthesis

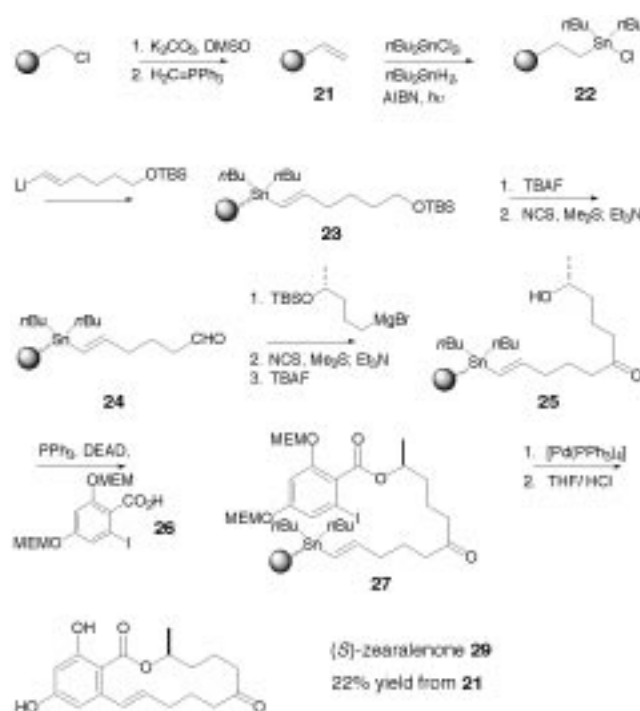
While the structure of the prostaglandins includes a convenient handle for attachment to the solid support, the synthesis of natural products lacking such functionality demands a different strategy. Nicolaou and co-workers have employed a series of cyclorelease approaches to the synthesis of the natural products zearalenone, muscone, and epothilone A, where cleavage from the resin results in concomitant ring formation. The solid-phase synthesis of zearalenone^[14] closely followed a solution approach by Hegedus et al. (Scheme 4) in which an intramolecular Stille reaction on **28** under high dilution conditions generated the 14-membered



Scheme 4. Solution-phase approach to the synthesis of (*S*)-zearalenone according to Hegedus et al.^[15] MEM = 2-methoxyethoxymethyl.

macrocyclic ring of the natural product (**29**).^[15] Nicolaou et al. aimed to employ the same disconnection; however, the macrocyclization precursor would be coupled to a solid support through the tin moiety.

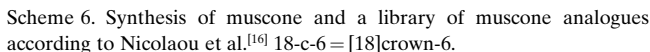
The starting point was the preparation of a polymer-supported tin reagent (Scheme 5). Elaboration of Merrifield resin (cross-linked polystyrene) to form alkene **21** was followed by the addition of *n*Bu₂SnHCl in the presence of AIBN. Reaction of the resulting functionalized resin (**22**) with a vinyl lithium reagent gave vinyltin intermediate **23**. The chain was further elaborated through Grignard addition to



Scheme 5. Synthesis of (*S*)-zearalenone according to Nicolaou et al.^[14] AIBN = 2,2'-azobisisobutyronitrile, DEAD = diethyl azodicarboxylate, NCS = *N*-chlorosuccinimide, TBAF = tetra-*n*-butylammonium fluoride.

aldehyde **24** followed by a series of functional group interconversions to form **25**. Esterification with **26** under Mitsunobu conditions resulted in the formation of the final resin-bound intermediate **27**, analogous to the solution-phase cyclization precursor (**28**, Scheme 4). The crucial intramolecular Stille coupling proceeded smoothly in the presence of a Pd⁰ catalyst, releasing the protected natural product into solution. Acid-mediated removal of the MEM groups resulted in the isolation of zearalenone (**29**) in approximately 22% overall yield (from **21**). Once the optimal link to the resin support had been established (through **23**), the solid-phase reactions were carried out under very similar conditions to those in solution, although a greater reagent excess was generally employed.

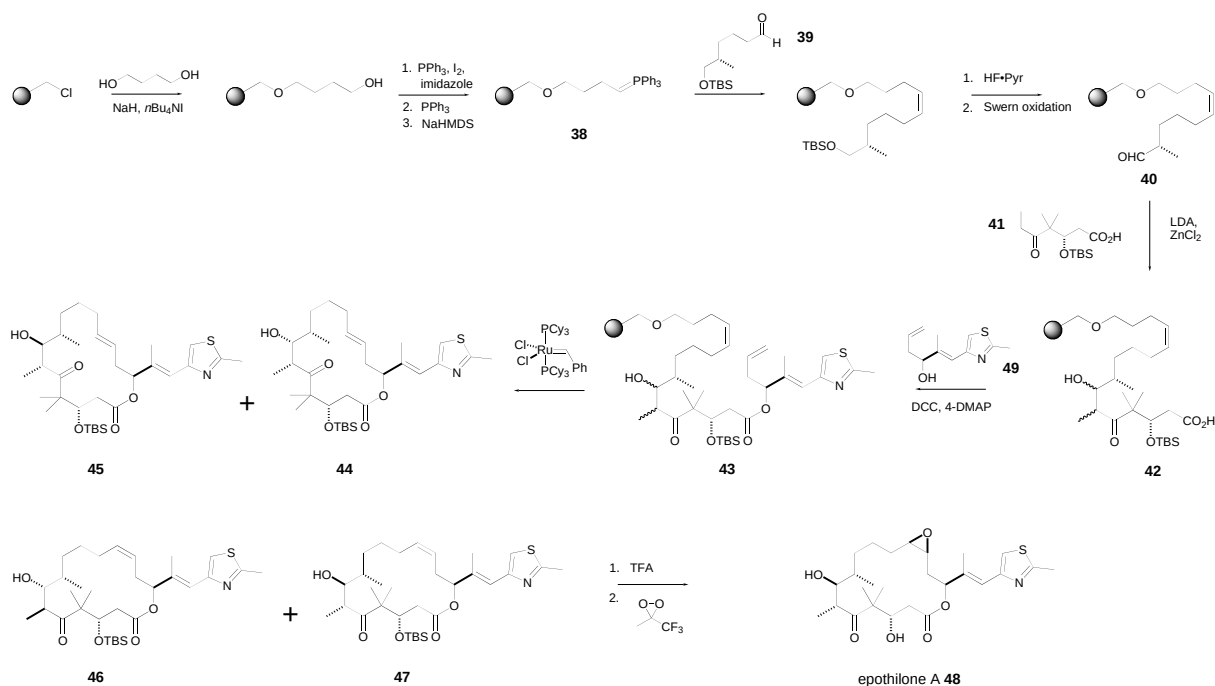
A Horner–Wadsworth–Emmons/cyclorelease strategy was employed by Nicolaou et al. in the synthesis of a racemic muscone library.^[16] A novel phosphonate-functionalized resin (**30**, Scheme 6) was prepared by coupling CH₃PO(OMe)Cl to Merrifield resin through a C₄ linker. The library of muscone analogues was generated very efficiently using standard combinatorial split-and-pool techniques where individual compounds were identified by radiofrequency tagging.^[17] Thus groups of functionalized resin beads were enclosed in individual SMART microreactors (SMART = single or multiple addressable radiofrequency tag) which contained a semiconductor unit capable of storing radiofrequency information. Each microreactor was subjected to a unique series of reagents, and the synthetic history was recorded in the radiofrequency code. The first combinatorial step involved the treatment of methylphosphonate **30** with *n*BuLi and a series of esters (**31–33**) to give β -oxophosphonates **34**. Another opportunity for diversity came in the olefin meta-



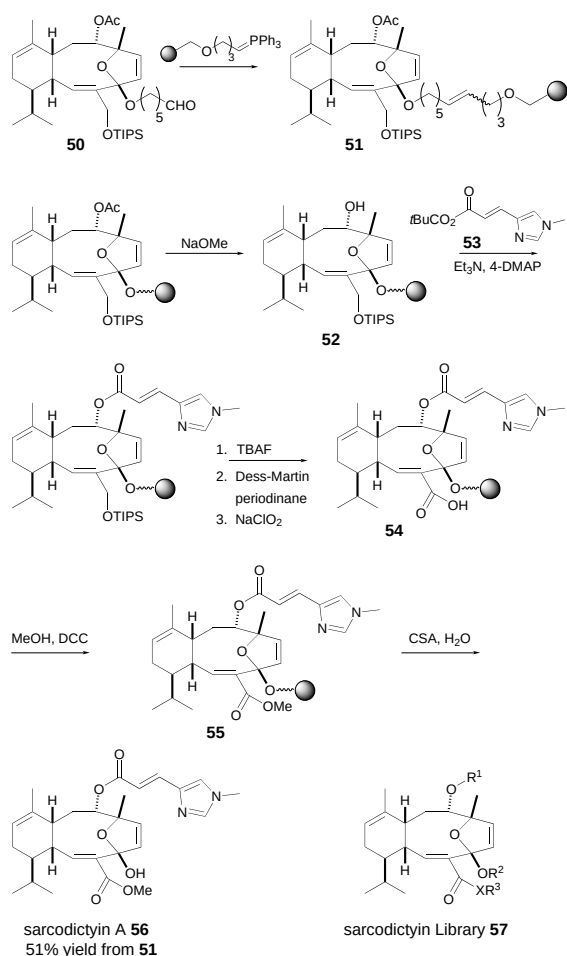
The epothilones, a group of highly cytotoxic natural products, have stimulated intense interest in the synthetic community, resulting in total syntheses by Danishefsky, Nicolaou, Schinzer, Mulzer, and their co-workers.^[19] The

The convergent nature of this synthesis made it very amenable to the preparation of a library of epothilone analogues.^[21] Thus simply varying the three key components (**39**, **41**, and **49**) would generate a large number of structures. Using split-and-pool methodology with the radiofrequency tagging system described previously, a 47-membered library was prepared through the reaction of a range of aldehydes, ethyl ketones, and heterocyclic alcohols.

More recently, Nicolaou and co-workers were able to demonstrate the solid-phase synthesis of sarcodictyin A along with a library of analogues (Scheme 8).^[22] Like the epothilones, this family of natural products has been the focus of substantial synthetic effort due to their potent cytotoxic



Scheme 7. Synthesis of epothilone A according to Nicolaou et al.^[19c] DCC = 1,3-dicyclohexylcarbodiimide, LDA = lithium diisopropylamide, NaHMS = sodium bis(trimethylsilyl)amide, TFA = trifluoroacetic acid.



Scheme 8. Synthesis of sarcodictyin A and a library of sarcodictyin analogues according to Nicolaou et al.^[22] CSA = 10-camphorsulfonic acid, TIPS = triisopropylsilyl.

activity and the difficulty associated with isolating significant quantities from natural sources.^[23] The approach to the solid-phase synthesis of sarcodictyin A started with attachment of advanced intermediate **50**, representing the core of the natural product, to a polystyrene support. After formation of **51** by a Wittig reaction, the acetate group was removed and the free alcohol (**52**) was acylated with mixed anhydride **53**. This would be the first opportunity for diversity in a sarcodictyin library. Conversion of the TIPS-protected primary alcohol into an acid led to the formation of **54**, which was esterified with methanol to form **55**. Again, the use of a range of nucleophiles at this stage would increase the number of possible library members. Finally, the natural product was released from the resin under acid conditions to give **56** in 51% yield (from **51**). Further diversity could be generated in this final step by transketalization with different alcohols. This route was employed to prepare a 66-membered library of sarcodictyin analogues (**57**). It should be noted that in some cases low yields were observed for the acylation reaction used to incorporate the heterocyclic side chain, and these compounds had to be prepared in solution.

It is evident from this synthesis that there are still major limitations on the utility of solid-phase chemistry. Construction of the core of the sarcodictyins was arguably the most challenging and stimulating aspect of the reported solution syntheses. Solid-phase chemistry could only be used at a late stage, after the assembly of the core had been accomplished in solution.

Conclusions

Solid-phase chemistry has progressed significantly since the days of Merrifield and peptide synthesis, such that the synthesis of complex organic molecules on polymer supports

is now a realistic goal. Of course enormous challenges still remain. The choice of resin and linker is crucial to the success of the synthesis, and new functionalized polymer supports would be a welcome addition to the solid-phase arsenal. One of the advantages of the liquid-phase approach reported by Janda is the ability to monitor reactions directly by NMR spectroscopy. The other syntheses required cleavage of intermediates before characterization, which takes considerable time and results in loss of material. Recent applications of magic angle spinning NMR spectroscopy and single-bead IR spectroscopy and mass spectrometry should simplify the characterization of resin-bound intermediates and accelerate the optimization of solid-phase reactions.^[24]

The extension of solid-phase natural product synthesis to the generation of libraries of complex organic molecules is even more demanding. It seems unlikely at this stage that the high yields and reliability seen in peptide couplings could be achieved for the range of reactions required in non-oligomeric natural product synthesis, making the preparation of large libraries extremely difficult. However the work presented here demonstrates that small libraries can be prepared efficiently on the solid phase, even in the case of very complex structures, allowing the discovery of novel compounds with interesting and varying activity.

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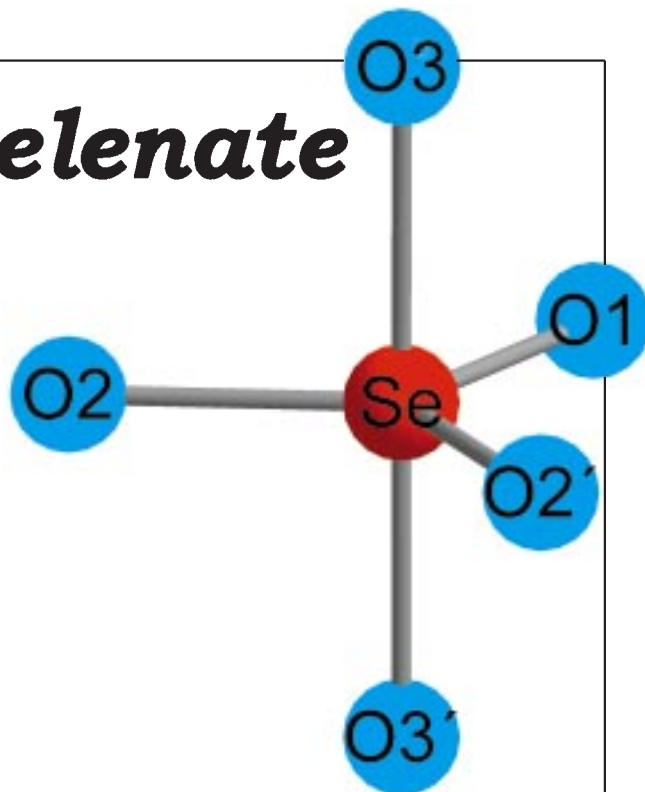
Keywords: combinatorial chemistry • natural products • solid-phase synthesis • total synthesis

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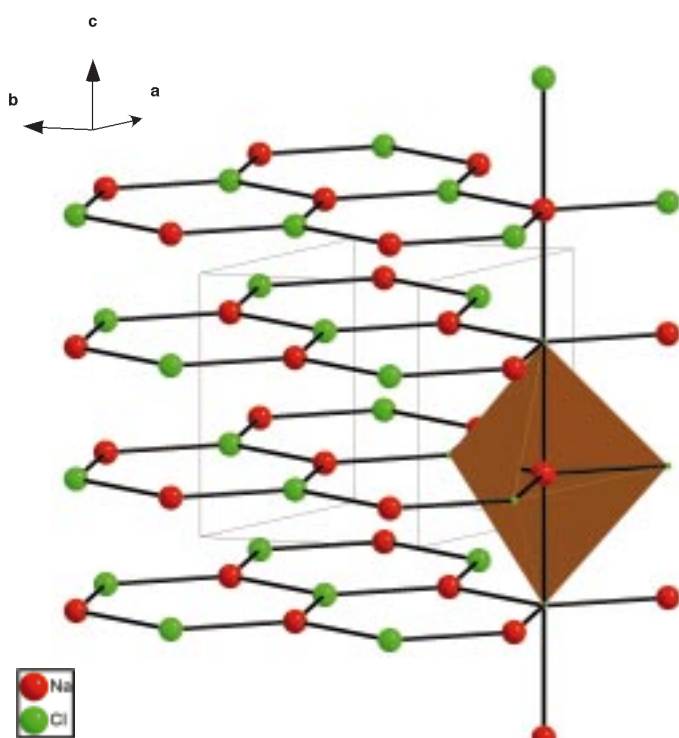
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Lithium orthoselenate

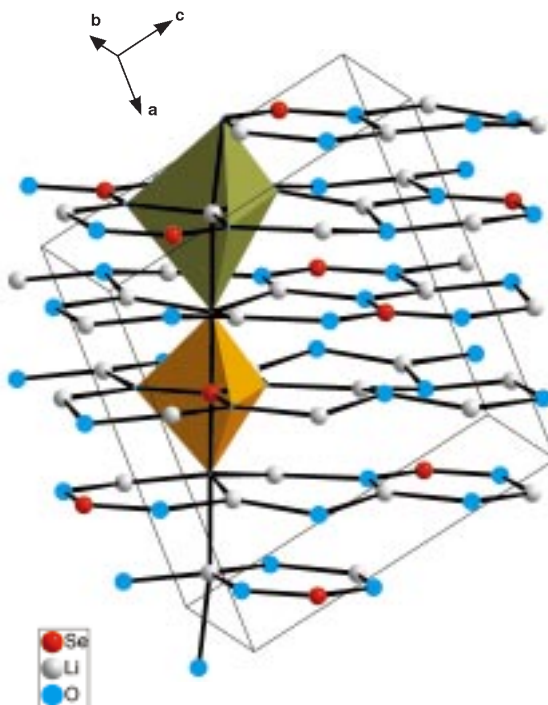
The first orthoselenate contains novel trigonal-bipyramidal complex anions.



It crystallizes as an order variant of a theoretically predicted AB structure ...



theoretically predicted AB structure



lithium orthoselenate

... in which the cations and anions are equally coordinated as $A^{[5]}B^{[5]}$.

More about this on the following pages.

Li₄SeO₅, the First Orthoselenate, Crystallizes as an Order Variant of a Theoretically Predicted A^[5]B^[5] Structure Type**

Helmut Haas and Martin Jansen*

Addition of water to nonmetal oxides results in the formation of oxoacids. The most water-rich form of an oxoacid and the anion derived from it are described by the prefix *ortho*. The maximum possible number of constituent water molecules is only found in the case of boron and tellurium, however. Even H₄CO₄ or H₄SiO₄ are unstable to intra- or intermolecular cleavage of water. In the case of fully deprotonated oxoanions, solid-state reaction with strong oxide bases provides access to further orthoanions, which are then only stable in the crystalline state. The most well known examples are the alkali metal orthonitrates.^[1] Although the existence of orthoesters does not guarantee that the corresponding orthoanions can also be prepared—the C(OR)₄/CO₄⁴⁻ pair confirms this—we have taken the existence of esters^[2] of the hypothetical orthoselenium acid, H₆SeO₆, as an incentive to attempt the synthesis of an orthoselenate. With this goal, we have systematically worked through the quasi-binary system Li₂O/Li₂SeO₄, initially under high isotactic pressures (piston-cylinder press).^[3] We discovered that a new phase is indeed formed, however, with the surprising Li₂O:Li₂SeO₄ ratio of 1:1 instead of the initially sought 2:1 product ("Li₆SeO₆"). We subsequently established that, under suitable temperature conditions, the new phase can also be formed under normal pressure. The colorless samples are only slightly sensitive to moisture and CO₂.

Since all attempts to grow single crystals failed, the *ab initio* crystal structure was determined from powder intensities and refined by profile fitting of the powder diffraction diagram^[4, 5] (Figure 1). The structure analysis revealed that the compound was indeed an orthoselenate,^[6] with the composition Li₄SeO₅. The selenium atom is coordinated in a trigonal-bipyramidal fashion by oxygen atoms (Figure 2). Since the bonding arrangement is without precedence, there are no comparable data to assess the Se–O bond lengths. It is clear, however, that the increase in the mean Se–O bond length of 6.7%, which results from the increase in the coordination number from four (SeO₄²⁻) to five, lies within the expected range. Despite integration into the crystal structure and the low site symmetry 2, the dimensions of the SeO₅⁴⁻ anion are remarkably regular. It displays, within the margins of error of the

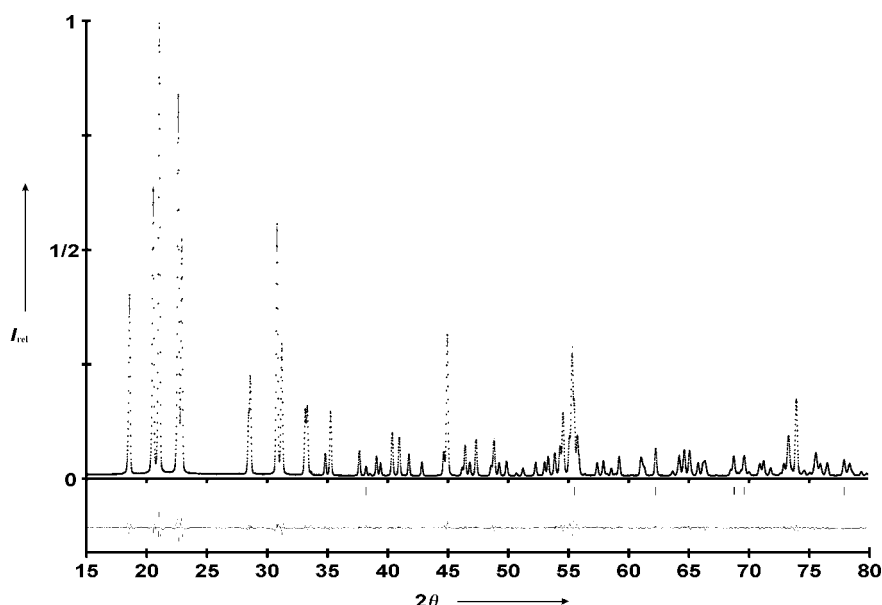


Figure 1. Powder diffraction diagram of Li₄SeO₅. Top: measured (data points) and calculated intensities (solid line), bottom: difference plot.

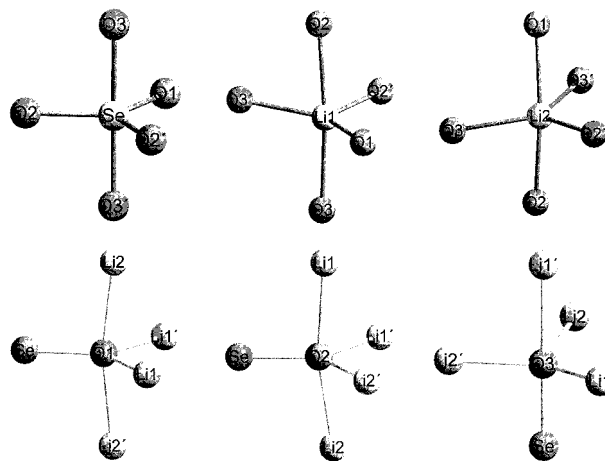


Figure 2. Coordination polyhedra in the crystal structure of Li₄SeO₅. Selected bond lengths [pm] and angles [°]: Se–O1 172.0(2), Se–O2 170.6(1) 2 ×, Se–O3 179.5(1) 2 ×; O1–Se–O2 120.4(2) 2 ×, O1–Se–O3 89.9(1), O2–Se–O2' 119.1(1), O2–Se–O3 90.1(1), O2'–Se–O3' 90.1(1), O2–Se–O3' 90.0(1), O2'–Se–O3 90.0(1), O3–Se–O3' 179.8(1).

structure determination, the point symmetry $\bar{6}2m$ (*D*_{3h}). The trigonal bipyramid is one of the coordination polyhedra in which the ligands cannot form bonds of equal length to the central atom and to each other. The compromise between these mutually exclusive requirements results, in this case, in an increase in the distance to the axial ligands (+4.9%, for comparison, in PF₅:^[7] +3.8%). The lithium and oxygen atoms also show trigonal-bipyramidal coordination (Figures 2 and 3). The mean Li–O distances of 204.6 (Li1) and 200.5 pm (Li2) lie between the typical values for tetrahedral and octahedrally coordinated lithium (196.3 and 212.5 pm, respectively). The coordination polyhedra around lithium are very distorted with respect to bond angles and distances (Figure 2; maximum deviation from the mean value and the ideal value: 12.8 pm and 12.9°). In the case of oxygen, the bond distances and angles (Figure 2) are dispersed over a

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comparable range. All deformations can be explained by the strong variation in the cation valences. The corresponding compensation in the bond distances can be quantitatively understood on the basis of bond length/bond strength relationships.^[8] The valence sums determined (Li1: 1.07, Li2: 1.19, Se: 5.65, O1: 2.07, O2: 2.06, O3: 1.98) lie within the acceptable tolerances of the expected values of 1, 6, and 2.

From the empirical formula and the coordination environments described above, it is clear that this is an order variant of an AB structure, in which the cations and anions are equally coordinated as A^[5]B^[5] (Figure 3). The aristotype^[9, 10]

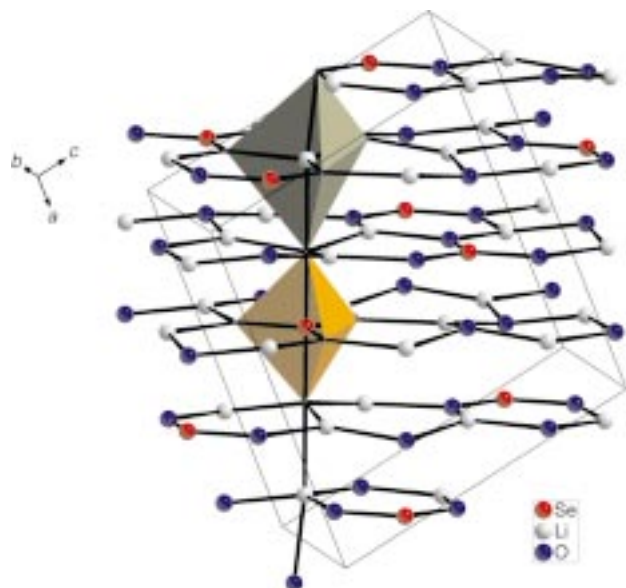


Figure 3. Crystal structure of Li₄SeO₅ (the layer motif is repeated after every tenth layer).

of Li₄SeO₅ can be regarded as a counterpart to the conventional sodium chloride structure (A^[6]B^[6]). This new structure type was first discovered as one of the viable modifications during an investigation of the energy landscape of NaCl (Figure 4).^[11] To our knowledge, this is the first example of a

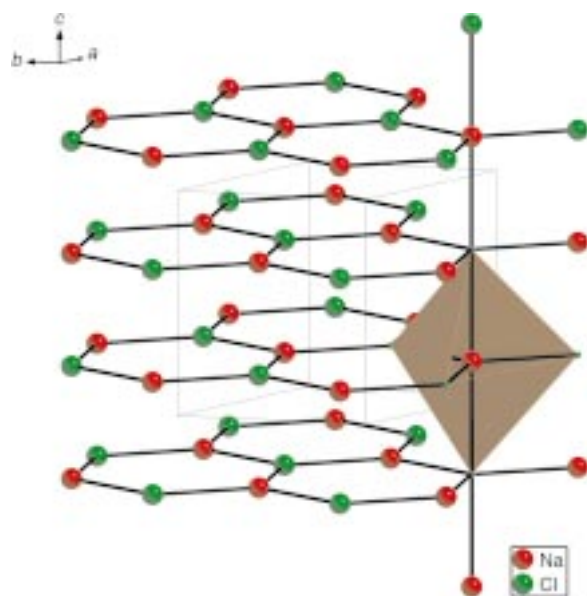


Figure 4. Hypothetical structure of Na^[5]Cl^[5].

virtually ab initio predicted structure type, which was then experimentally realized.

Experimental Section

Li₄SeO₅ was prepared from the solid-state reaction of Li₂O and Li₂SeO₄ in closed silver crucibles which were sealed in glass ampoules. The reaction time was two days, and the reaction temperature was 500 °C. Li₂O was produced by dehydration of LiOH, and Li₂SeO₄^[12] by the reaction of Li₂CO₃ with H₂SeO₄. H₂SeO₄ was obtained by oxidation of SeO₂ with H₂O₂ under an oxygen atmosphere.^[13]

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Helical Crystallization of Proteins on Carbon Nanotubes: A First Step towards the Development of New Biosensors**

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Since their discovery in 1991^[1] carbon nanotubes have attracted considerable interest because of their peculiar shape: they are several micrometer long tubes with diameters as small as a few nanometers. Their outstanding mechanical and electronic properties^[2] have made them very promising nanomaterials for new applications in chemistry and physics, particularly for the development of new nanotechnologies. However, few applications have so far been reported in biology. Small proteins have been introduced into the inside hollow of opened nanotubes thus forming natural nano test tubes.^[3] It should also be of great interest to decorate the outer surface of these carbon nanotubes with biological macromolecules, such as oligonucleotides^[4] or proteins. Such devices could promote the development of new biosensors and bioelectronic nanomaterials, which could take advantage of the specific biomolecular recognition properties associated with the bound macromolecules.

For such purposes the specific recognition function has to be densely packed on the outer surface of the carbon nanotubes and the protein has to be functional. A good criterion for the conservation of the functional properties of the protein is its ability to form ordered arrays. In fact, the cylindrical shape, the perfect straightness, and the exceptional rigidity^[5] of the carbon nanotubes make their surfaces good candidates for the growth of helical crystals of proteins (Figure 1).

These nanotubes are easy to handle and can be prepared in gram quantities by the arc-discharge method.^[2] This method leads to the formation of multi-walled nanotubes (MWNTs) that are made of several coaxial graphite layers. The tube diameter shows a broad distribution ranging from 2 to 30 nm. Such a polydisperse material could increase the chance of crystallization since the protein has the opportunity to select

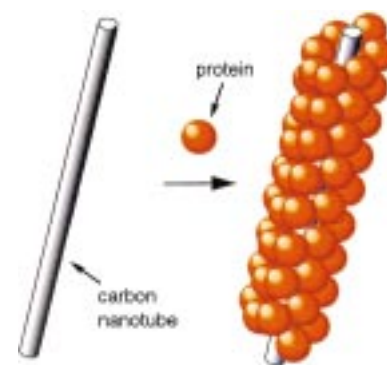


Figure 1. Schematic representation of the helical crystallization of proteins on the outer surface of a carbon nanotube.

the nanotube of suitable diameter for helical self-organization.

Streptavidin, a water-soluble protein synthesized by *Streptomyces avidinii*,^[6] was chosen to study the interaction of proteins with carbon nanotubes. Streptavidin has become very useful in many biochemical assays, such as labeling and affinity chromatography because of its high affinity for (+)-biotin ($K_a \approx 10^{15}$).^[7] Upon incubation of streptavidin with MWNTs the protein molecules were found to strongly interact with the carbon tubes. In appropriate conditions the tubes were almost completely covered by streptavidin whereas only a few free molecules could be seen in the background of the electron microscope grid. This observation was not surprising since streptavidin can be purified on hydrophobic resins such as benzyl-DC beads of cellulose,^[8] can form 2D crystals on nonspecific hydrophobic lipid films,^[9] and binds strongly to plain carbon-coated electron microscopy grids. These properties probably reflect the fact that streptavidin displays hydrophobic domains within its structure. While most of the streptavidin molecules bound stochastically to the nanotubes and did not form any ordered arrangements (Figure 2a) we noticed that in some instances the nanotubes showed lateral striations that were regularly spaced at 6.4 nm with an angle of 71° to the tube axis (Figure 2b). Perpendicular striations were also visible, which suggests that the streptavidin molecules were organized in a square lattice.

The helical organization of the streptavidin molecules on the nanotube surface was evidenced by the optical diffraction pattern, which exhibited a characteristic layer line distribution (Figure 3a). Direct observation of the images and the analysis of the optical diffraction pattern, which showed a layer line spacing of $1/12.8 \text{ nm}^{-1}$, indicated that the helical repeat consisted of two striations. This distance between the layer lines is in good agreement with the size of the streptavidin molecule as revealed by X-ray analysis.^[10] A noise-free view of the helical repeat was obtained by averaging 25 sub-images of the streptavidin-coated nanotube. The sub-images were extracted at regular spacings that corresponded to the repeat distance along a 300-nm long nanotube image (Figure 3b). The indexation of the Fourier transform revealed that the order of the Bessel functions were multiples of two, which indicated a two-start helical organization formed by two protein helices that wind around the nanotubes and that each

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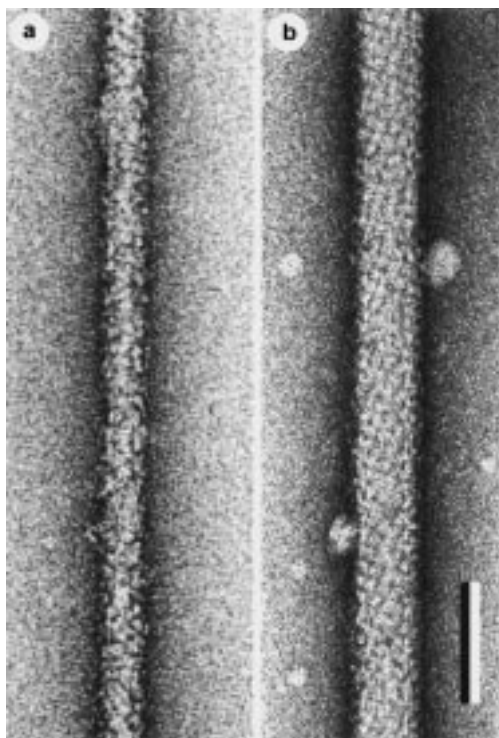


Figure 2. Electron micrographs showing multi-walled nanotubes (MWNTs) coated with streptavidin molecules and negatively stained with uranyl acetate. a) Stochastic binding of streptavidin molecules on a MWNT with a diameter smaller than 15 nm. b) Helical organization of streptavidin molecules on a carbon nanotube with a suitable diameter of 16 nm. The bar represents 50 nm.

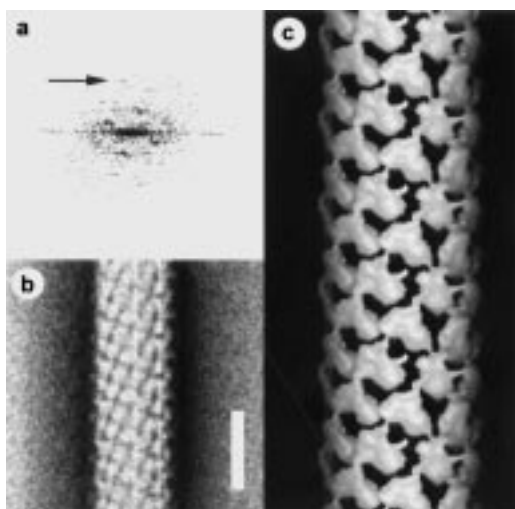


Figure 3. Analysis of the streptavidin helices formed on MWNTs. a) Computed power spectrum of the Fourier transform of a helical array of streptavidin molecules. The helical repeat is 12.8 nm and the arrow indicates $1/2.5 \text{ nm}^{-1}$. b) Noise-free view of the helical repeat obtained by correlation. c) Three-dimensional model of streptavidin assemblies calculated by retroprojecting the noise-free helical repeat shown in (b) along the Euler angles deduced from the analysis of the diffraction pattern. The bar represents 25 nm in (b) and 12.5 nm in (c).

helix consisted of eight streptavidin molecules per turn. The diffraction pattern showed structural information down to a resolution of $1/2.5 \text{ nm}^{-1}$ on the fifth layer line (arrow in Figure 3a). The observation of a large number of striated

tubes indicated that the outer diameter of the structure was constant at $27 \pm 2 \text{ nm}$, which showed that the size of the tube was an essential parameter for helical organization. The diameter of the underlying carbon nanotube, which could occasionally be measured, was about 16–17 nm, which indicated that the protein layer had a thickness of 5 nm. This thickness is consistent with the size of a streptavidin molecule and demonstrates that a single protein layer coats the nanotubes. We further noticed that all the observed crystalline arrays exhibited the same handedness and the same helical repeat unit.

The helical parameters determined from the analysis of the optical diffraction pattern were used to reconstruct a 3D model of the assemblies by retroprojection (Figure 3c). The structure indicates that the orientation and the molecular contacts are similar to those found in 2D crystals with $C222$ symmetry.^[11] The streptavidin-coated nanotubes should thus show biotin-binding properties since it was demonstrated for 2D crystals that two biotin binding sites face the aqueous phase and are accessible for interaction with biotinylated molecules. It was previously shown that crystals of streptavidin formed on biotinylated lipid monolayers^[11] or tubules^[12] can be decorated with polybiotinylated ferritin molecules from horse spleen or biotinylated Fab-fragments, respectively. Thus carbon nanotubes covered with streptavidin molecules could be used as a bioreactive docking matrix. These results provide carbon nanotubes with new and very powerful applications in biotechnology, since many biotinylated compounds, such as proteins or DNA fragment, are now available.

The binding of streptavidin to the carbon nanotubes was efficient in a wide range of conditions tested including various temperatures, incubation times, pH values, and ionic strengths. However, the formation of highly ordered helical arrangements in a quality suitable for structural investigations was poorly reproducible. The best results were obtained in the presence of a buffer containing 20% of methanol and we hypothesize that methanol reduces the strength of the hydrophobic interactions involved in the binding of the protein on to the carbon nanotubes and thus favors protein–protein interactions that lead to helical arrangements.

To further demonstrate the potential of carbon nanotubes in structural biology and biotechnology we also investigated the interaction and crystallization of HupR, another water-soluble protein. In the photosynthetic bacterium *Rhodobacter capsulatus* the DNA binding protein HupR is involved in the cell response to hydrogen and belongs to the subfamily of nitrogen-regulatory proteins (NtrC).^[13] Like streptavidin, HupR was found to interact strongly with the carbon surface of MWNT and some nanotubes presented striations that made an angle close to 81° with the tube axis. The helical organization of HupR was more reproducible than for streptavidin and importantly occurred in the absence of methanol. We noticed that, unlike streptavidin, HupR protein formed ordered arrays on a wider range of MWNT diameters although a minimum value of about 12 nm was required (Figure 4). The optical diffraction pattern showed a lattice line spacing of $1/5.4 \text{ nm}^{-1}$ and revealed structural details down to a resolution of $1/2.7 \text{ nm}^{-1}$ (Figure 5a). The distance between the layer lines is in good agreement with the size of

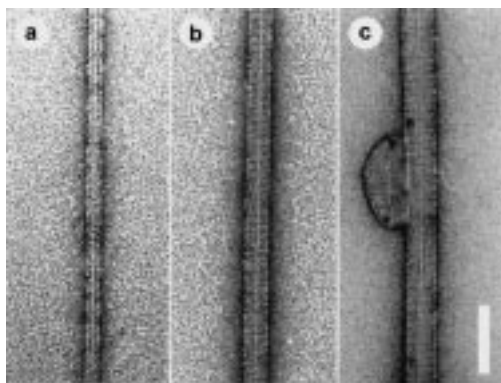


Figure 4. Electron micrograph showing MWNTs incubated with HupR protein and negatively stained with uranyl acetate. a) Stochastic binding of HupR proteins on a MWNT whose diameter of 8 nm is too small to accommodate a helical organization. b) and c) Helical organization of HupR proteins on carbon nanotubes with a diameter of 13 and 18 nm, respectively. The protrusion on the MWNT shown in (c) arises from contaminating amorphous carbon particles stacked on the tube. The bar represents 50 nm.

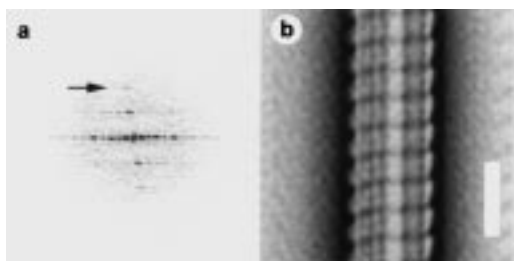


Figure 5. Analysis of the helices formed by the HupR protein on MWNTs. a) Computed power spectrum of the Fourier transform of a helical crystalline array. The repeat is 5.4 nm and the arrow indicates the second layer line at $1/2.7 \text{ nm}^{-1}$. b) Noise-free view of the helical organization obtained by a correlation over an average of 40 repeated units. The bar represents 15 nm.

the HupR molecule as revealed by recent electron microscopy observations of 2D crystals (Figure 5).^[14]

Although the interaction and self-organization of proteins on the carbon tubes are still poorly understood, we have demonstrated the feasibility of organizing soluble proteins on the surface of multi-walled carbon nanotubes. These nanotubes thus appear capable of acting as new supports for protein crystallization and which are suitable for structural investigations by electron microscopy. In fact, analysis of self-organized protein arrays by electron microscopy and image processing has become a powerful method for such a purpose since a periodic signal can be readily extracted from noise. The natural propensity of membrane proteins to form 2D crystals allowed their structure determination by electron crystallography close to atomic resolution. To extend this concept to soluble proteins, functionalized lipid molecules were designed to allow the 2D crystallization of various systems.^[15] However, a major drawback of the structure determination of 2D crystals is the limited possibility of tilting the specimen, a feature that is required to access the third dimension.^[16] In this respect supramolecular protein assemblies with helical symmetry are of particular interest since several molecular views can be recorded in a single image^[17]

thus giving direct access to information on the three-dimensional structure without the need for tilting the specimen. Several biological macromolecules, such as viruses,^[18] membrane proteins,^[19] or filamentous components of the cytoskeleton such as microtubules,^[20] exhibit helical symmetry suitable for studies by electron microscopy. However, naturally occurring helical assemblies of biological macromolecules present one or more of the following drawbacks that limit their general use or the attainable resolution: they may contain a limited number of coherently ordered repeating units, they may flatten themselves during specimen preparation, or their formation may be very specific. It is therefore of interest to control the formation of perfectly ordered helical arrangements of a sufficiently large size for a large variety of proteins.

Assemblies of lipids were shown to form tubular structures upon hydration and to induce the helical crystallization of proteins.^[12] Multi-walled carbon nanotubes appear now as general and promising alternative supports for the helical crystallization of biological macromolecules. Carbon nanotubes can be prepared on a large scale and display properties such as straightness, rigidity, and conductivity that are not shared by lipid tubes. We anticipate that these supports will flatten to a lesser extent and will improve the attainable resolution when observed under appropriate preservation conditions such as in a frozen hydrated state.

The use of protein coated carbon nanotubes should extend well beyond the described applications in structural biology. Carbon nanotubes are likely to be amenable to industrial production and progresses in related fields will soon allow the manipulation of these nanostructures. A step towards this direction was witnessed by the recent application of carbon nanotubes as tips for scanning probe microscopes.^[21] Our experiments demonstrate that it is possible to functionalize the nanotubes in order to modify their recognition properties. New applications of such nanomaterials now appear conceivable in the emerging fields of bio-nanotechnology.

Experimental Section

Multi-walled carbon nanotubes were prepared by the arc-discharge method^[2] and stored as a suspension in methanol (2 mg mL^{-1}). In the case of the incubation with streptavidin molecules, 100 μL of the MWNT suspension were dried under an ethane gas flow and resuspended in 20 μL of a 40% aqueous solution of methanol. This suspension was sonicated to disperse the MWNTs prior to the addition of 20 μL streptavidin solution at a concentration of $10 \mu\text{g mL}^{-1}$ in a buffer containing 10 mM Tris (pH 8) and 50 mM NaCl, and allowed to stand at room temperature for 45 minutes. The HupR protein was stored in a buffer containing 10 mM Tris (pH 8) and 350 mM NaCl. The same protocol was followed for the interaction with the MWNT except that the nanotubes were resuspended in pure water and in the absence of methanol prior to the addition of the protein. Five microliters of these preparations were placed on a 10 nm thick carbon film previously treated by a glow discharge in air. After two minutes of adsorption, the grid was negatively stained with a 2% uranyl acetate solution.

The images were recorded at a magnification of 45 000 times with a Philips CM120 transmission electron microscope operating at 100 kV. Three times enlarged prints of the electron micrographs were digitized using a Umax powerlook II scanner at a resolution of $375 \mu\text{m}$, which corresponds to $5 \text{ \AA}$ at the specimen level. The images were processed with the IMAGIC software package to calculate Fourier transforms, self- and cross-correla-

tion functions, to average images of the helical repeat, and to reconstruct a three-dimensional model from the weighted back projection algorithm.^[22]

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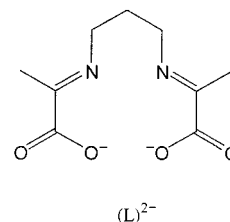
Keywords: electron microscopy • helical structures • nanotubes • proteins • self-organization

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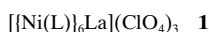
Template Assembly of Metal Aggregates by Imino-Carboxylate Ligands**

Dan M. J. Doble, Colin H. Benison, Alexander J. Blake, Dieter Fenske, Matthew S. Jackson, Richard D. Kay, Wan-Sheung Li, and Martin Schröder*

The aggregation of soft metal centers to form covalently bonded clusters is well known,^[1–3] and there are well-established theories regarding the structure and bonding in these polynuclear complexes, for example Ru– and Os–carbonyl^[2] and Au clusters.^[3] In contrast, hard metal cations do not readily form metal–metal bonds, and usually require bridging ligands such as carboxylate,^[4] oxide,^[5] or hydroxide ligands^[5] to induce aggregation. These ligands have enabled one-pot syntheses of some spectacular high-nuclearity aggregates, often with interesting magnetic and electronic properties.^[4, 5] However, such ligands can adopt a variety of coordination modes, which, together with the often unpredictable coordination geometries of the metals concerned, makes it very difficult to rationalize the structures of the resulting aggregates. Recent developments in self-assembly have shown how metal–ligand frameworks can be successfully templated about a substrate anion^[6] or cation.^[7] Our approach has been to use the tetradentate ligand (L)^{2–}^[8] to chelate the four equatorial sites of a potentially octahedral metal ion M^{II} (M^{II} = Ni^{II}, Mn^{II}), leaving two free axial sites at the metal center for aggregation by interaction with the carboxylate oxygen donors of neighboring [M(L)] units.^[9] Thus, each planar [M(L)] moiety has available two acceptor sites on M^{II} to form a 180° junction, and two carboxylates donors forming 90° junctions to one another and to the acceptor sites on M^{II}.



Slow codiffusion of methanolic solutions of [Ni(L)]^[10a] and La(ClO₄)₃ · 6H₂O results in the growth of brown, columnar crystals of **1**. A single-crystal X-ray structure determination^[11]



reveals a highly unusual^[12] heptanuclear [(Ni(L))₆La]³⁺ cluster (Figure 1) in which a La^{III} center is located at the center of the

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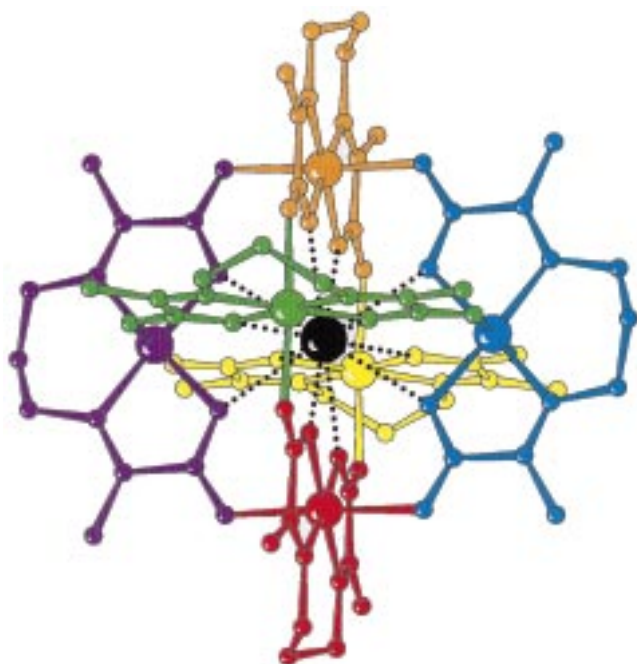


Figure 1. Crystal structure of **1**. The [Ni(L)] units are shown in different colors. The interstitial La^{III} cation is represented by a black sphere, and its icosahedral coordination sphere is indicated by dotted lines. The ClO₄⁻ anions and all hydrogen atoms are omitted for clarity.

octahedral cage formed by six [Ni(L)] moieties, and is bound by an icosahedron of twelve carboxylate groups (La–O 2.665(5), 2.710(5) Å), two from each [Ni(L)] unit. The La^{III} center lies on a crystallographic $\bar{3}$ axis so that all six [Ni(L)] units are equivalent. The octahedral Ni^{II} centers are bound equatorially by (L)²⁻ (Ni–O 1.997(5), 2.002(5); Ni–N 1.976(6), 1.997(5) Å). The axial sites are occupied by the carboxylate oxygen atoms of two neighboring [Ni(L)] units (Ni–O 2.122(6), 2.160(5) Å). Thus, each [Ni(L)] unit is joined to four other [Ni(L)] units such that the six Ni^{II} centers lie at the vertices of a near-perfect octahedron with La^{III} at its center (Figure 2 a).

By contrast, crystallization of [Ni(L)] from MeOH/Et₂O in the presence of NaClO₄ yields purple crystals of **2**, containing



a highly novel Ni₉Na₄ cluster in which four Na⁺ cations are encapsulated within a nine-membered [[Ni(L)]₉] cage^[11] (Figure 3). The cage is made up of [Ni(L)] units which are related by an approximate, noncrystallographic, threefold axis. As in **1**, all the Ni^{II} centers are octahedral, with the equatorial sites bound by (L)²⁻ (Ni–O 2.010(3)–2.077(3), Ni–N 1.996(3)–2.054(3) Å) and the axial sites bound by the carboxylate oxygen atoms of neighboring complexes (Ni–O 2.051(3)–2.134(3) Å). The Ni^{II} centers are arranged in a tricapped trigonal prism, although connection of the Ni^{II} centers of adjoining [Ni(L)] units gives a polyhedron with three square faces and eight triangular faces, with each [Ni(L)] moiety connected to four other [Ni(L)] fragments (Figure 2 b). All the carboxylate oxygen atoms are orientated inwards, encapsulating a core of four Na⁺ cations (Na–O 2.265(3)–2.459(3) Å), of which two are five-coordinate and two are

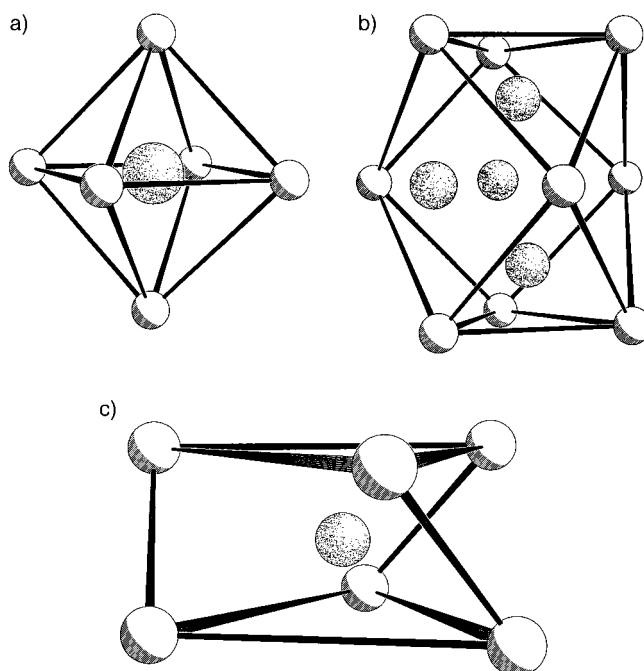


Figure 2. Polyhedra formed by connection of the metal centers of adjoining [M(L)] complex units in a) **1**, b) **2**, and c) **3**. Interstitial atoms are shown as speckled spheres.

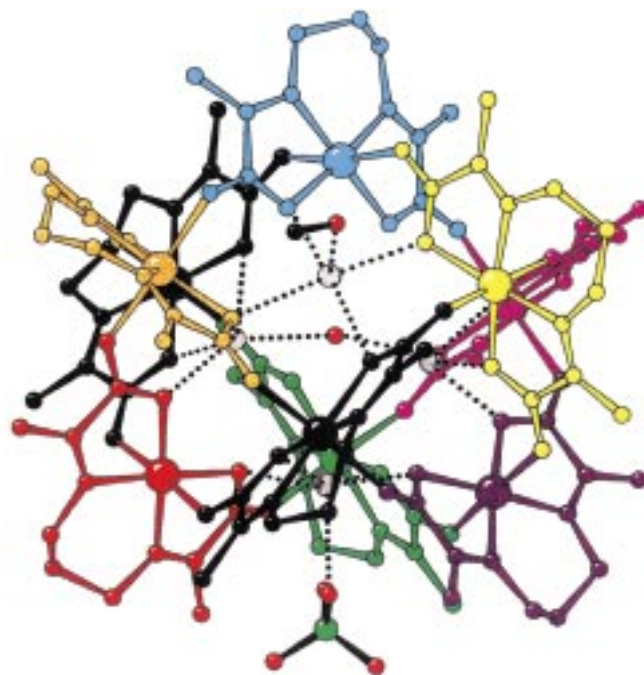


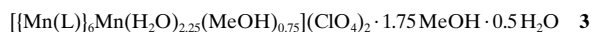
Figure 3. Crystal structure of **2**. The [Ni(L)] units are shown in different colors. The interstitial Na⁺ cations are represented by grey spheres, and their coordination spheres are indicated by dotted lines. All hydrogen atoms, noncoordinated ClO₄⁻ anions, and solvent molecules are omitted for clarity.

four-coordinate. A water molecule is also bound within the Na₄ core (Na–O 2.392(3)–2.404(4) Å), and a ClO₄⁻ anion and a MeOH molecule protrude through two of the square faces of the [[Ni(L)]₉] cage to bind to the Na⁺ core (Na–O(ClO₄⁻) 2.607(6)–2.774(8), Na–O(MeOH) 2.367(4) Å). The cation in

2 is, to our knowledge, the first complex cage to have been assembled around a template of four metal centers.

Therefore, [Ni(L)] units aggregate into an octahedral cage in the presence of $\text{La}(\text{ClO}_4)_3 \cdot 6\text{H}_2\text{O}$ and into a tricapped trigonal-prismatic cage in the presence of NaClO_4 . Since the same anion and solvent are present in each case, it appears that the difference in aggregation state results from a templating effect of the interstitial metal cations. A related example has been reported recently by Saalfrank and co-workers in which Group 1 metal ions were found to template the formation of a surrounding cage of [Fe(triethanolamine)] complexes.^[7a] In this case, Na^+ templates the formation of a six-membered cage, whereas the larger Cs^+ cation templates the formation of an eight-membered cage. In contrast, [Ni(L)] forms a larger aggregate in the presence of Na^+ than in the presence of the larger La^{III} cation. We believe that the [Ni(L)] units adopt a cage geometry with an internal hole size that best matches the ionic radius of the metal ions available for encapsulation. Thus, while the rigid icosahedral cavity in the $[\{\text{Ni}(\text{L})\}_6]$ octahedron accommodates a La^{III} center, it is too large for occupation by a single Na^+ center and too small to accommodate two Na^+ centers. Thus, the [Ni(L)] units assemble into a larger, nine-membered cage around four Na^+ centers.

Crystallization of [Mn(L)]^[10b] from $\text{MeOH}/\text{Et}_2\text{O}$ in the presence of $\text{Mn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ gave colorless, cube-shaped crystals of **3**. Single-crystal X-ray diffraction analysis^[11] reveals a novel heptanuclear $[\{\text{Mn}(\text{L})\}_6\text{Mn}]^{2+}$ cluster lying



on a crystallographic threefold axis (Figure 4). All seven Mn^{II} centers are approximately octahedral with six outer Mn^{II} centers bound by $(\text{L})^{2-}$ in the equatorial plane ($\text{Mn}-\text{O}$ 2.163(9)–2.200(9), $\text{Mn}-\text{N}$ 2.220(12)–2.269(12) Å) and an interstitial Mn^{II} center bound by six carboxylate oxygen atoms from surrounding [Mn(L)] units ($\text{Mn}-\text{O}$ 2.187(9), 2.232(10) Å). Unlike **1** and **2**, in which each [Ni(L)] unit is joined to four others through carboxylate bridges, the [Mn(L)] units in **3** are only joined to three neighboring units, with the axial sites on the Mn^{II} centers bound either by carboxylate oxygens atoms of neighboring [Mn(L)] complexes ($\text{Mn}-\text{O}$ 2.137(9)–2.138(10) Å) or terminal H_2O molecules (disordered with MeOH ; $\text{Mn}-\text{O}$ 2.185(9) Å). Connection of the Mn^{II} centers of adjoining [Mn(L)] units gives a twisted Mn_6 trigonal-prismatic cage with a twist angle of 42.23° in which only one carboxylate O-donor from each [Mn(L)] unit binds to the interstitial Mn^{II} ion (Figure 2c).

In conclusion, we have shown that the aggregation of [M(L)] into polynuclear cages can be templated by main group, lanthanide, and transition metal ions. The nuclearity and geometry of these cages is influenced by the size and coordination requirements of the template metal centers. Thus, in **1** an icosahedral La^{III} ion templates an octahedral cage and binds to two carboxylate donors from each [M(L)] moiety; in **2** four Na^+ centers template a tricapped trigonal prismatic cage with four- and five-coordinate Na^+ centers; and in **3** an octahedral Mn^{II} ion templates the formation of a twisted trigonal prismatic cage and binds to one carboxylate

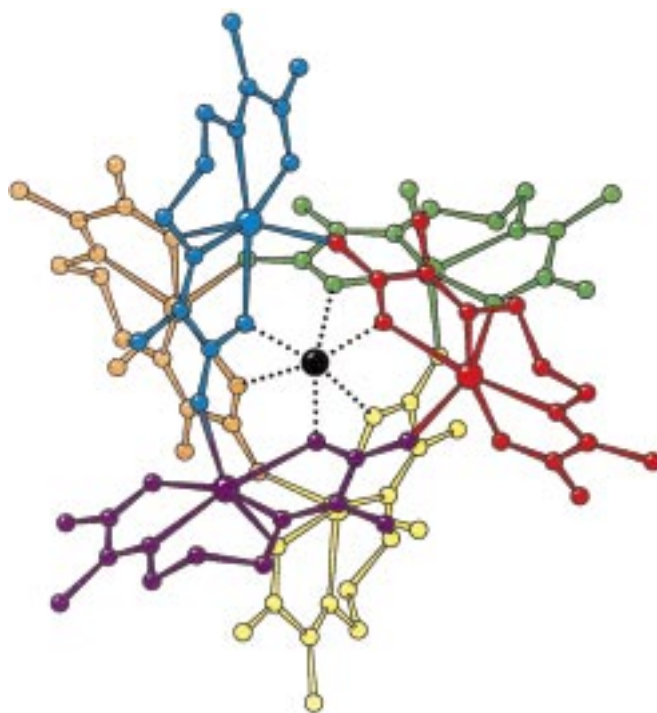


Figure 4. Crystal structure of **3**, viewed down the crystallographic threefold axis. The [Mn(L)] units are shown in different colors. The interstitial Mn^{II} center is represented by a black sphere, and its distorted trigonal-prismatic coordination sphere is indicated by dotted lines. All hydrogen atoms, ClO_4^- anions, and noncoordinated solvent molecules are omitted for clarity.

donor from each [M(L)] fragment. Preliminary experiments confirm the self-assembly of [M(L)] units at Ba^{2+} , Cs^+ , and K^+ .

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- [10] a) Synthesis of [Ni(L)]: Reaction of 1,3-diaminopropane (0.074 g, 1 mmol) with sodium pyruvate (0.220 g, 2 mmol) and Ni(BF₄)₂·6H₂O (0.340 g, 1 mmol) in MeOH (40 mL) under reflux for 2 h resulted in a blue-green solution. This was reduced in volume and eluted through a Sephadex LH-20 column. Slow diffusion of Et₂O vapor into the resulting solution yielded green crystals of [Ni(L)(MeOH)₂]·2MeOH. The structure of this complex has been confirmed by single-crystal X-ray diffraction.^[11b] b) The method for the synthesis of [Ni(L)] was also used in the synthesis of [Mn(L)].
- [11] a) Satisfactory elemental analyses were obtained for **1**, **2**, and **3**. Single-crystal X-ray analyses: All fully occupied non-hydrogen atoms were refined using anisotropic displacement parameters, and disordered atoms were refined isotropically over partially occupied sites. All hydrogen atoms were allowed to ride on their respective parent atoms. **1**: C₅₄H₇₂N₁₂O₃₆Cl₃Ni₆La, *M_r* = 2062.76, trigonal, space group *P*3̄, *a* = 12.4549(11), *c* = 12.736(2) Å, *V* = 1711 Å³, *T* = 150(2) K, *ρ*_{calcd} = 2.002 g cm⁻³, *Z* = 1. Data were collected at 200(2) K on a Stoe Imaging Plate Diffractometer System (IPDS). Of the 2031 data collected (2θ_{max} = 50°, -14 ≤ *h* ≤ 12, 0 ≤ *k* ≤ 14, 0 ≤ *l* ≤ 15), 1988 were unique (*R*_{int} = 0.140) and 1670 had *F* ≥ 4σ(*F*). All non-hydrogen atoms were located using heavy-atom and difference-Fourier methods.^[13] *R*₁ = 0.0748 (*F* ≥ 4σ(*F*)), and *wR*₂ = 0.226 (all data) for 179 parameters, (Δ/*σ*)_{max} = 0.02, the largest difference Fourier extrema of 3.04 and -1.53 e Å⁻³ both lay near the La atom. **2**: C_{92.5}H_{150.3}N₁₈O_{61.65}Cl₃Na₄Ni₉, *M_r* = 3663.16, monoclinic, space group *P*2₁/*n*, *a* = 17.735(4), *b* = 27.149(5), *c* = 28.938(6) Å, β = 100.22(3)°, *V* = 13711(5) Å³, *T* = 200(2) K, *ρ*_{calcd} = 1.581 g cm⁻³, *Z* = 4. Data were collected as for **1**. Of the 78620 data collected (2θ_{max} = 52.26°, -21 ≤ *h* ≤ 21, -33 ≤ *k* ≤ 31, -35 ≤ *l* ≤ 35), 25638 were unique (*R*_{int} = 0.109) and 20268 had *F* ≥ 4σ(*F*). All non-hydrogen atoms were located using direct and difference Fourier methods.^[13] *R*₁ = 0.0549 (*F* ≥ 4σ(*F*)), and *wR*₂ = 0.1634 (all data) for 1752 parameters, (Δ/*σ*)_{max} = 0.08, largest Fourier extrema: 0.84 and -0.95 e Å⁻³. **3**: C₅₈H₉₃N₁₂O_{38.5}Cl₂Mn₆, *M_r* = 2029.92, cubic, space group *Pa*3̄, *a* = 25.523(3) Å, *V* = 16625.9 Å³, *T* = 150(2) K, *ρ*_{calcd} = 1.622 g cm⁻³, *Z* = 8. Data were collected as for **1**. Of the 8599 data collected (2θ_{max} = 50°, 0 ≤ *h* ≤ 30, 0 ≤ *k* ≤ 21, 0 ≤ *l* ≤ 30), 4467 were unique (*R*_{int} = 0.138) and 2195 had *F* ≥ 4σ(*F*). All non-hydrogen atoms were located using direct and difference Fourier methods.^[13] *R*₁ = 0.1053 (*F* ≥ 4σ(*F*)), and *wR*₂ = 0.249 (all data) for 358 parameters, (Δ/*σ*)_{max} = 0.02, largest Fourier extrema: 0.87 and -0.88 e Å⁻³. b) 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-104533 ([Ni(L)(MeOH)₂]·2MeOH), CCDC-104534 (**1**), CCDC-104535 (**2**), and CCDC-104536 (**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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Dendritically Cross-Linking Chiral Ligands: High Stability of a Polystyrene-Bound Ti-TADDOLate Catalyst with Diffusion Control**

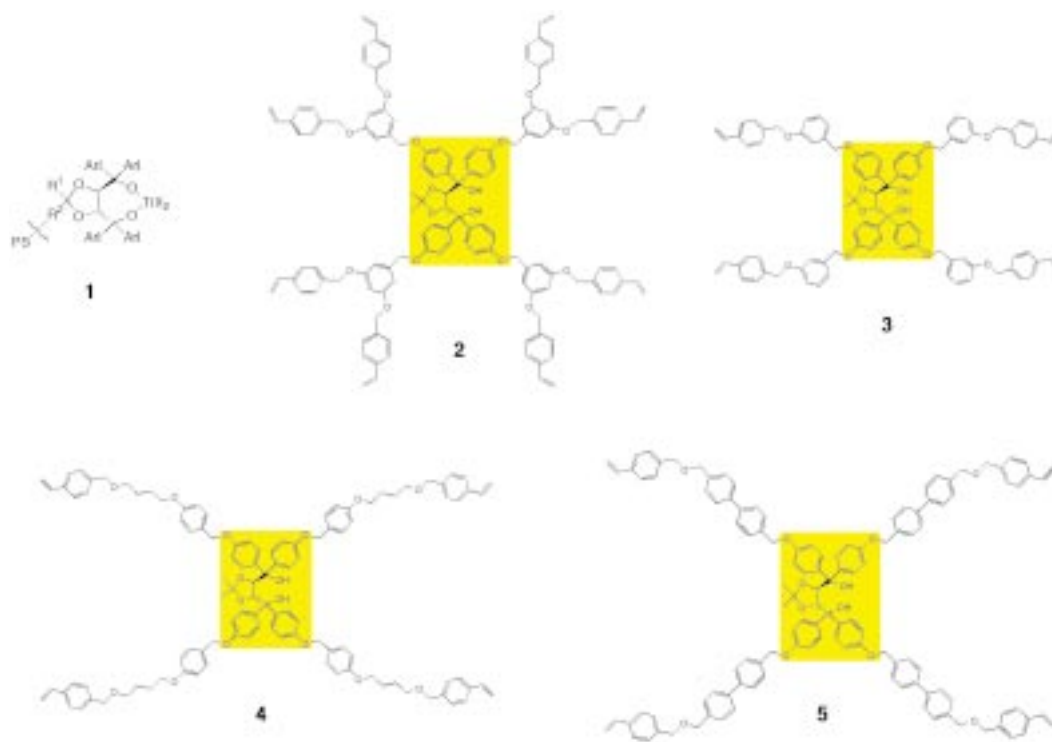
Holger Sellner and Dieter Seebach*

Polymer-bound reagents and catalysts^[1] have received renewed attention in connection with combinatorial synthetic methods.^[2] Besides the classical approach of grafting a functional group or ligand to a given polymer (e.g. the Merrifield resin), there have recently also been examples of incorporation of the groups of interest through copolymerization.^[3] Both methods have already been applied to TADDOL, a versatile ligand or ligand precursor for EPC synthesis^[4] (see **1** in Scheme 1).^[5] Two years ago we incorporated for the first time a dendritically modified TADDOL (**2**) with copolymerizable groups at the periphery as cross-linker in polystyrene, and obtained an enantioselective catalytic efficiency with the resulting Ti complex which was similar to that of the homogeneous analogues.^[6] Before starting to test this surprising effect with other ligands, we had to investigate its origin, and the stability of this new type of catalyst in multiple applications had to be analyzed.

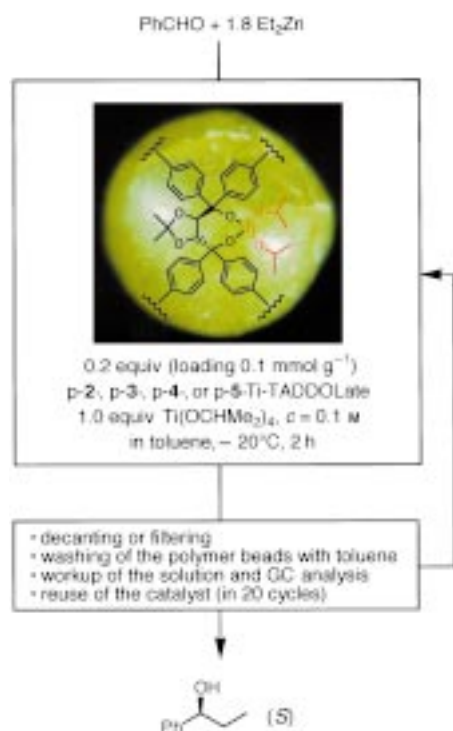
By cross-linking suspension copolymerization with styrene of dendrimer **2** and—for comparison—of TADDOLs **3–5**, bearing shorter and longer, more or less flexible spacers, we have generated beads of p-**2**, p-**3**, p-**4**, and p-**5** (Scheme 1),^[7] having a diameter of about 400 μm in the non-solvent-swollen state. Upon treatment with Ti(OCHMe₂)₄, polymer-bound diisopropoxy-Ti-TADDOLates were obtained, as illustrated in Scheme 2. Elementary analysis of the p-**2** derivative with a loading of 0.1 mmol g⁻¹ showed that 85% of the chiral diol moieties within the polymer had been complexed with Ti ions.

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Scheme 1. Polymer-bound Ti-TADDOLate **1** and styryl-substituted TADDOL derivatives **2–5**, which as cross-linkers were radically suspension-copolymerized with styrene to give **p-2**, **p-3**, **p-4**, and **p-5**. Loading: 0.1–0.6 mmol g⁻¹.^[7]



Scheme 2. Enantioselective addition of diethylzinc to benzaldehyde using polymer-bound Ti-TADDOLates from **p-2**–**p-5** (Scheme 1) under standard conditions. With 0.20, 0.05, and 0.02 equiv of **p-2**·Ti-TADDOLate the *S/R* selectivity in the formation of 1-phenylpropanol is 98/2, 96/4, and 90/10, respectively (GC analysis^[5a]). For results of 20 cycles, see Figure 1.

The polymer-bound Ti-TADDOLates were employed 20 times in the catalysis of the nucleophilic addition of diethylzinc to benzaldehyde^[4] under the conditions specified in Scheme 2. The results are presented in Figure 1.

The following features are remarkable:

- 1) While the enantioselectivity is above 9:1 with all polymers of low loading (0.1 mmol TADDOL per g polymer), only the dendritic polymer gives rise to a constant selectivity of 98:2 in 20 sequential applications (Figure 1 a, b).
- 2) The catalytic performance drops from **p-3** to **p-4** to **p-5**, that is, with increasing chain length of the spacers between the TADDOL core and polymer backbone. Besides the decline in enantioselectivity there are erratic results with respect to both kinetics and conversion with the simply elongated derivatives (Figure 1 a).^[8]
- 3) The same is true for polymers of higher degree of loading (Figure 1 b).
- 4) The low-loaded dendritic **p-2** beads keep their high swelling properties (ca. 2.5-fold) even after 20 runs, while all others do not swell as well after multiple reuse.
- 5) The rate of reaction is the same with and without stirring when **p-2** beads are employed (which fill the whole reaction volume under standard conditions). Thus, free diffusion of reactants and products to and from the active center is obtained.
- 6) The monomeric, soluble dendritic diisopropoxy-**2**·Ti-TADDOLate and the derived polymeric **p-2**·Ti-TADDOLate show virtually identical kinetics; it even looks like the polymer is slightly faster (Figure 1 c).^[9]

Conclusion: It is worthwhile to dendritically modify other *preferred* ligands with peripheral styryl groups, to incorporate them in a cross-linking manner into polymers, and to investigate the performance of the resulting immobilized catalysts.

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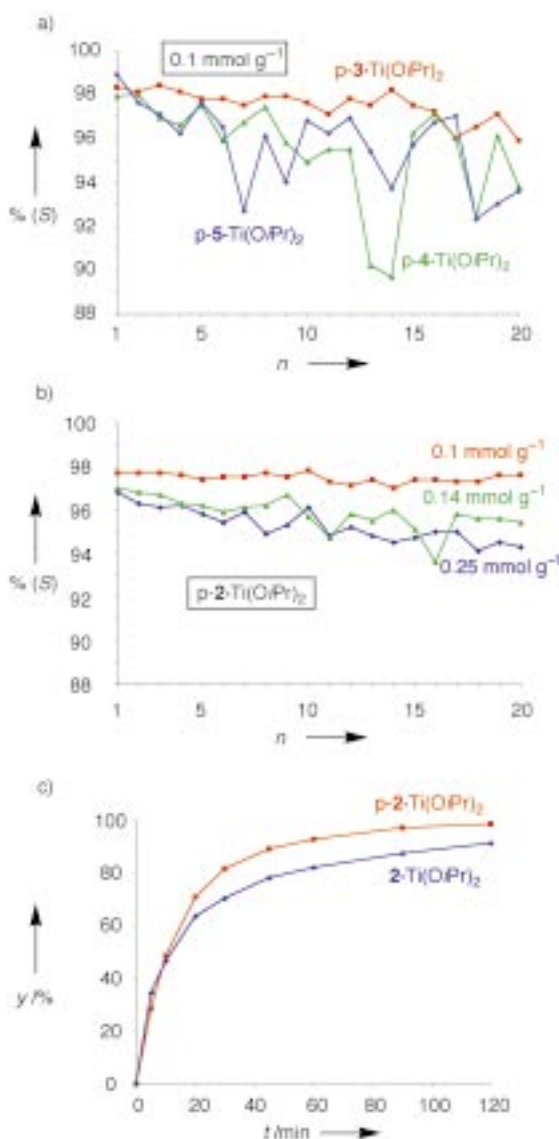


Figure 1. Enantioselectivities in the formation of (*S*)-1-phenylpropanol in *n* catalytic cycles a) with *p*-3-Ti-TADDOLate, *p*-4-Ti-TADDOLate, and *p*-5-Ti-TADDOLate under standard conditions (Scheme 2) and b) with *p*-2-Ti-TADDOLate using loadings of 0.1, 0.14, and 0.25 mmol g⁻¹; c) comparison of reaction kinetics with polymer-bound (red) and with homogeneous (blue) dendritic Ti-TADDOLate (*y* = conversion).^[9]

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- [7] The TADDOL core unit was employed as hexahydroxy derivative, which was etherified with the corresponding benzyl bromide.^[6] For the copolymerization, see references [5a, b, 6].
- [8] The polymers obtained with the previously used simple styryl TADDOL derivatives also showed such a decline.^[5a, b]
- [9] Although the curves in Figure 1 c have been reproduced several times, we choose this careful phrasing: The measurements are difficult, mainly due to a strong dependance of the rate upon the Ti(OCHMe₂)₄ concentration. Also, the 0.5-mmol scale of these kinetic measurements gives rise to difficulties in correct addition of substrates and sampling.

Chelated Bisphosphites with a Calix[4]arene Backbone: New Ligands for Rhodium-Catalyzed Low-Pressure Hydroformylation with Controlled Regioselectivity

Rocco Paciello,* Lorenz Siggel, and Michael Röper*

Dedicated to Professor Dr. Hans-Jürgen Quadbeck-Seeger on the occasion of his 60th birthday

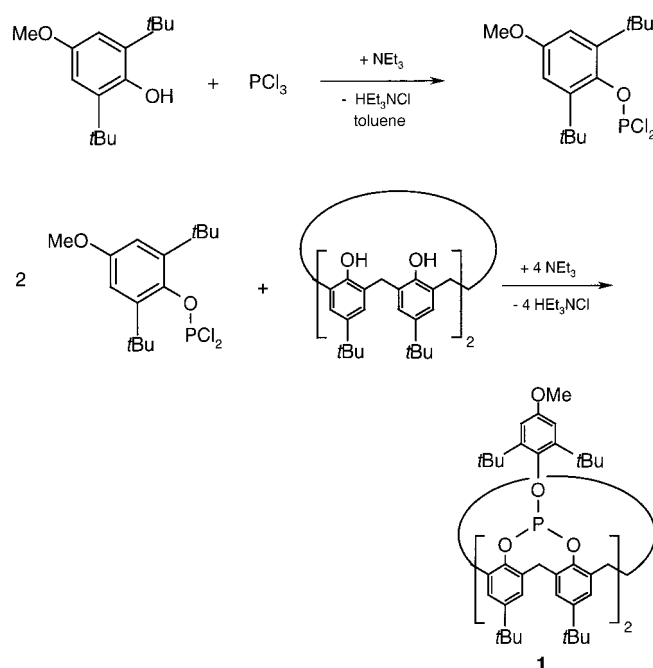
The rhodium-catalyzed low-pressure hydroformylation of olefins is, in terms of production volume, one of the most important technical applications of homogeneous catalysis.^[1] Rhodium complexes with ligands such as triphenylphosphane lead to aldehydes and especially to their linear isomers in very high yields. Furthermore, they allow the synthesis to be performed under low-pressure conditions, which is advantageous during processing.^[2] Industrial laboratories in particular are constantly searching for catalysts with improved properties. At the center of attention are chelating ligands such as bisphosphanes^[3] and sterically hindered bisphosphites,^[4] which provide active rhodium complexes of a defined structure in a predictable and controlled fashion.

We have been looking for polyols that can be readily prepared and that contain backbones with a defined con-

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formation for the synthesis of new chelate bisphosphites. During this search we came across calix[4]arenes, which are available in a one-pot reaction in acceptable yields from commercially available precursors such as *p*-*tert*-butylphenol and formaldehyde.^[5] Calix[4]arenes are macrocycles that possess a conical molecular form with four, symmetrically located OH groups at the narrow edge of the cone.^[6] Phosphorus-containing ligands based on calix[4]arenes, especially those with phosphane groups, have already been prepared.^[7] Recently chelate phosphanes have been reported in which the phosphorus atoms are located at the wide edge of the cone. These ligands induce *trans* configurations in square-planar and octahedral complexes of Pd²⁺, Pt²⁺, and Ru²⁺.^[8]

We obtained **1** (Scheme 1), the first compound of a new class of chelate phosphites in which two phosphorus atoms each are bonded to two neighboring oxygen atoms of the



Scheme 1. Synthesis of the calixarene bisphosphite **1**.

calix[4]arenes, from the reaction of *p*-*tert*-butylcalix[4]arene with two equivalents of P(OAr)Cl₂ (Ar = 2,6-di-*tert*-butyl-4-methoxyphenyl) in the presence of the base triethylamine.^[9] This results in molecular pincers made up of the rigid calix[4]arene backbone and two phosphorus atoms as bonding sites which are further blocked by the sterically demanding aryloxy substituent. The structure of a rhodium complex formed from **1**, as obtained from molecular modeling calculations, is illustrated in Figure 1 (see below).^[10]

The hydroformylation of 1-octene with [Rh(CO)₂(acac)]/1 (acac = acetylacetonate) at 20 bar CO/H₂ proceeded relatively slowly (Table 1). A reaction time of eight hours resulted in 63 % conversion and an aldehyde selectivity of 61 %. The regioselectivity in favor of *n*-nonanal was surprisingly high with 99.5 %.^[10] GC analysis showed only trace amounts of 2-methyloctanal which could just barely be determined quantitatively. This selectivity corresponds to a ratio of

Table 1. Hydroformylation of 1-octene with Rh/calix[4]arene bisphosphite.^[a]

Ligand	Conversion of 1-octene [%]	Selectivity [%]			Nonanal regio-selectivity ^[10] [%]
		nonanals	<i>n</i> -octenes	octane	
1	63 (8 h)	61	12	27	99.5
2	65 (4 h)	75	13	12	96.4
3	53 (4 h)	71	16	13	92.4
4	90 (4 h)	12	82	6	70.0

[a] Conditions: rhodium as [Rh(CO)₂acac], molar ratio olefin:Rh = 4000:1; L:Rh = 5:1; ligand **1**: 20 bar, 100 °C; ligand **2–4**: 5 bar, 80 °C.

200:1 between linear and branched aldehydes; to our knowledge, the highest regioselectivities observed to date.

The unusual properties of the catalyst become clear upon analysis of the structure obtained from molecular modeling calculations (see Figure 1).^[11] The rhodium atom located at the front side is blocked by the two sterically demanding 2,6-di-*tert*-butylphenoxy substituents in such a manner that even small molecules such as 1-octene are hindered in their attempt to coordinate to the metal center. This results in a relatively low reaction rate. Owing to the steric hindrance, however, the 1-octyl–rhodium intermediate which leads to *n*-nonanal is formed almost exclusively, whereas the sterically more demanding 2-octyl–rhodium intermediate either does not form or does not react to provide 2-methyloctanal.

These results imply that it should be possible to significantly increase the catalytic activity without seriously affecting the regioselectivity by decreasing the steric demand in the ligand structure. Replacement of the two *tert*-butyl substituents of the aryloxy group in **1** with isopropyl (ligand **2**), methyl (ligand **3**), and hydrogen (ligand **4**) in molecular modeling calculations resulted in a gradual opening of the structure (Figure 1). A drastic and unexpected structural change occurred upon the transition to **4**: The phenoxy group bends to the side and thus allows an uninhibited approach to the active center.

Based on these results, rhodium complexes with **2** and **3** should be significantly more active than those with the lead structure **1**, while maintaining a very high regioselectivity in

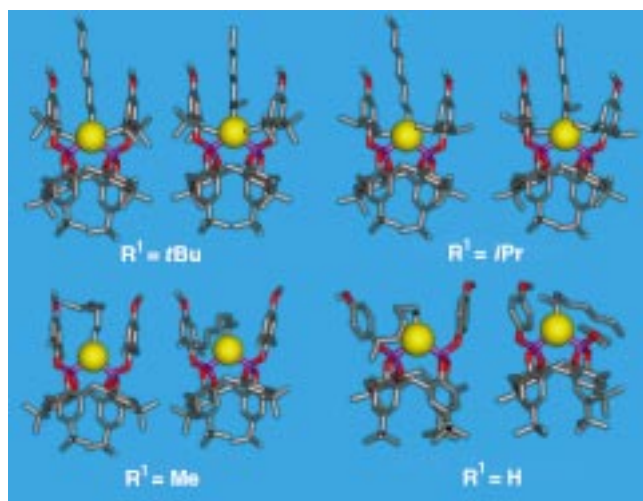
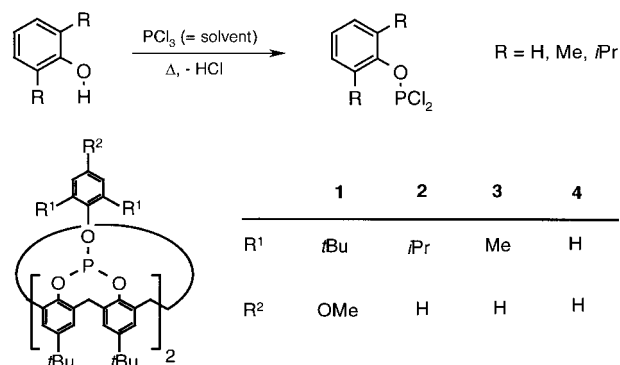


Figure 1. Molecular modeling structures of the rhodium alkyl complexes with **1–4**; the structure with the lowest energy is depicted for both the 1-octyl (left) and the 2-octyl adducts (right).

favor of *n*-nonanal. The highest activity is expected for **4**, albeit accompanied by a very low regioselectivity.

During the synthesis of **2–4** the preparation of the precursor P(OAr)Cl₂ was changed in order to obtain uniform products.^[12] Reaction of the phenols in PCl₃ as the solvent, removal of resulting HCl in an argon stream, and removal of excess PCl₃ by distillation resulted in a product (Schema 2) which, without further isolation, could be treated with *p*-*tert*-butylcalix[4]arene to yield the desired ligands **2–4**.



Scheme 2. Synthesis of P(OAr)Cl₂ and the calixarene bisphosphites **1–4**.

The hydroformylation results with **2–4** are summarized in Table 1. Owing to the significantly higher activity, we were able to employ milder conditions than those for the reaction with **1** (5 bar CO/H₂, 80 °C), and also cut the reaction time in half. The ligands **2** and **3** still result in improved aldehyde selectivities, and the regioselectivities of 96.4 % (**2**) and 92.4 % (**3**) are still high.

The calculated structural change upon the transition to ligand **4** manifested itself in catalysis experiments by a high activity for the olefin isomerization, poor aldehyde selectivity, and a very poor regioselectivity of 70 %. Whereas the transition from **1** to **2** and **3** results in only gradual differences, such as a slight decrease in the regioselectivity and a significant improvement in activity, the sterically less demanding ligand **4** leads to a qualitatively different catalyst.

In the rhodium-catalyzed hydroformylation of 1-octene, chelate bisphosphites with a *p*-*tert*-butylcalix[4]arene backbone lead to *n*-nonanal with surprisingly high regioselectivities.^[13] By variation of the structure and use of molecular modeling calculations, it was possible to influence the activity and regioselectivity of the reaction in a controlled fashion. Thus, we have taken a big step towards the idea of rational catalyst design. To be able to treat the electronic properties of ligands in an active complex, and not only the steric factors^[14] with molecular modeling calculations as presented herein, we are developing an ab initio process for the catalyst core made up of the rhodium atom and the neighboring atoms in combination with a simultaneous molecular mechanical treatment of the ligand periphery (“embedding” method).

Experimental Section

Syntheses: All reactions were carried out with dry reagents and solvents in flame-dried glassware under an argon atmosphere. Substituted phenols and

p-*tert*-butylcalix[4]arene were obtained from Aldrich. They were dried by azeotropic distillation with toluene.

1: First step: 3,5-Di-*tert*-butyl-4-hydroxyanisole (23.6 g, 0.1 mol) was dissolved in toluene (500 mL). The solution was cooled and after addition of triethylamine (68.2 mL, 0.5 mol) transferred into an addition funnel. This solution was added dropwise within 15 min to a solution of toluene (1 L) and PCl₃ (8.8 mL, 0.1 mol) cooled to –40 °C. The mixture was slowly warmed to room temperature, stirred for 1 h at room temperature and then for 10 h at 100 °C. Second step: Powdered *p*-*tert*-butylcalix[4]arene (32.4 g, 0.05 mol) was added to the solution obtained in the first step, and the mixture was cooled to –40 °C. A solution of triethylamine (136.5 mL, 1 mol) in toluene (500 mL) was added dropwise within 15 min. The solution was slowly warmed to room temperature and stirred for 1 h at room temperature and then for 10 h at 100 °C. The NEt₃HCl precipitate was isolated by filtration through a glass frit and washed with toluene (3 × 250 mL). The filtrate and wash solutions were concentrated at 60 °C and 80 mbar. The light brown, viscous residue was washed with pentane (3 × 100 mL), dried and subsequently extracted with acetonitrile (400 mL) under reflux. After a portion had been dissolved, the remaining powder was filtered, washed with pentane (3 × 200 mL) and dried in vacuo. This procedure afforded 5 g of a white powder (yield 22.5 %). Elemental analysis (%): found: C 75.5, H 8.3, O 10.9, P 5.3; calcd: C 76.2, H 8.9, O 9.9, P 5.1; ³¹P NMR: δ = 121 (one signal); MS (EI, direct injection): *m/z*: 1176 [*M*⁺].

2: First step: At room temperature, PCl₃ (21.0 mL, 0.24 mol) was slowly added dropwise to 2,6-diisopropylphenol (7.13 g, 0.04 mol). The solution was subsequently stirred for 5 h under reflux, and excess PCl₃ was removed by distillation. Conversion approx. 94 % (GC). Second step: *p*-*tert*-Butylcalix[4]arene (13.0 g, 0.02 mol) was dissolved in toluene (1.2 L), triethylamine (55 mL, 0.4 mol) was added and the resulting solution cooled to –20 °C, after which the solution from the first step was added dropwise. The mixture was slowly warmed to room temperature and stirred for 1 h at room temperature and then for 10 h at 100 °C. The NEt₃HCl precipitate was isolated by filtration through a glass frit and washed with toluene (3 × 250 mL). The filtrate and wash solutions were worked up as for **1**. Yield: 11.3 g of a white powder (56 %). Elemental analysis (%): found: C 77.3, H 8.5, O 9.2, P 5.6; calcd: C 77.0, H 8.1, O 9.1, P 5.9; ³¹P NMR: δ = 120 (one signal).

3: Synthesis as described for **2**, yield 12.4 g of a white powder (65 %). Elemental analysis (%): found: C 76.4, H 7.3, O 10.2, P 6.3; calcd: C 76.0, H 7.4, O 10.1, P 6.5; ³¹P NMR: δ = 119 (one signal).

4: Synthesis as described for **2**, yield 4.5 g of a white powder (23 %). Elemental analysis (%): found: C 75.2, H 7.1, O 10.9, P 7.2; calcd: C 75.3, H 7.0, O 10.8, P 7.0; ³¹P NMR: δ = 115 (one signal).

Hydroformylation experiments: A 350-mL autoclave equipped with a gas dispersion turbine was charged with 1-octene (56.9 g, 508 mmol), texanol (70 mL, 2,2,4-trimethyl-1,3-pentanediol isobutyrate), rhodium in the form of [Rh(CO)₂(acac)] (0.032 g, 0.124 mmol), and **1** (0.710 g, 0.604 mmol). After purging with a gas mixture of 50 vol. % CO and H₂ each, the reaction mixture was heated to 100 °C and a pressure of 20 bar applied with this gas mixture. During the reaction the pressure in the reactor was maintained at 20 bar with a pressure controller. After a reaction time of 8 h, analysis of the reaction mixture yielded the following composition (mass proportions, GC, with internal standard and correction factors): 1-octene: 16.6 % (0.188 mol), internal *n*-octenes: 3.41 % (0.039 mol), octane: 7.84 % (0.087 mol), 2-propylhexanal: 0.00 % (0.0 mol), 2-ethylheptanal: 0.00 % (0.0 mol), 2-methyloctanal: 0.11 % (0.001 mol), *n*-nonanal: 21.84 % (0.195 mol), texanol: 45.8 %, other: 0.0 %; conversion (based on 1-octene): 63 %; yields: nonanal: 39 %, octane: 17 %; selectivity (based on 1-octene): nonanals: 61 %, octane: 27 %, internal *n*-octenes: 12 %; regioselectivity^[10] *n*-nonanal/nonanals: 99.5 %. The approximate conditions applied for the analogous hydroformylations in the presence of **2–4** were a temperature of 80 °C and a total pressure of 5 bar.

Molecular modeling calculations: All calculations were performed using a Silicon Graphics Indigo2 Workstation under a CHARMm21 force field as implemented in the program Quanta (Molecular Simulations Inc., 9685 Scranton Road, San Diego, CA 92121, USA). No restrictive assumptions were made for the geometric environment of the rhodium atom. The chelate bisphosphite and the 1-octyl- or the 2-octyl substituent were bonded to the rhodium atom. Vibrational terms for the bonds between the

metal center and the attached atoms were set based on crystallographic data, the angle and torsional terms were set to zero. Parameters for the non-bonding terms (Lennard-Jones 6–12) were set by Quanta. For further details see reference [11].

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Organic Transformations at a Group 4 Metallocene Framework: Formation of a Rigid ansa-Metallocene by Mannich-type Carbon–Carbon Coupling

Stephanie Knüppel, Gerhard Erker,* and Roland Fröhlich

Group 4 ansa-metallocenes have been prepared in great structural variety owing to their key importance in homogeneous Ziegler catalysis. In the vast majority of the cases reported the specific ansa bridge and the chosen substitution pattern was built up or incorporated into the preformed organic ligand system before final attachment to the electrophilic Group 4 metal center.^[1] Examples for selective organic reactions at the Group 4 metallocene framework, especially those involving conventional organic functional group chemistry, are close to nonexistent,^[2] probably because of a pronounced incompatibility of the majority of the air- and moisture-sensitive Group 4 metallocene complexes with the typical conditions involved in many classical C–C coupling procedures in organic synthesis.

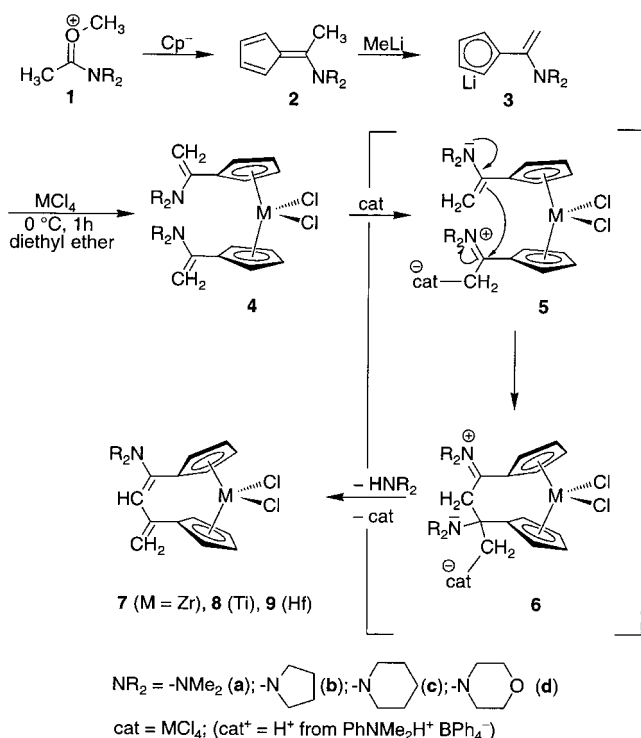
We and a few others had previously investigated a number of ways for dealing with functional groups at the bent metallocene nucleus.^[3] We have now, to our knowledge for the first time, found a simple way to close an ansa bridge at the preformed Group 4 metallocene complex using a variant of the Mannich reaction, a classical synthetic organic method for forming a carbon–carbon bond.

Our simple route starts with rather conventional fulvene chemistry. O-methylated *N,N*-dimethylacetamide (**1a**) was treated with sodium cyclopentadienide according to the procedure developed by Hafner et al.^[4] to yield 6-(dimethylamino)-6-methylfulvene (**2a**, Scheme 1). Amine exchange by treatment with, for example, the cyclic secondary amines pyrrolidine, piperidine, or morpholine gave the corresponding 6-amino-substituted fulvenes **2b–d**. In some cases the direct route employing the acetamides derived from these cyclic amines proved to be advantageous.

Deprotonation of the fulvene **2a** readily takes place upon treatment with methylolithium (1 molar equiv) in diethyl ether to give **3a**, which was isolated (ca. 90% yield) and characterized spectroscopically (¹³C NMR ([D₆]benzene/[D₈]THF 8/1): δ = 42.2 (NMe₂), 157.6 and 81.6 (C=CH₂), 119.1 (*ipso*-C), 104.6 and 103.7 (CH of C₅H₄); ¹H NMR: δ = 4.33 and 3.91 (²J = 1 Hz, =CH₂)). The enamino-substituted cyclopentadienides **3b–d** were obtained accordingly by treatment of **2b–d** with methylolithium. The reagents **3a–d** were then treated with titanium, zirconium, or hafnium tetrachloride in a 2:1 molar ratio under carefully selected conditions. The reaction

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Scheme 1.

of (1-pyrrolidinoethenyl)cyclopentadienide (**3b**) with ZrCl₄ may serve as a representative example.

Zirconium tetrachloride (1 molar equiv) was added at 0 °C in one portion to a solution containing **3b** (2 molar equiv) in diethyl ether. After the mixture was stirred for 1 h at 0 °C, the precipitate was removed by filtration through Cellite. The bis[(1'-pyrrolidinoethenyl)cyclopentadienyl]ZrCl₂ metallocene (**4b**, Scheme 1) was obtained from the filtrate as a solid in greater than 50% yield. The ¹H NMR spectrum shows the characteristic signals of the monosubstituted η⁵-C₅H₄ moiety at δ = 6.67 and 6.18 (in CDCl₃) and the typical resonances of the intact enamino substituent (δ = 4.29, 4.07 (=CH₂)). Treatment of **3c** and **3d** with ZrCl₄ under analogous conditions gave the complexes **4c** and **4d**, respectively.

We then changed the conditions for the reaction of **3** with ZrCl₄ slightly and obtained a different type of product. As a typical example, reagent **3b** was again treated with ZrCl₄ in diethyl ether, but this time the reaction mixture was allowed to remain at ambient temperature for 12 h with stirring before it was worked up. In this case the open metallocene **4b** was not found, but instead the ansa-metallocene **7b** was obtained in good yield (ca. 70% isolated). The product **7b** shows the ¹H NMR signals of two nonequivalent monosubstituted η⁵-cyclopentadienyl rings (δ = 6.72, 6.67, 6.17, and 6.08 in CDCl₃) and a typical ABX spin pattern of the condensed ansa bridge at δ = 5.44 (-CH=), 4.92 and 4.84 (=CH₂) (¹³C NMR: δ = 99.5 (-CH=), 109.3 (=CH₂)) as well as a single pyrrolidyl residue.

Treatment of the enamino-substituted cyclopentadienides **3a**, **3c**, and **3d** with ZrCl₄ under analogous conditions (diethyl ether, room temperature, 12 h) gave the analogous C₃-bridged ansa-zirconocenes **7a**, **7c**, and **7d**, respectively, mostly in good yield. Similarly, we have also prepared the corresponding

ansa-titanocene **8a** and the ansa-hafnocene dichlorides **9a** and **9b** by treatment of the respective enaminocyclopentadienides **3a** and **3b** with TiCl₄ or HfCl₄ under the standard conditions.

Single crystals suitable for crystal structure analyses were obtained for the ansa-zirconocene complex **7a** and the hafnium analogue **9a**. The two structures are very similar, aside from the expected changes resulting from the two different metals. Therefore, only the structure of the zirconium complex **7a** is briefly described here (Figure 1).^[5]

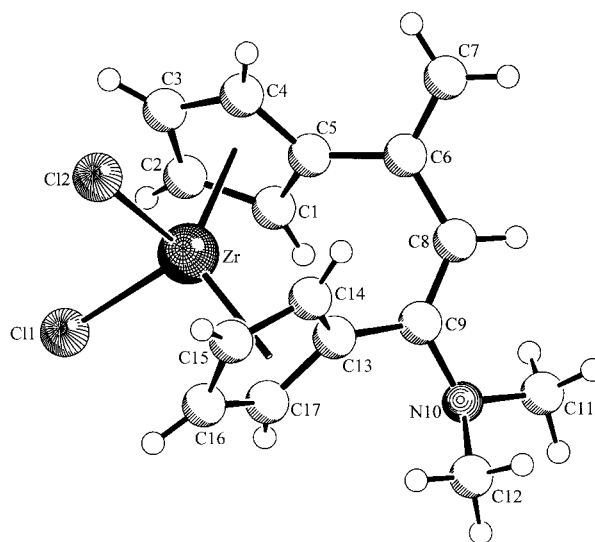


Figure 1. Molecular structure of the ansa-zirconocene complex **7a**. Selected bond lengths [Å] and angles [°]: Zr–Cl1 2.432(1), Zr–Cl2 2.441(1), Zr–C₆ 2.506(5), C5–C6 1.490(5), C6–C7 1.333(6), C6–C8 1.464(6), C8–C9 1.353(6), C9–N10 1.399(5), C9–C13 1.481(5), N10–C11 1.455(5), N10–C12 1.468(6); Cl1–Zr–Cl2 97.40(5), C5–C6–C7 119.9(4), C5–C6–C8 117.5(4), C7–C6–C8 122.2(4), C6–C8–C9 125.1(3), C8–C9–N10 122.9(3), C8–C9–C13 123.1(3), N10–C9–C13 114.0(3).

The X-ray crystal structure analysis of **7a** shows that a very rigid ansa-metallocene framework has been formed. The ansa bridge consists of a conjugated *N,N*-dimethylaminobutadiene framework to which the Cp moieties are bonded at the positions C6 and C9 (Figure 1). The diene-amine unit shows a typical bond alternation (C7–C6 1.333(6), C6–C8 1.464(6), C8–C9 1.353(6) Å).^[6] This unit is itself planar. The nitrogen atom N10 is also oriented in the diene-amine plane; it is slightly pyramidalized but clearly in electronic conjugation with the adjacent diene π system.^[6a] This orientation brings the two methyl substituents at N10 in *E* and *Z* positions at the N10–C9–C8 moiety.

The resulting C₃ ansa bridge is located at the narrow back side of the bent metallocene. There it adopts a “skew” arrangement that brings it substantially away from an expected perpendicular orientation relative to the Cp ring planes. The angle between, for example, the Cp ring (C1–C5) and the plane of the ansa bridge (C6–C8–C9) amounts to approximately 119°. The angle between the Cp planes is 126°, which is about 3° smaller than that observed in an open Group 4 bent metallocene complex. Thus, complex **7a** (and its congeners) exhibits a very rigid ansa-metallocene frame-

work.^[7] In the crystal it is actually chiral, but inverts rapidly in solution.

In a series of experiments it was demonstrated that the ansa-metallocene complexes **7** were formed via **4** by an acid-catalyzed intramolecular condensation reaction. Treatment of, for example, the (1'-piperidinoethenyl)-substituted zirconocene dichloride **4c** with a catalytic amount of ZrCl₄ in diethyl ether for 12 h at room temperature resulted in clean formation of the condensation product **7c** (84% isolated). The **4d**→**7d** condensation was similarly catalyzed by the Brønsted acid dimethylanilinium tetraphenylborate (ca. 30 mol %, CD₂Cl₂, 24 h, room temperature, quantitative conversion). We conclude that the **4**→**7** condensation represents an example of an acid-catalyzed Mannich-type reaction (see Scheme 1 for a plausible mechanistic formulation). It must be assumed that residual ZrCl₄ has served as the Lewis acid catalyst for the condensation step in the initially described one-pot preparations of complexes **7**.

Activation of the rigid ansa-metallocenes **7–9** by treatment with excess methylalumoxane (MAO) in toluene^[8] results in the formation of active homogeneous Ziegler catalyst systems. Table 1 contains a compilation of characteristic data

These scouting polymerization experiments show that the acid-catalyzed intramolecular Mannich-type coupling reaction (**4**→**7**) has resulted in the formation of rigid ansa-metallocene systems that are suitable precursors for the generation of active homogeneous Ziegler catalysts. The catalyst systems based on **7–9** exhibit characteristics similar to those typically shown by the “constrained-geometry” Ziegler catalysts based on half-sandwich silyl-bridged Cp*/amido zirconium complexes.^[9] Moreover, our study shows that variants of carbon–carbon bond forming reactions originating from the classical synthetic organic repertoire can be adopted and successfully used in the chemistry of even quite sensitive organometallic Group 4 metal complexes, provided that a few precautions are taken to protect these sensitive systems from undergoing undesired side reactions. We are confident that further adaptations of classical organic C–C coupling protocols will allow an extension of the scope of transformations in this area of organometallic chemistry and will help to make a variety of novel organometallic catalysts and reagents readily available.

Experimental Section

The preparation of the hafnium complex **9a** serves as a typical example: Compound **2a**^[4] was cleanly deprotonated by treatment with methylolithium (1 molar equiv) in THF at –78 °C. The lithium compound **3a** was isolated in 89% yield. ¹H NMR ([D₆]benzene/[D₈]THF 8/1, 200 MHz): δ = 6.26, 6.05 (m, each 2 H, C₅H₄), 4.33, 3.91 (d, ²J = 1 Hz, each 1 H, =CH₂), 2.77 (s, 6 H, CH₃); ¹³C NMR ([D₆]benzene/[D₈]THF 8/1, 50 MHz): δ = 157.6, 81.6 (C=CH₂), 119.1 (*ipso*-C), 104.6, 103.7 (CH of C₅H₄), 42.2 (CH₃). A sample of **3a** (1.50 g, 10.6 mmol) was suspended in diethyl ether (60 mL) and cooled to –78 °C. Solid HfCl₄ (1.70 g, 5.3 mmol) was added in one portion with stirring. The mixture was allowed to warm to room temperature and stirred for 12 h. A precipitate was removed by filtration, and washed with a small amount of diethyl ether and dichloromethane. Solvent was removed from the combined filtrates. Recrystallization from diethyl ether gave 1.48 g (59%) of the metallocene **9a**. M.p. 174 °C (decomp., DSC); elemental analysis calcd for C₁₆H₁₇NCl₂Hf (472.7): C 40.65, H 3.62, N 2.96; found: C 40.56, H 4.14, N 3.34; ¹H NMR (CDCl₃, 200 MHz): δ = 6.60 (m, 4H), 6.03 (m, 2H), 5.94 (m, 2H, C₅H₄), 5.58 (s, 1H, –CH=), 4.96, 4.91 (brs, each 1H, =CH₂), 2.60 (s, 6H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ = 147.4 (N=C=), 138.9, 135.0, 124.5 (*ipso*-C of C₅H₄ and C=CH₂), 122.5, 121.9, 112.1, 108.9 (CH of C₅H₄), 111.1 (=CH₂), 104.6 (–CH=), 41.7 (CH₃); IR (KBr): $\tilde{\nu}$ = 1604 cm^{–1}. Complex **9a** was also characterized by an X-ray crystal structure analysis.^[5b]

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Table 1. Polyethylene and polypropylene formation with the catalyst systems **7**/MAO–**9**/MAO.^[a]

Cat.	M	Al:M ^[b]	[7] [mmol]	PE ^[c] [g]	PP ^[d] [g]	T [°C]	a ^[e]	M.p. [°C]	$\bar{M}$ ^[f] [kgmol ^{–1}]
7a	Zr	750	0.044	29	–	20	320	127	
7a	Zr	670	0.049	55 ^[g]	–	60 ^[h]	840	125	
8a	Ti	600	0.055	8	–	60	70	130	
9a	Hf	670	0.049	41	–	60 ^[h]	420	126	
7d	Zr	1180	0.028	10	–	20	175	132	
7d	Zr	1100	0.030	62	–	60 ^[h]	1040	125	
7a	Zr	530	0.062	–	36 ^[i]	20	90	185	
7b	Zr	760	0.043	–	1.4 ^[j]	0	5	230	
7d	Zr	890	0.037	–	1.2 ^[j]	–5	8	370	
7d	Zr	1030	0.032	–	2.8	20	14	–	– ^[k]
7d	Zr	1100	0.030	–	7.3	60	121	–	– ^[k]

[a] Reactions in toluene (Büchi glass autoclave) at 2 bar, reaction time 60 min, unless indicated otherwise. [b] M = Ti, Zr, Hf. [c] Yields of polyethylene. [d] Yields of polypropylene. [e] Integral catalyst activity *a* in g(polymer)/[mmol(cat)]^{–1}bar^{–1}h^{–1}. [f] Molecular weight (PP). [g] After 40 min. [h] Initial temperature, increased by 30–40 °C during the exothermic reaction. [i] After 180 min. [j] After 120 min. [k] Not determined.

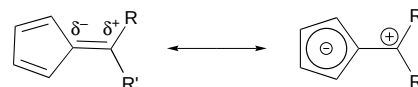
from selected examples of ethene and propene polymerization reactions with these ansa-metallocene catalyst systems. The zirconium-based catalysts produce near to atactic polypropylene as expected. The **7a**/MAO and **8a**/MAO catalyst systems can also be used for ethene/norbornene and ethene/1-hexene copolymerizations. With the **7a**/MAO catalyst (Al:Zr ≈ 700) the ethene/norbornene copolymer was formed with an activity *a* of about 220. From the ¹H NMR analysis an ethene/norbornene ratio of approximately 57:1 was deduced (m.p. 116 °C). The norbornene incorporation was slightly less with the titanium-based catalyst system **8a**/MAO (70:1, m.p. 102 °C, *a* = 92). A random ethene/1-hexene copolymer (ratio ca. 60:1) was formed with the **7a**/MAO catalyst system at 60 °C in toluene (*a* = 800).

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- [5] a) X-ray crystal structure analysis of **7a**: $C_{16}H_{17}NCl_2Zr$, $M_r = 385.43$, yellow crystal, $0.50 \times 0.40 \times 0.15$ mm, $a = 10.774(1)$, $b = 10.501(1)$, $c = 13.930(1)$ Å, $\beta = 101.52(1)^\circ$, $V = 1544.3(2)$ Å³, $\rho_{\text{calc}} = 1.658$ g cm⁻³, $F(000) = 776$ e, $\mu = 10.47$ cm⁻¹, empirical absorption correction with φ scan data ($0.945 \leq C \leq 0.999$), $Z = 4$, monoclinic, space group $P2_1/n$ (no. 14), $\lambda = 0.71073$ Å, $T = 223$ K, $\omega/2\theta$ scans; of 3261 reflections collected ($\pm h, -k, -l$), $[(\sin\theta)/\lambda] = 0.62$ Å⁻¹, 3132 were independent and 2744 observed [$I \geq 2\sigma(I)$]; 183 refined parameters, $R = 0.045$, $wR^2 = 0.165$, max./min. residual electron density $0.76/-0.97$ e Å⁻³, hydrogen atoms calculated and refined as riding atoms.^[5c] b) X-ray crystal structure analysis of **9a**: $C_{16}H_{17}NCl_2Hf$, $M_r = 472.70$, yellow crystal, $0.25 \times 0.20 \times 0.20$ mm, $a = 10.756(2)$, $b = 10.458(2)$, $c = 13.909(3)$ Å, $\beta = 101.71(2)^\circ$, $V = 1532.0(5)$ Å³, $\rho_{\text{calc}} = 2.049$ g cm⁻³, $F(000) = 904$ e, $\mu = 71.46$ cm⁻¹, empirical absorption correction with φ scan data ($0.737 \leq C \leq 0.998$), $Z = 4$, monoclinic, space group $P2_1/n$ (no. 14), $\lambda = 0.71073$ Å, $T = 223$ K, $\omega/2\theta$ scans; of 3244 reflections collected ($\pm h, -k, -l$), $[(\sin\theta)/\lambda] = 0.62$ Å⁻¹, 3116 were independent and 2903 observed [$I \geq 2\sigma(I)$]; 184 refined parameters, $R = 0.028$, $wR^2 = 0.087$, max./min. residual electron density $1.58/-1.95$ e Å⁻³, hydrogen atoms calculated and refined as riding atoms.^[5c] c) Data sets were collected with Enraf Nonius CAD4 or MACH3 diffractometers, equipped with sealed tube or rotating anode generators. Programs used: data reduction MolEN, structure solution SHELXS-86, structure refinement SHELXL-97, graphics SCHAKAL-92. 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-112078 (**7a**) and -112077 (**9a**). 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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Intramolecular NMe₂H Elimination and Fulvene Coupling Leading to Novel Allyl-Bridged Zirconocene and Hafnocene Complexes**

Sheng-Di Bai, Xue-Hong Wei, Jian-Ping Guo, Dian-Sheng Liu,* and Zhong-Yuan Zhou

Fulvenes can be described by several resonance structures^[1] that support the presence of significant dipole character (Scheme 1).^[2] Therefore the exocyclic double bond is susceptible to nucleophilic addition (by, for example, alkylmetal



Scheme 1. Resonance structures of fulvene.

compounds such as LiMe, ClMgC₃H₅, LiC₆H₅, and KCH₂C₆H₅),^[3, 4] reduction,^[5] reductive coupling,^[6] or deprotonation at the 6-methyl group (R = CH₃) upon exposure to organometallic compounds of Group 1 or 2.

Recently there has been increased study of Group 4 aminofulvene complexes. For example, introduction of an amino group at C6 of fulvene and subsequent reaction with an organolithium compound led to chiral ligands, which upon reaction with ZrCl₄ provided the corresponding substituted zirconium complexes.^[7] Starting from 6-(dimethylamino)-6-methylfulvene (**1**, see Scheme 1), a C₁-bridged anionic C₃H₄CR₁⁻NR₂ ligand was obtained with which a constrained-geometry Ziegler catalyst had been synthesized.^[8, 9] We report here that an unusual intramolecular NMe₂H elimination and a fulvene coupling take place when Group 4 chlorides are treated with (1-dimethylaminoethenyl)cyclopentadienyllithium (**2**), resulting in the novel allyl-bridged metallocene complexes **3** or **4** (Scheme 2).

The preparation of **2** involved the deprotonation of **1**. This type of reaction had been previously described with the use of different organolithium reagents. It was found that the deprotonation did not depend on the base strength of the

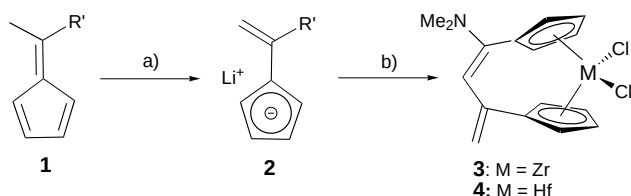
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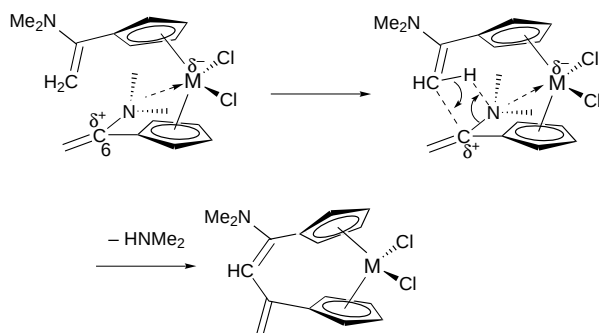


Scheme 2. Synthesis of **2–4**. a) $\text{LiCH}(\text{SiMe}_3)_2$, THF, 2 h, 0–25 °C; b) MCl_4 (M = Zr or Hf), THF or Et_2O , 12 h, –78 °C → room temperature. R' = NMe_2 .

organolithium compound, but rather the weak nucleophilic behavior of, for example, LiNiPr_2 (LDA),^[10] LiNMe_2 , or, in our case, $\text{LiCH}(\text{SiMe}_3)_2$. Alkyl- and aryllithium compounds normally cause an addition or reductive coupling reaction. Lithium naphthalenide, a reductive reagent, reacted with aminofulvene **1**, leading to bridged and cyclic bridged bifulvenes.^[11] However, $\text{LiCH}(\text{SiMe}_3)_2$ did not prove to be a strong nucleophilic reagent like alkyllithium, possibly due to its steric effect. It may be used as a relatively stronger bulky base than lithium amides.

Subsequent treatment of **2** with MCl_4 (M = Zr or Hf) in THF or Et_2O at –78 °C proceeded, unexpectedly, by an intramolecular NMe_2H elimination that resulted in the coupling of fulvenyl groups to give complexes **3** and **4** (Scheme 2). When the reaction of **2** with ZrCl_4 was carried in a sealed NMR tube, two ^1H resonances were observed at $\delta = 2.26$ and 1.50, consistent with the presence of free NMe_2H . The ^1H NMR data of **3** and **4** are in accord with their X-ray structures (see below).

The reaction discussed is a useful synthetic pathway to allyl-bridged ansa-metalloenes, especially those with a conjugated 14π electron system (three sp^2 carbon bridging atoms). We propose the following mechanism for the reaction (Scheme 3): 1) primary formation of a metallocene with a



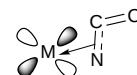
Scheme 3. Proposed mechanism for the formation of **3** and **4**.

$\text{N} \rightarrow \text{M}$ donor bond from the dimethylamino group (which is more or less similar to the formation of a tertiary amine in the Mannich reaction), 2) nucleophilic attack on C6 of one of the fulvenyl ligands to form a transition state, and 3) loss of one molecule of dimethylamine, maybe by a concerted reaction, to give complex **3** or **4**.

The $\text{N} \rightarrow \text{Zr}$ donor bond was observed for a zirconocene cation derived from aminofulvene **1**.^[7] However, the question of importance for the mechanism is whether this donor bond

would occur for the 16-electron configuration of the zirconium or hafnium atom. Our experiments have shown that there was no intramolecular NMe_2H elimination and fulvene coupling during the deprotonation of **1** (see the ^1H NMR data of **2**). We believe that in polar solvents, such as THF or Et_2O , formation of an ionized zirconium atom enhances the $\text{N} \rightarrow \text{Zr}$ interaction and induces the positive charge on C6, which is probably crucial for the subsequent nucleophilic attack. This thermodynamically favorable coupling reaction may also result from formation of a conjugated 14π electron system including two Cp fragments and the bridging allyl group (for carbon).

According to this mechanism the positive charge on the N atom will influence the intramolecular NMe_2H elimination, which should be related to Lewis acidic and, in our view, d-electron backbonding effects of the central metal. Since the $\text{C}=\text{C}-\text{N}$ moiety in the enamino group could form a three-centered 4π electron system, the C–N bond would preferably act as a p donor to the central metal and cause a backbonding effect (Scheme 4). Thus the early transition metal might easily steer the intramolecular NMe_2H elimination to give the allyl-bridged metallocene.



Scheme 4. Schematic representation of the p donor bonding in **3** and **4**.

The X-ray structure of **3** is shown in Figure 1. The average $\text{Cp}(\text{centroid})-\text{Zr}$ bond distances are 2.205 and 2.192 Å, and the corresponding $\text{Cp}(\text{centroid})-\text{Zr}-\text{Cp}'(\text{centroid})$ angle is

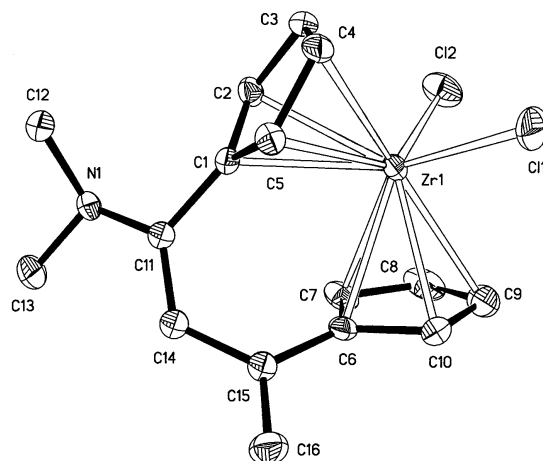


Figure 1. The molecular structure of **3**. Selected bond lengths [Å] and angles [°]: Zr1–C1 2.5266(11), Zr1–C2 2.5020(12), Zr1–C3 2.5068(11), Zr1–C4 2.5135(11), Zr1–C5 2.4903(12), C1–C11 1.495(2), C11–C14 1.345(2), C14–C15 1.471(2), C6–C15 1.470(2), C15–C16 1.336(2), N1–C11 1.390(2), N1–C13 1.460(2); C12–Zr1–C11 97.33(2), C1–Zr1–C6 73.96(3), C14–C11–C1 122.99(10), C11–C14–C15 125.37(11), C6–C15–C14 118.10(10), C16–C15–C6 119.83(11).

128.0°, which is smaller than that of the propyl-bridged zirconocene $[(\eta^5\text{-Cp})_2\text{C}_3\text{H}_6]\text{ZrCl}_2$ (129.5°).^[12] The single and double bonds of the bridging allyl group are 1.471(2) and 1.345(2) Å long, respectively, and the N1–C11 bond length of 1.390(2) Å means that the N atom engages in the conjugate system with the allyl and cyclopentadienyl fragments.

The X-ray structure of **4** (Figure 2) is quite similar to that of **3**. The average $\text{Cp}(\text{centroid})-\text{Zr}$ bond distances are 2.189

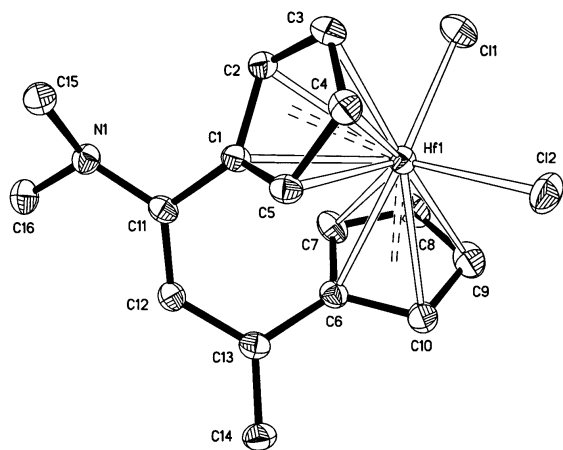


Figure 2. The molecular structure of **4**. Selected bond lengths [Å] and angles [°]: Hf1–C1 2.509(4), Hf1–C2 2.486(4), Hf1–C3 2.495(5), Hf1–C4 2.513(4), Hf1–C5 2.479(4), C1–C11 1.476(6), C11–C12 1.363(6), C12–C13 1.459(6), C6–C13 1.492(6), C13–C14 1.354(6), N1–C11 1.389(6), N1–C16 1.414(7); C12–Hf1–C11 96.31(5), C1–Hf1–C6 73.77(14), C12–C11–C1 123.0(4), C11–C12–C13 124.6(4), C6–C13–C12 117.9(4), C14–C13–C6 119.4(4).

and 2.163 Å, and the corresponding Cp(centroid)–Zr–Cp'(centroid) angle is 128.4°. The single and double bonds of the bridging allyl group are 1.476(6) and 1.363(6) Å long, respectively, and the N1–C11 bond length is 1.389(6) Å.

Experimental Section

2: In a typical run a solution of $\text{LiCH}(\text{SiMe}_3)_2$ (7.4 mmol, 1.23 g) in THF (ca. 10 mL) was added dropwise to a solution of **1** (1 g, 7.4 mmol) in THF (ca. 20 mL) at 0 °C with stirring. The mixture was allowed to warm to room temperature for 2 h. The solvent was removed in vacuo to give **2** as a light brown powder (1.02 g, 98 %). ^1H NMR (300 MHz, $[\text{D}_6]\text{benzene}$): δ = 6.76, 6.54 (d, 4H; Cp), 4.48, 4.05 (d, 2H; CH_2), 2.94 (s, 6H; NMe_2).

3: ZrCl_4 (0.85 g, 3.6 mmol) was added in several portions with stirring to a solution of **2** (1.02 g, 7.2 mmol) in THF (ca. 20 mL) at –78 °C. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed in vacuo. The residue was extracted with dichloromethane, and the extract filtered. The solvent was slowly removed from the filtrate in vacuo to give yellow crystalline **3** (1.30 g, 91 %). Elemental analysis calcd for $\text{C}_{16}\text{H}_{17}\text{Cl}_2\text{NZr}$ (385.43): C 49.81, H 4.41, N 3.63; found: C 48.71, H 4.28, N 3.41; ^1H NMR (300 MHz, CDCl_3): δ = 6.74, 6.14, 6.08 (m, 8H; Cp), 5.62 (s, 1H; CH), 5.03, 4.93 (d, 2H; CH_2), 2.62 (s, 6H; NMe_2); ^{13}C NMR (75 MHz, CDCl_3): δ = 148.7, 140.2, 138.0, 126.9 (*ipso*-C), 125.0, 124.5, 114.9, 112.0 (CH of Cp), 111.6 (CH_2), 105.8 (CH), 42.7 (NMe_2).

4: The procedure is similar to that of **3**, except that Et_2O was used as the solvent instead of THF. The reaction of HfCl_4 (1.20 g, 3.74 mmol) and **2** (1.05 g, 7.44 mmol) resulted in yellow crystalline **4** (1.10 g, 77 %). Elemental analysis calcd for $\text{C}_{16}\text{H}_{17}\text{Cl}_2\text{NHf}$ (472.70): C 40.65, H 3.62, N 2.96; found: C 40.64, H 3.39, N 3.00; ^1H NMR (300 MHz, CDCl_3): δ = 6.65, 6.07, 5.99 (m, 8H; Cp), 5.62 (s, 1H; CH), 4.99, 4.95 (d, 2H; CH_2), 2.64 (s, 6H; NMe_2); ^{13}C NMR (75 MHz, CDCl_3): δ = 147.3, 138.8, 134.5, 124.6 (*ipso*-C), 122.1, 121.7, 111.9, 108.9 (CH of Cp), 110.7 (CH_2), 104.3 (CH), 41.4 (NMe_2).

Crystal data for **3**: $\text{C}_{16}\text{H}_{17}\text{Cl}_2\text{NZr}$, M_r = 385.43, monoclinic, space group $P2_1/n$, a = 10.8208(9), b = 10.5382(12), c = 13.9891(10) Å, β = 101.380(5)°, V = 1563.8(2) Å³, $F(000)$ = 776; Z = 4, ρ_{calcd} = 1.637 g cm^{–3}, $\mu(\text{MoK}\alpha)$ = 10.33 cm^{–1}, T = 294(2) K, crystal size 0.38 × 0.36 × 0.08 mm, 3504 unique reflections for $2.18 < \theta < 25.00^\circ$, 2722 reflections with $I > 2\sigma(I)$; R = 0.0269, R' = 0.0380, S = 0.917.

Crystal data for **4**: $\text{C}_{16}\text{H}_{17}\text{Cl}_2\text{NHf}$, M_r = 472.70, monoclinic, space group $P2_1/n$, a = 10.830(2), b = 10.516(2), c = 13.965(3) Å, β = 101.51(3)°, V = 1558.5(2) Å³, $F(000)$ = 904; Z = 4, ρ_{calcd} = 2.015 g cm^{–3}, $\mu(\text{MoK}\alpha)$ = 10.33 cm^{–1}, T = 294(2) K, crystal size 0.40 × 0.38 × 0.20 mm, 2827 unique

reflections for $2.18 < \theta < 25.55^\circ$, 2813 reflections with $I > 2\sigma(I)$; R = 0.0446, R' = 0.0482, S = 0.933.

Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-114199 (**3**) and CCDC 114200 (**4**). 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 Convenient and Versatile Route to Hydroquinolines by Inter- and Intramolecular Aza-Diels–Alder Pathways**

Henning Steinhagen and E. J. Corey*

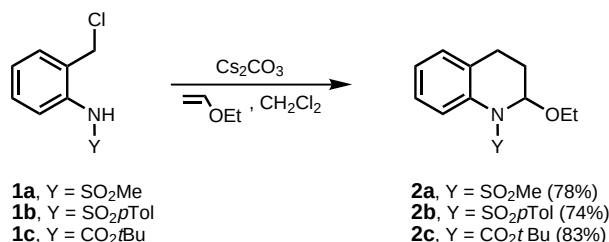
Described herein is a practical method for the generation of *o*-azaxylylenes and their application to the synthesis of a variety of hydroquinoline derivatives. The formation of *o*-azaxylylenes has previously been reported by photochemical fragmentation,^[1,2] fluoride induced elimination of *o*-trime-

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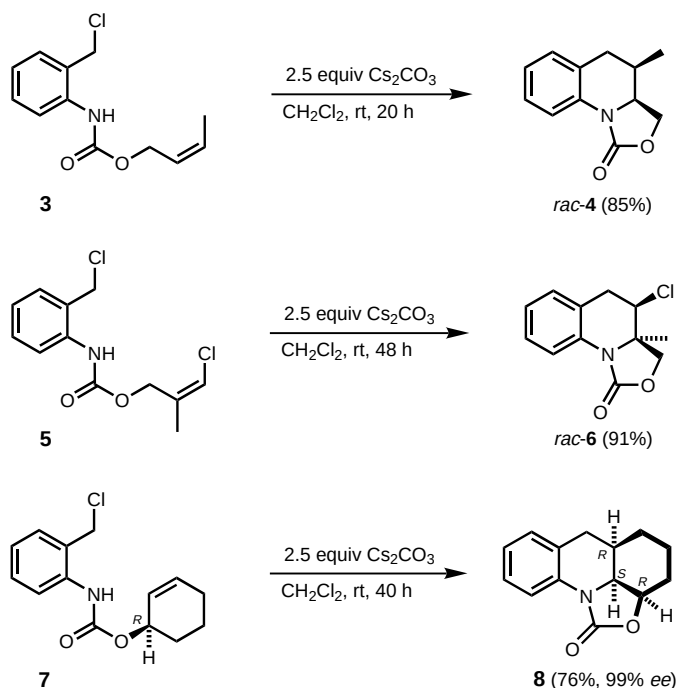
thylsilylaminobenzyltrimethylammonium salts,^[3] and by pyrolytic elimination of *o*-hydroxymethylanilines at 140–180 °C.^[4] Surprisingly, the simplest method possible for *o*-azaxylylene production, base-induced elimination of hydrogen chloride from amide or sulfonamide derivatives of *o*-chloromethylaniline, has never been reported. We have found that this process is highly effective for the generation of *o*-azaxylylenes and that these intermediates are readily trapped by olefins, especially π -electron-rich olefins, to form hydroquinoline derivatives as exemplified in Scheme 1.^[5] The



Scheme 1. Generation and trapping by ethyl vinyl ether of *o*-azaxylylenes.

synthesis of hydroquinolines **2a** and **2b** was conducted at –78 °C with addition of the chloromethylaniline derivative to the reaction mixture containing ethyl vinyl ether (2 equiv) and cesium carbonate (2.5 equiv) in dichloromethane over 4 h by syringe pump.^[6] The hydroquinoline **2c** was prepared in a similar manner but with addition by syringe pump at 23 °C over 24 h.

This new approach to hydroquinolines is especially powerful in the intramolecular version. As shown in Scheme 2, these intramolecular reactions proceed under mild conditions and provide hydroquinolines **4**, **6**, and **8** stereospecifically by a



Scheme 2. Intramolecular [2+4] cycloaddition reactions of *o*-azaxylylenes to form tri- and tetracyclic hydroquinolines.

suprafacial (*cis*) cycloaddition. The structures of the products were unambiguously determined in each case by X-ray crystallographic analysis.^[7] In each case NMR analysis of the crude reaction mixture indicated the absence of diastereomeric product.

The formation of the 3-chlorotetrahydroquinoline derivative **6** is especially interesting because this structure represents the core of the potent antiviral agent virantmycin,^[8] previously accessible only by lengthy multistep routes.^[9] The enantiospecific synthesis of the chiral tetracyclic tetrahydroquinoline **8** ($[\alpha]_D^{25} = +82$, $c = 0.95$ in CHCl₃, 99% *ee* by chiral HPLC analysis)^[10] from the readily available precursor **7**^[11] is also noteworthy and illustrative of the potential of this method for the production of enantiomerically pure ($\geq 99\%$ *ee*) products. X-ray crystallographic analysis of **8** provided proof of the stereochemistry and demonstrated a molecular conformation in which the chair-form cyclohexane ring is approximately orthogonal to the planar tricyclic core (Figure 1).

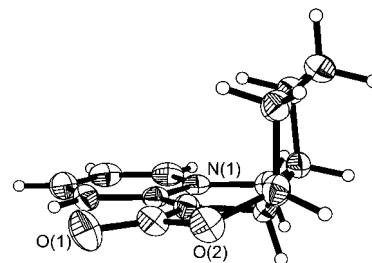
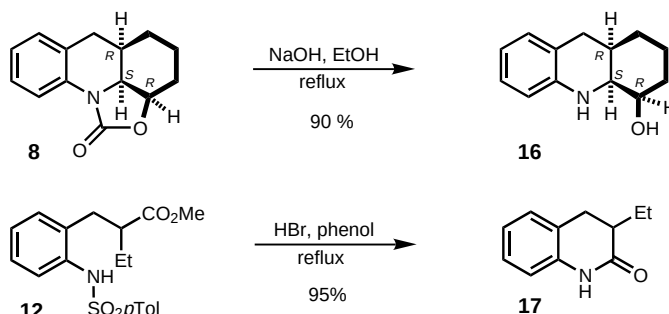


Figure 1. ORTEP plot of **8** as determined by X-ray crystallography.^[7c]

A variety of other trapping experiments with in situ generated *o*-azaxylylenes are summarized in Table 1. Vinyl ether substrates generally gave the products of [2+4] cycloaddition (**9**, **10**, **11**). On the other hand, ketene acetals afforded the conjugate adduct (**12** with **1b**) and the cycloadduct (**13** with **1c**). The π -electron-rich substituted acetylenes *N,N*-diethyl-1-propynylamine and 1-ethoxy-1-propyne furnished the dihydroquinoline derivatives **14** and **15**. In contrast, the π -electron-deficient dienophile ethylpropiolate did not afford Diels–Alder products with the *o*-azaxylylenes from **1b** or **1c**.



The various products described above can be transformed into a variety of compounds. For example, the tetracyclic carbamate **8** is easily cleaved upon treatment with ethanolic NaOH to the corresponding chiral tricyclic aminoalcohol **16**

Table 1. Trapping reactions of *o*-azaxylylenes with π -electron-rich dienophiles.

Substrate	Dienophile	Conditions ^[a]	Product	Yield [%] ^[b]
1c		C	9	95
1b		A	10	91
1c		B	11	90
1b		A	12	90
1c		B	13	40
1c		B	14	60
1c		B	15	95

[a] **A**: 2 h, -78°C , 10 equiv dienophile, addition of substrate over 15 min; **B**: 24–48 h, rt, 10 equiv dienophile, addition of substrate over 1 min; **C**: 24 h, rt, 2 equiv dienophile, addition of substrate by syringe pump over 24 h; [b] yields of isolated products after chromatography on silica gel with mixtures of hexane/EtOAc (+1% NEt_3 for **14** and **15**) for elution.

($[\alpha]_{\text{D}}^{25} = +53$, $c = 0.9$ in CHCl_3), and the sulfonamide **12** underwent cyclization with HBr/phenol to the bicyclic lactam **17** in 95% yield.

The reactions described herein define a practical and versatile method for the generation and use of *o*-azaxylylenes in aza-Diels–Alder reactions which proceed from readily available precursors and under mild conditions.

Experimental Section

All reactions were carried out under an inert atmosphere with anhydrous solvents.

7: A solution of (*R*)-2-cyclohexen-1-ol^[10] (147 mg, 1.5 mmol) in diethyl ether (1 mL) was added to a solution of phosgene (3 mmol) in toluene (1.6 mL) at -78°C . The mixture was stirred at -15°C for 3 h and at 0°C for

an additional 30 min. This solution of chloroformate was transferred by cannula to a solution of *o*-aminobenzyl alcohol (Alfa, 185 mg, 1.5 mmol) and pyridine (0.14 mL, 1.7 mmol) in dichloromethane (5 mL) over 15 min at 0°C . The reaction mixture was warmed to 23°C and stirred for an additional 2 h. Aqueous workup and chromatography on silica gel with hexane/ethyl acetate (6/1) afforded the hydroxy carbamate coupling product as a colorless crystalline solid (282 mg, 76%). M.p. $91-92^{\circ}\text{C}$; $[\alpha]_{\text{D}}^{25} = +150$ ($c = 0.77$ in CHCl_3); IR (film): $\tilde{\nu} = 3348, 3344, 2939, 1731, 1702, 1593, 1529, 1454, 1305, 1231, 1219, 1192, 1040, 1008, 938\text{ cm}^{-1}$; $^1\text{H NMR}$ (400 MHz, CDCl_3 , 25°C): $\delta = 7.94$ (d, $J = 8\text{ Hz}$, 1H), 7.82 (brs, 1H, NH), 7.32 (t, $J = 7.4\text{ Hz}$, 1H), 7.17 (d, $J = 7.4\text{ Hz}$, 1H), 7.03 (t, $J = 7.4\text{ Hz}$, 1H), 5.95–6.00 (m, 1H), 5.78–5.83 (m, 1H), 5.26–5.29 (m, 1H), 4.69 (d, $J = 5.2\text{ Hz}$, 2H), 2.16 (t, $J = 5.6\text{ Hz}$, 1H, OH), 1.60–2.14 (m, 6H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3 , 25°C): $\delta = 153.98, 137.82, 132.70, 129.25, 129.00, 128.96, 126.00, 123.38, 121.12, 69.04, 64.28, 28.57, 24.94, 18.94$; HR-MS (EI): calcd (found) for $\text{C}_{14}\text{H}_{17}\text{NO}_3$ [M^+]: 247.1208 (247.1218). A solution of thionyl chloride (102 μL , 1.40 mmol) in dichloromethane (4 mL) was added over 15 min to a solution containing the hydroxy carbamate (240 mg, 0.97 mmol) and triethylamine (195 μL , 1.40 mmol) in dichloromethane (4 mL). The solution was stirred for 2 h and the solvent was removed in vacuo. Chromatography of the crude product on silica gel with hexane/ethyl acetate (6/1) afforded **7** as a colorless crystalline solid (230 mg, 89%). M.p. $120-121^{\circ}\text{C}$; $[\alpha]_{\text{D}}^{25} = +141$ ($c = 0.95$ in CHCl_3); IR (film): $\tilde{\nu} = 3285, 3037, 3029, 2945, 2913, 1687, 1590, 1527, 1456, 1296, 1248, 1049\text{ cm}^{-1}$; $^1\text{H NMR}$ (400 MHz, CDCl_3 , 25°C): $\delta = 7.87$ (d, $J = 7.8\text{ Hz}$, 1H), 7.37 (t, $J = 1.4, 7.8\text{ Hz}$, 1H), 7.28 (d, $J = 1.4, 7.6\text{ Hz}$, 1H), 7.09 (t, $J = 7.5\text{ Hz}$, 1H), 6.87 (brs, 1H, NH), 5.98–6.02 (m, 1H), 5.79–5.83 (m, 1H), 5.29–5.31 (m, 1H), 4.62 (s, 2H), 1.65–2.14 (m, 6H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3 , 25°C): $\delta = 153.66, 136.89, 132.94, 130.14, 130.08, 127.19, 125.83, 124.30, 122.82, 69.40, 44.03, 28.56, 24.94, 18.92$; HR-MS (EI): calcd (found) for $\text{C}_{14}\text{H}_{16}\text{NO}_2\text{Cl}$ [M^+]: 265.0869 (265.0878).

8: A suspension of **7** (53 mg, 0.2 mmol) and cesium carbonate (163 mg, 0.5 mmol) in dichloromethane (5 mL) was stirred for 40 h at 23°C . The reaction mixture was filtered through a pad of celite and the solvent was evaporated in vacuo. Chromatography of the crude product on silica gel with hexane/ethyl acetate (6/1) gave **8** as a colorless crystalline solid (35 mg, 76%). M.p. $149-150^{\circ}\text{C}$; $[\alpha]_{\text{D}}^{25} = +82$ ($c = 0.95$ in CHCl_3); IR (film): $\tilde{\nu} = 2929, 1744, 1494, 1459, 1390, 1364, 1349, 1339, 1324, 1293, 1272, 1209, 1185, 1135, 1104, 1060, 994, 946\text{ cm}^{-1}$; $^1\text{H NMR}$ (400 MHz, CDCl_3 , 25°C): $\delta = 8.40$ (d, $J = 8.4\text{ Hz}$, 1H), 7.21 (t, $J = 7.8\text{ Hz}$, 1H), 7.10 (d, $J = 7.5\text{ Hz}$, 1H), 6.98 (t, $J = 7.4\text{ Hz}$, 1H), 4.64 (dt, $J = 6.6, 8.6\text{ Hz}$, 1H), 4.26 (dd, $J = 3.8, 6.6\text{ Hz}$, 1H), 3.12 (dd, $J = 5.9, 16.7\text{ Hz}$, 1H), 2.65 (d, $J = 16.4\text{ Hz}$, 1H), 2.08–2.22 (m, 2H), 1.71–1.77 (m, 1H), 1.44–1.61 (m, 2H), 1.31–1.36 (m, 1H), 1.15–1.22 (m, 1H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3 , 25°C): $\delta = 154.28, 134.50, 129.47, 127.06, 122.74, 122.09, 116.31, 73.06, 56.03, 32.55, 29.19, 27.14, 24.44, 19.66$; HR-MS (FAB): calcd (found) for $\text{C}_{14}\text{H}_{15}\text{NO}_2\text{Na}$ [$M + \text{Na}^+$]: 252.1000 (252.1008).

16: A solution of **8** (31.5 mg, 0.137 mmol) in 10% ethanolic NaOH (2 mL) was heated at reflux for 1 h. After evaporation of the solvent in vacuo the mixture was suspended in water (5 mL) and extracted with chloroform (10 mL $\times$ 3). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed in vacuo. Chromatography of the crude mixture on silica gel with hexane/ethyl acetate (6/1) afforded the chiral aminoalcohol **16** as a colorless crystalline solid (25 mg, 90%). M.p. $134-135^{\circ}\text{C}$; $[\alpha]_{\text{D}}^{25} = +53$ ($c = 0.9$ in CHCl_3); IR (film): $\tilde{\nu} = 3419, 3405, 3378, 3371, 3300, 3235, 3225, 3201, 3017, 2936, 2920, 2895, 2855, 1606, 1504, 1496, 1486, 1444, 1374, 1289, 1104, 1054, 1043, 1008\text{ cm}^{-1}$; $^1\text{H NMR}$ (500 MHz, CDCl_3 , 25°C): $\delta = 6.97$ (t, $J = 7.6\text{ Hz}$, 1H), 6.93 (d, $J = 7.4\text{ Hz}$, 1H), 6.57 (t, $J = 7.3\text{ Hz}$, 1H), 6.49 (d, $J = 8\text{ Hz}$, 1H), 4.21 (brs, 1H, NH), 3.81–3.85 (m, 1H), 3.64 (t, $J = 3\text{ Hz}$, 1H), 3.03 (dd, $J = 5.6, 16.2\text{ Hz}$, 1H),

2.50 (dd, $J = 1.6, 16.2$ Hz, 1H), 1.89–1.93 (m, 1H), 1.73–1.81 (m, 2H), 1.60–1.63 (m, 1H), 1.55 (brs, 1H, OH), 1.28–1.40 (m, 3H); ^{13}C NMR (100 MHz, CDCl_3 , 25 °C): $\delta = 143.47, 129.73, 126.68, 119.18, 116.49, 113.43, 72.67, 53.93, 33.16, 32.96, 27.80, 25.31, 23.35$; HR-MS (CI): calcd (found) for $\text{C}_{15}\text{H}_{17}\text{NO}$ [M^+]: 204.1388 (204.1397).

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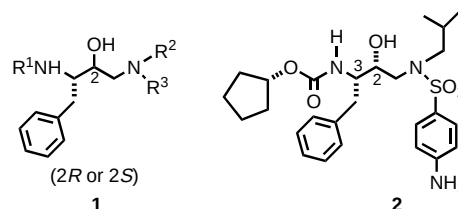
Keywords: antiviral agents • azaxylidenes • cycloadditions • heterocycles • stereocontrol

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- [6] The substrates **1a–1c** of Scheme 1 were prepared from *o*-amino-benzyl alcohol by the sequence: 1) *N*-acylation or *N*-sulfonylation, and 2) conversion of the resulting benzyl alcohol into the corresponding chloride by reaction with thionyl chloride in dichloromethane.
- [7] a) Crystal structure data for **4**: $\text{C}_{12}\text{H}_{13}\text{NO}_2$; monoclinic; $P2_1c$; $a = 14.972(7)$, $b = 5.599(3)$, $c = 13.403(5)$ Å; $\alpha = 90$, $\beta = 114.93(3)$, $\gamma = 90^\circ$; $Z = 4$; R_1 [$I > 2\sigma(I)$] = 0.0386; b) crystal structure data for **8**: $\text{C}_{14}\text{H}_{15}\text{NO}_2$; monoclinic; $P2_1c$; $a = 17.7653(11)$, $b = 9.1414(6)$, $c = 15.4356(10)$ Å; $\alpha = 90$, $\beta = 115.5670(10)$, $\gamma = 90^\circ$; $Z = 8$; R_1 [$I > 2\sigma(I)$] = 0.0481; c) crystal structure data for **6**: $\text{C}_{12}\text{H}_{12}\text{NO}_2\text{Cl}$; monoclinic; $P2_1n$; $a = 6.2128(5)$, $b = 12.1974(12)$, $c = 14.4788(13)$ Å; $\alpha = 90$, $\beta = 98.188(3)$, $\gamma = 90^\circ$; $Z = 4$; R_1 [$I > 2\sigma(I)$] = 0.0601. 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-113239/113240/113241. 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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- [10] Chiralpak AD column (Daicel Chemical Industries); flow rate: 0.7 mL min $^{-1}$; eluent: 6% *i*PrOH in hexane; UV detection at 254 nm; t_R [(+)-**8**] = 26.4 min, t_R [(-)-**8**] = 30.0 min.
- [11] The substrates **3**, **5**, and **7** were prepared starting from the corresponding allylic alcohols by the sequence: 1) conversion into chloroformates by reaction with phosgene, 2) treatment of the chloroformates with *o*-aminobenzyl alcohol, and 3) conversion of the resulting hydroxy carbamates into the corresponding chlorides with thionyl chloride (see experimental section for details). 3-Chloro-2-methyl-2-propen-1-ol was prepared according to A. Mooradian, J. B. Cloke, *J. Am. Chem. Soc.* **1946**, *68*, 785–789; L. F. Hatch, J. J. Russ, L. B. Gordon, *J. Am. Chem. Soc.* **1947**, *69*, 2614–2616. Enantiomerically pure (*R*)-2-cyclohexen-1-ol was prepared by kinetic resolution with lipase as described by T. Fukazawa, T. Hashimoto, *Tetrahedron: Asymmetry* **1993**, *4*, 2323–2326.

re- and *si*-Face-Selective Nitroaldol Reactions Catalyzed by a Rigid Chiral Quaternary Ammonium Salt: A Highly Stereoselective Synthesis of the HIV Protease Inhibitor Amprenavir (Vertex 478)**

E. J. Corey* and Fu-Yao Zhang

The development of therapeutically useful HIV protease inhibitors has been one of the major contributions of synthetic and medicinal chemistry to human well-being during this decade.^[1,2] Since several of these agents possess chiral substituted 1,3-diamino-2-hydroxypropyl segments as a central structural subunit, for example, the phenylalanine-related subunit **1**, considerable research effort has been directed at methods for their synthesis.^[3,4] Described herein is a new strategy for stereocontrol in the synthesis of subunits of type **1** and a specific application with a practical synthesis of amprenavir (**2**),^[5,6] an important second generation HIV

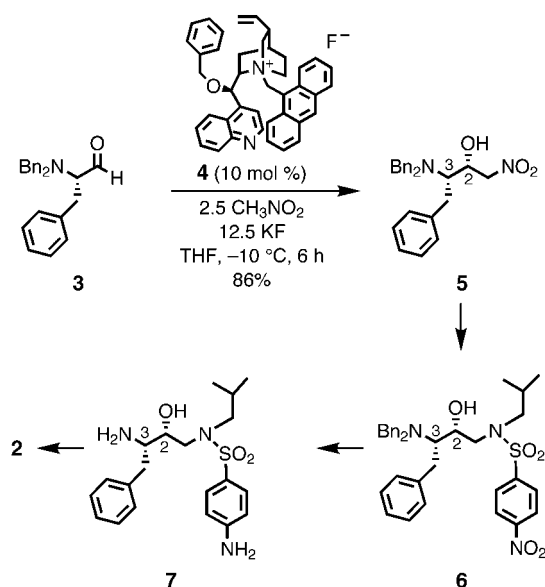


protease inhibitor with a number of clinical advantages over first generation agents. The method currently being used to produce **2** involves the activation of the carboxyl group of *N*-*tert*-butoxycarbonylphenylalanine and transformation to the corresponding diazomethyl ketone with diazomethane as a key element.^[7] Because of the intrinsic hazards of this step a safer alternative seems preferable.

The sequence utilized for the synthesis of amprenavir is summarized in Scheme 1. *N,N*-Dibenzyl-(*S*)-phenylalaninal (**3**)^[8] in tetrahydrofuran (THF) was added to a mixture of quaternary ammonium salt **4**,^[9,10] nitromethane, and finely divided potassium fluoride in THF with stirring. After 6 h the nitro alcohol **5** was isolated in 86 % yield by flash chromatography on silica gel.^[10] The more polar C(2) diastereomer of **5** was isolated in 5 % yield, which indicated a 17:1 diastereoselectivity for the nitroaldol reaction.^[11] In contrast, the nitroaldol reaction of **3** in the presence of tetra-*n*-butylammonium fluoride^[8c] afforded a mixture of **5** and the C(2) diastereomer with only 4:1 diastereoselectivity under the same reaction conditions. Treatment of nitro alcohol **5** with 2.5 equivalents of NiCl_2 and 25 equivalents of NaBH_4 in methanol at 0 °C for 10 min with vigorous agitation provided

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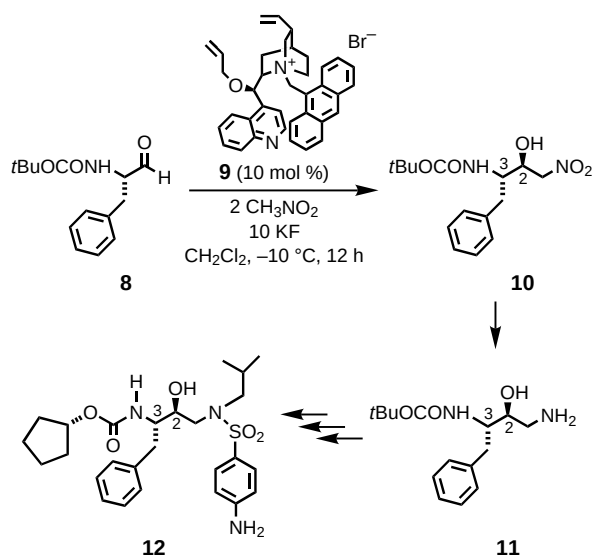
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Scheme 1. Application of the stereoselective nitroaldol reaction promoted by a chiral quaternary ammonium salt to the synthesis of amprenavir (**2**).

the corresponding amino alcohol (85%), which was reductively alkylated by reaction with isobutyraldehyde/MgSO₄ to form the Schiff base, which was subsequently exposed to sodium borohydride in ethanol at 0–23 °C for 4 h. Reaction of the resulting secondary amine (82% yield)^[12] with *p*-nitrobenzenesulfonyl chloride/Et₃N in CH₂Cl₂ at 23 °C for 2 h produced sulfonamide **6** (94%). Amprenavir (**2**) was synthesized from **6** in 95% overall yield by: 1) catalytic hydrogenation (1 atm H₂, Pd(OH)₂/C, MeOH, 23 °C for 4 h) to form diamine **7** and 2) reaction of **7** with (*S*)-3-tetrahydrofuranyl-*N*-oxysuccinimidyl carbonate and Et₃N in CH₂Cl₂ at 23 °C for 1 h. The overall yield of **2** from amino aldehyde **3** was 50%. Comparison of the spectroscopic data of the product with those for authentic amprenavir confirmed the identity of our synthetic **2**.

The nitroaldol reaction promoted by chiral quaternary ammonium fluoride was also applied to the stereoselective synthesis of the 2*S*,3*S*-diastereomeric series starting from the *N*-*tert*-butoxycarbonyl derivative of *S*-phenylalaninal (**8**, Scheme 2).^[9b] A mixture of **8**, 10 mol% of quaternary ammonium salt **9**,^[13] and 10 equivalents of finely powdered KF in CH₂Cl₂ was treated with 2 equivalents of nitromethane at –10 °C with stirring. After 12 h the organic product, which consisted of a 9:1 mixture of the 2*S*,3*S* nitro alcohol **10** and the 2*R*,3*S* diastereomer 2-*epi*-**10** (88% total yield), was purified by flash chromatography on silica gel to give pure samples of **10** and 2-*epi*-**10**. Reduction of **10** (1 atm H₂, Pd/C, EtOH, 23 °C, 5 h) produced amino alcohol **11** (87%) and similarly reduction of 2-*epi*-**10** afforded 2-*epi*-**11**. The latter was identified by comparison with an authentic sample.^[7b, 14] Amino alcohol **11** was transformed to the C(2) diastereomer of amprenavir (**12**) by using the methods described above for the synthesis of amprenavir. In contrast to the stereoselectivity observed for the conversion **8**→**10** under the influence of the chiral salt **9**, the reaction of **8** and nitromethane in the presence of *n*-Bu₄NF or achiral amines afforded approximately 1:1 mixtures of **10** and the C(2) diastereomer.



Scheme 2. Application of the stereoselective nitroaldol reaction promoted by chiral quaternary ammonium salts to the synthesis of the C(2) diastereomer **12** of amprenavir.

The synthetic utility of the chiral quaternary ammonium salts for enantioselective alkylation and Michael addition reactions, as described previously, depends on the three-dimensional rigidity of the cation in solution and the preference for one particular conformation.^[9] This conformation suggests a working mechanistic model that provides a plausible explanation for the novel face-selective nitroaldol reactions reported herein. In the case of the *re*-face-selective nitroaldol reaction **3**→**5**, there is one particular assembly of the aldehyde **3** with cation **4** that places the formyl oxygen atom in proximity to the sole accessible face^[9] of the ammonium nitrogen atom (leading to a contact ion pair, –O^{δ-}⋯N⁺, in the transition state for addition of CH₂NO₂⁻ to the formyl group), while affording maximum van der Waals contact between **3** and **4**. A graphical rendering of the three-dimensional structure of this assembly of **3** and **4** is shown in Figure 1. The carbon atoms of the aldehyde **3** are darkened relative to the carbon atoms of the cation **4**. In the front view the *re* face of the formyl group is exposed, the *si* face is behind, and the formyl oxygen atom sits right above the ammonium N⁺ ion (not visible). One of the benzyl groups of **3** makes a face–face contact with the anthracene subunit of **4** (see back view) and the other contacts the quinoline ring of **4**. As a result of the snug fit between **3** and **4** in the assembly shown in Figure 1 only the *re* face of **3** is exposed to attack by CH₂NO₂⁻ and the proximity of the N⁺ ion to the formyl oxygen atom of **3** promotes the carbonyl addition through a charge acceleration.

Figure 2 depicts an assembly of the aldehyde **8** and ammonium cation **9** that allows van der Waals contact between the *tert*-butyl and phenyl groups of **8** and the 9-anthracenylmethyl subunit of **9**, while placing the formyl oxygen atom of **8** in proximity to the open face of the N⁺ center of **9**. The attack of CH₂NO₂⁻ on the formyl carbon atom in this assembly must occur at the *si* face of the CHO group, a reaction pathway that leads to the predominating nitroaldol product **10**.

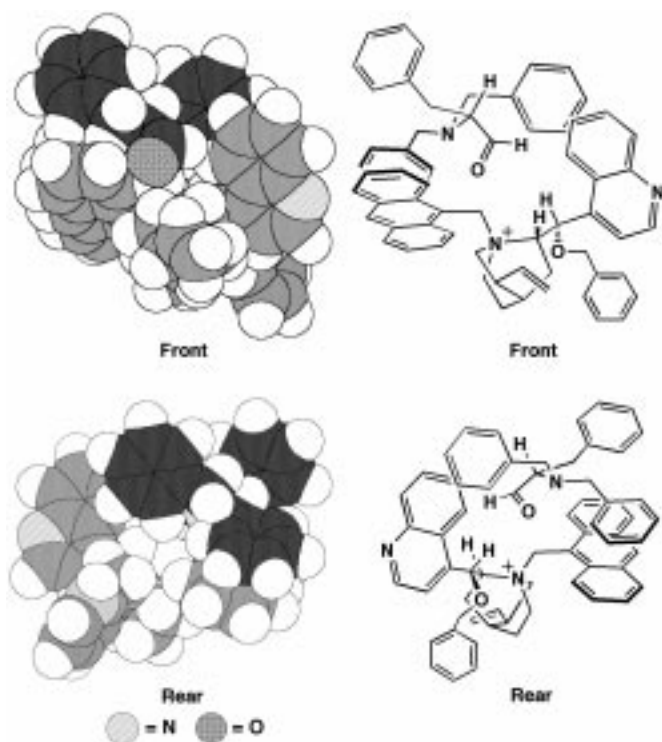


Figure 1. Two views of the assembly of **3** and **4** that could lead to nitroaldol product **5**. The front and rear views are related by a rotation of 180° about the vertical axis.

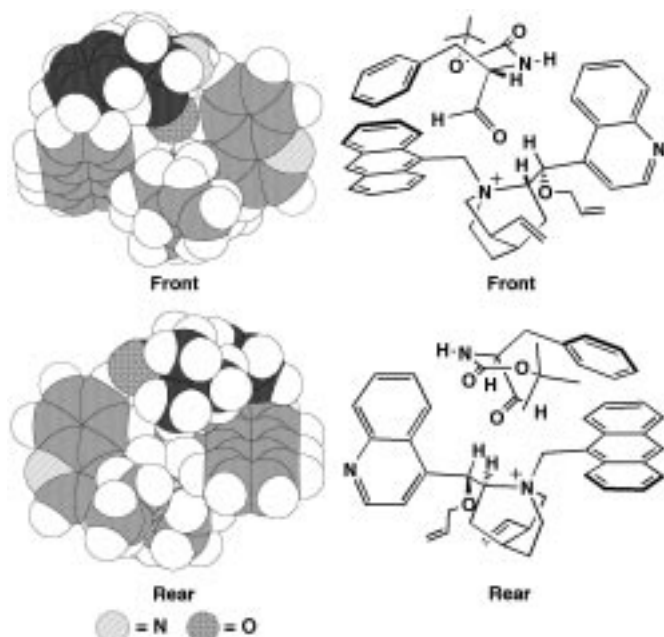


Figure 2. Two views of the assembly of **8** and **9** that could lead to nitroaldol product **10**. The two views are related by 180° rotation about the vertical axis.

Alternative pathways involving the reaction of a contact ion pair^[9] of CH_2NO_2^- and the chiral quaternary ammonium ion with external aldehyde cannot be excluded. However, such a mechanism does not lead to a clear explanation of the high stereoselectivities observed for the nitroaldol reactions **3** → **5** and **8** → **10**.

The use of the rigid chiral quaternary ammonium cations **4** and **9** to control the face selectivity in nucleophilic addition to aldehydes **3** and **8**, *re* or *si* depending on the N-protecting group of the *S*-phenylalaninal moiety, suggests a new strategy for stereocontrol of such reactions. Although further research is required to establish the scope and to validate the stereochemical/mechanistic model, the results reported herein and previously^[9] (and the experimental simplicity) provide ample incentive for additional study. The experimental procedure for these stereoselective nitroaldol reactions is exceedingly simple (see Experimental Section).

Experimental Section

5: A well-stirred mixture of **4** (12 mg, 0.02 mmol), potassium fluoride (145 mg, 2.5 mmol), tetrahydrofuran (0.3 mL), and nitromethane (27 μL , 0.5 mmol) was cooled to -10°C and treated with a solution of (*S*)-*N,N*-dibenzylphenylalaninal (66 mg, 0.2 mmol) in THF (0.4 mL). After stirring the mixture at -10°C for 6 h the mixture was filtered to remove KF and concentrated. The solid quaternary ammonium salt **4** was precipitated with diethyl ether/hexane (7/3) and the product-containing soluble fraction was evaporated in vacuo to give an oil, which was purified by flash chromatography (silica gel, hexanes/ethyl acetate (10/1)) to give the 2*R*,3*S* nitro alcohol **5** (66 mg) and the 2*S* diastereomer (4 mg). **5**: $[\alpha]_D^{25} = +12.5$ ($c = 1.1$ in CHCl_3); IR (film): $\tilde{\nu} = 3565.7, 3490.4, 3085.7, 2929.1, 2843.0, 2808.6, 1602.5, 1552.3, 1494.7, 1454.1, 1381.8, 1264.5, 1181.9 \text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3 , 25°C): $\delta = 7.29\text{--}7.10$ (m, 15H), 4.72 (dd, $J = 13.4, 2.0 \text{ Hz}$, 1H), 4.42 (m, 1H), 3.93 (dd, $J = 9.7, 13.4 \text{ Hz}$, 1H), 3.72 (d, $J = 13.5 \text{ Hz}$, 2H), 3.44 (d, $J = 13.5 \text{ Hz}$, 2H), 3.09 (m, 1H), 2.96 (dd, $J = 6.2, 10.5 \text{ Hz}$, 2H), 2.29 (d, $J = 5.5 \text{ Hz}$, 1H); ^{13}C NMR (100 MHz, CDCl_3 , 25°C): $\delta = 140.2, 138.8, 129.3, 128.8, 128.5, 128.4, 127.2, 126.2, 79.9, 70.6, 61.6, 54.6, 32.3$. HRMS (CI): calcd for $\text{C}_{24}\text{H}_{28}\text{N}_2\text{O}_3$ [$M + \text{H}^+$]: 391.2022, found: 391.2030.

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- [14] We are grateful to Dr. Peter Cheng of the Bristol-Myers Squibb Research Institute (Princeton, NJ) for an authentic sample of 2-*epi*-**11**^[7b] and spectral data.

A Three-Dimensional Coordination Polymer with an Expanded NbO Structure**

Tianyan Niu, Xiqu Wang, and Allan J. Jacobson*

Coordination chemistry allows a systematic approach to the synthesis of extended lattices.^[1] Many structures have been reported with unprecedented lattice types, while others are based on frameworks that are found in simple inorganic structures, for example, diamond,^[2] PtS,^[3] and quartz.^[4]

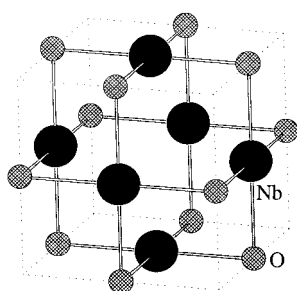


Figure 1. The framework of NbO.

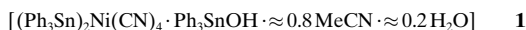
Several coordination polymers have been described^[5] containing square-planar centers based on the NbO net (6⁴8²), the tetragonal CdSO₄ structure (6⁵8, B), and the “dense” net (7⁵9, C). An example of an NbO net^[6] (Figure 1) is the compound formed by cyanuric acid and biuret containing two interpenetrated hydrogen-bonded

networks.^[7,8] Examples of the other two structure types are the interpenetrated networks formed by copper(II) nitrate with 1,2-bis(4-pyridyl)ethane (B)^[9] and 1,2-bis(4-pyridyl)ethyne (C).^[5a] Schindler and Baur^[10] have shown that several non-interpenetrated inorganic frameworks can be related to the NbO-type structure.

For example sodalite, [(Me₄N)_{1.3}(H₂O)_{0.7}{Mo₄O₈(PO₄)₂}] · 2H₂O,^[11] and [Cs₃{V₅O₉(PO₄)₂}] · xH₂O^[12] frameworks can be constructed by connecting four rings of silicate tetrahedra Si₄O₄O_{8/2}, Mo₄O₈(PO₄)_{4/2} cubes, and V₅O₉(PO₄)_{4/2} “helmets”, respectively, with 90° rotations between adjacent groups imposed by the shared tetrahedral bridging units.

We and others have investigated the syntheses and properties of coordination polymers formed by linking cyanometalate anions with trialkyl- or triaryltin cations.^[13–18] The compounds in this class have the general formula [(R₃Sn^{IV})_nM(CN)_m] and contain polymeric –M–C≡N–Sn–N–C≡M– chains which are connected to give frameworks of various topologies. A summary of the transition metals and organic R groups that have been used in the previous studies is given elsewhere.^[16] This class of compounds has the potential for use in molecular separations^[19] because large cavities are formed in some examples. Intercalation of large molecules, for example, ferrocene in [(Me₃Sn)₃Fe(CN)₆]^[20] has been reported.

In compounds formed by square-planar [Ni(CN)₄]^{2−} anions and R₃Sn⁺ cations in a 1:2 ratio, two possible structure types can be anticipated. The first is a two-dimensional layer structure in which all Ni(CN)₄ planes are parallel. The second is a hypothetical framework that can be thought of as being related to the NbO structures discussed above, but expanded by the bridging R₃Sn⁺ cations. This would entail alternating parallel and perpendicular Ni(CN)₄ planes. Here we report the synthesis of **1**, to our knowledge the first three-dimensional



sional cyanometalate coordination polymer with this expanded NbO-type structure. The framework of **1** is not interpenetrated, and the large central cavity in the structure is filled by inclusion of Ph₃SnOH and solvent molecules during synthesis.

Compound **1** was prepared in single-crystal form by slow interdiffusion of solutions of Ph₃SnCl in acetonitrile and K₂[Ni(CN)₄] in water. The structure of **1** was determined by single-crystal X-ray diffraction,^[21] and the composition was determined by elemental analysis. The local coordination environments of Ni and Sn atoms are shown in Figure 2. The

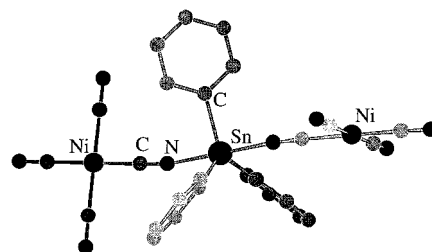


Figure 2. The local coordination environments of the nickel and tin atoms in **1**.

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$\text{Ni}(\text{CN})_4$ unit remains square planar with the expected bond lengths for $\text{Ni}-\text{C}$ and $\text{C}\equiv\text{N}$. Coordination of nitrogen atoms from cyanide groups in adjacent $\text{Ni}(\text{CN})_4$ units gives a trigonal-bipyramidal coordination environment for the Sn atoms. The $\text{N}-\text{Sn}$ distance (2.25 Å) is somewhat shorter than distances observed in similar compounds, for example, 2.37 Å (av) in $[(n\text{Bu}_3\text{Sn})_3\text{M}(\text{CN})_6]$ ($\text{M} = \text{Fe}, \text{Co}$),^[16] 2.34 Å in $[(\text{Me}_3\text{Sn})_4\text{Mo}(\text{CN})_8]$,^[17] and 2.33 Å in $[(\text{Ph}_3\text{Sn})_3\text{Fe}(\text{CN})_6 \cdot \text{H}_2\text{O} \cdot 2\text{MeCN}]$.^[18]

The building unit shown in Figure 2 is connected to similar units to form a low-density open framework structure with cubic symmetry $Fd\bar{3}c$ (Figure 3). Each $\text{Ni}(\text{CN})_4$ unit is

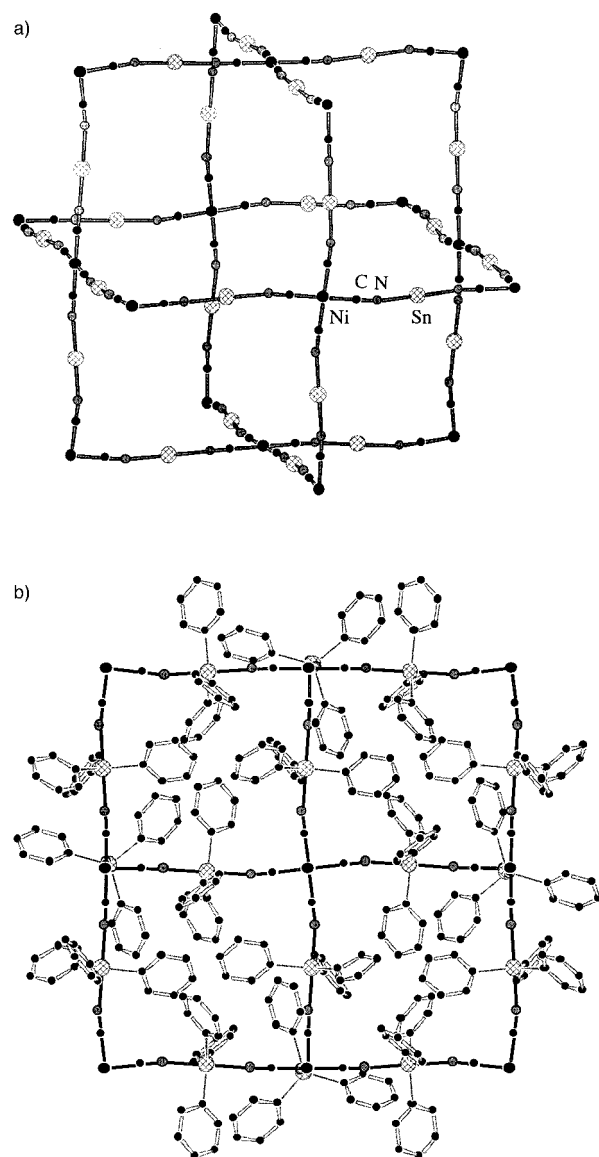


Figure 3. Views of the $(\text{Ph}_3\text{Sn})_2\text{Ni}(\text{CN})_4$ framework in **1**. a) Phenyl groups and solvent molecules are omitted. b) Solvent molecules are omitted, and only one orientation of each disordered phenyl ring is shown.

connected through Ph_3Sn bridges to similar units that are rotated by 90° to form an NbO -like arrangement that is expanded by the bridging units ($\text{C}-\text{N}-\text{Sn}$ 169.6°). The phenyl groups in the framework are disordered about a twofold axis. The unit shown in Figure 3 has dimensions of $a/2$ Å on each

side. The doubling is a consequence of the relative rotations of adjacent $\text{Ni}(\text{CN})_4$ units that are in the same orientation.

A very large cavity is present in the cubic array (see Figure 3). The free diameter of the cavity, allowing for the van der Waals dimensions of the phenyl groups, is about 12 Å, and the volume is thus 1728 Å^3 . The central cavity in **1**, as synthesized, contains Ph_3SnOH , acetonitrile, and water molecules in a disordered arrangement (Figure 4). The Ph_3SnOH

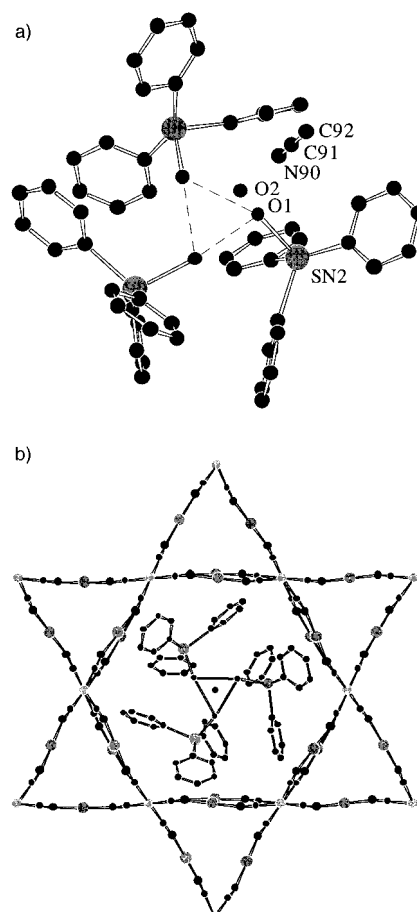


Figure 4. a) Model for the arrangement of the $(\text{Ph}_3\text{SnOH})_3$ trimer and the solvent molecules in the central cavity in **1**. b) The arrangement of the guest molecules in the framework, viewed down the (111) direction; phenyl groups on the framework are omitted for clarity.

molecule is disordered about a threefold axis. Three Ph_3SnOH molecules, together with water and acetonitrile, are disordered about a tetrahedral site. The occupancy of Ph_3SnOH was determined from elemental analysis to be one molecule per formula unit. The occupancies of the acetonitrile and water molecules were refined with C, N, and O isotropic thermal parameters constrained to be equal. The refined occupancies correspond to about 0.8MeCN and $0.2\text{H}_2\text{O}$ per formula unit. The presence of water molecules in the structure was confirmed by infrared spectroscopy.

The infrared spectrum of **1** shows a $\text{C}\equiv\text{N}$ stretching band at 2150 cm^{-1} which is blue shifted from the corresponding band for $\text{K}_2[\text{Ni}(\text{CN})_4]$, as observed in other organotin–cyanometalate compounds.^[16,22,23] Thermogravimetric analysis of **1** shows a weight loss beginning immediately on heating due to

rapid loss of water and acetonitrile molecules (see Experimental Section). The weight remains constant from 100 °C to about 200 °C, and powder X-ray diffraction data indicate that the structure is maintained. Reabsorption of water/acetonitrile occurred at room temperature for samples that had been heated to 160 °C. Above 200 °C complete decomposition of the framework and loss of the included nonframework molecules occurred in a single step. The residue was shown by powder X-ray diffraction to be a mixture of NiO and SnO₂.

Microcrystalline **1** was also obtained by direct reaction of Ph₃SnCl and K₂[Ni(CN)₄] in water/acetonitrile. The purity of the product was confirmed by comparison of its X-ray powder diffraction pattern with a pattern simulated using the parameters from the single-crystal X-ray structure analysis. Changing the ratio of Ph₃SnCl to K₂[Ni(CN)₄] from 3:1 to 2:1 in the reaction had no effect on composition and purity, indicating that **1** is the stable phase. The microcrystalline form of **1** and the larger crystals formed by diffusion are both stable under ambient conditions.

Compound **1** is the only example reported to date of a three-dimensional framework structure formed by [Ni(CN)₄]²⁻ anions and R₃Sn⁺ cations. Two-dimensional layer structures are formed with R groups other than phenyl. For example, our preliminary results show that the compounds [(R₃Sn)₂Ni(CN)₄] (R = ethyl, *n*-butyl) have layered structures. A different layered structure is adopted by [Ni(CN)₂ · 2Me₃SnCN · (*n*Bu₄N)OH].^[24] The compounds [(R₃Sn)₂M(CN)₄] (M = Pd, Pt; R = Me, Bu, Ph) have been reported also to have layered structures,^[25] but were not completely characterized.

The inclusion of Ph₃SnOH molecules appears to be necessary for the formation of **1**. The elemental composition and X-ray refinement indicate that each cavity contains three Ph₃SnOH molecules in a disordered arrangement. The inclusion of a fourth Ph₃SnOH molecule in the remaining tetrahedral position would lead to a very short O–O distance. Consequently, the remaining space in the cavity is occupied by acetonitrile and water molecules. The arrangement of the three Ph₃SnOH molecules shown in Figure 4 can be stabilized by the formation of hydrogen bonds with O–O atom separations of 2.86 Å. It is significant that **1** is recovered upon use of different reactant ratios, confirming the stability of the (Ph₃SnOH)₃ hydrogen-bonded trimer. In contrast, crystalline Ph₃SnOH forms infinite (Sn–O–Sn)_n chains with five-coordinate Sn atoms.^[26] The inclusion of the hydrogen-bonded (Ph₃SnOH)₃ trimer probably accounts for the formation of a three- rather than a two-dimensional framework. The presence of the guest molecules also prevents the formation of an interpenetrated lattice. Further studies are in progress to investigate the effects of other combinations of guest molecules and R₃Sn ligands on the preferential formation of three- rather than two-dimensional frameworks.

Experimental Section

1: K₂[Ni(CN)₄] (0.052 g, 0.216 mmol) was dissolved in distilled water (10 mL) and placed in a 30-mL screw-capped tube. A mixture of water/acetonitrile (1/1, 5 mL) was applied as a buffer layer, and a solution of Ph₃SnCl (0.25 g, 0.648 mmol) in acetonitrile (10 mL) was layered on top. Cubic colorless crystals of **1** were observed at the interface after 10 d of

interdiffusion. Elemental analysis calcd: C 56.54, H 3.88, N 5.31, Sn 28.13, Ni 4.64; found: C 56.23, H 3.68, N 4.80, Sn 28.39, Ni 4.61; IR (KBr; Galaxy FTIR 5000): $\tilde{\nu}$ = 3443 (m), 3049 (m), 2150 (s), 1481 (m), 1431 (m), 1076 (m), 997 (m), 729 (m), 694 cm⁻¹ (s); TGA (TA Instruments 2100, N₂ atmosphere, 5 °C min⁻¹): 5 % solvent loss, 58.3 % loss upon total decomposition, 36.7 % residue (calcd 37.7 %).

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- [21] a) X-ray crystal structure analysis of **1**: colorless cubic crystal of size 0.3 × 0.3 × 0.35 mm, cubic, space group *Fd3̄c*, *a* = 41.972(2) Å, *V* =

73938(4) Å³, $Z = 48$, $\rho = 1.365 \text{ g cm}^{-3}$, $2\theta_{\text{max}} = 41.22^\circ$, $\mu = 1.542 \text{ mm}^{-1}$, $T = 223(2) \text{ K}$; Siemens Smart CCD diffractometer; $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ Å}$); of 25 657 reflections measured, 692 were independent, $R(\text{int.}) = 0.0323$. The structure was solved by direct methods and refined by a full-matrix least-squares procedure against F^2 (hydrogen atoms were not refined) with SHELXTL (Version 5.03, G. M. Sheldrick); $R = 0.0558$, $wR^2 = 0.1547$ for 679 reflections with $I > 4\sigma(I)$, 68 parameters, $\text{GOF} = 1.164$; max./min. residual electron density $0.687/-0.501 \text{ e Å}^{-3}$. Semiempirical absorption correction applied. All other F -centered space groups (centro and non-centrosymmetric) were investigated, but none remove any of the disorder. b) 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-112388. 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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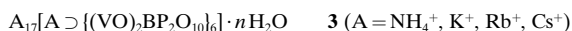
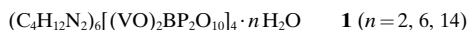
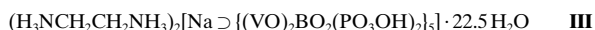
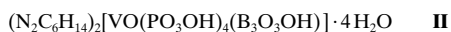
Templated Synthesis of Vanadium Borophosphate Cluster Anions**

Ranko P. Bontchev, Junghwan Do, and Allan J. Jacobson*

Early transition metals are well known to form complex polyoxoanions with ring and cage structures,^[1] including some exceptionally large ring-shaped molybdenum oxide clusters that have recently been reported.^[2] Other cluster compounds contain arsenate, organophosphonate, and borate anions in addition to metal oxo species.^[3] Several of these cluster anions encapsulate anions,^[4] molecules,^[5] or cations,^[6] and in some cases the size and shape of the cage has been shown to depend on the size of a specific ion present in the synthesis. For example, the Preyssler anion, $[\text{NaP}_5\text{W}_3\text{O}_{110}]^{14-}$, is not formed in the absence of Na^+ .^[7] Similarly, ClO_4^- and N_3^- anions

apparently act as templates in the formation of $[\text{HV}_{22}\text{O}_{54}(\text{ClO}_4)]^{6-}$ and $[\text{H}_2\text{V}_{18}\text{O}_{44}(\text{N}_3)]^{5-}$.^[8]

In the last few years a number of metal borophosphates have been synthesized and structurally characterized.^[9] Three new vanadium borophosphates have been described recently: the three-dimensional framework **I**^[10] and two compounds containing cluster anions, **II**^[11] and **III**.^[12] Here we report the



synthesis and structures of the new vanadium borophosphates **1**, **2**, and **3** containing cluster anions with different ring sizes, and show that the ring size of the anion is determined by the size of the cation used in the synthesis. Compound **III**, which was recently obtained in a low-yield synthesis,^[12] contains a cluster anion similar to that found in **2**.

Hydrothermal reactions of V_2O_5 , H_3BO_3 , and the appropriate phosphate salt at 180°C for three days gave crystals of **1**, **2**, and **3**. The compounds were characterized by single-crystal X-ray diffraction. The structure of **1** ($n = 6$) contains the cluster anion $[(\text{VO})_2\text{BP}_2\text{O}_{10}]_4^{12-}$ (Figure 1, top).^[13] Each tetra-valent vanadium atom in the cluster anion is coordinated by five oxygen atoms in a square-pyramidal arrangement with one short $\text{V}=\text{O}$ distance characteristic of a vanadyl group. Two VO_5 pyramids share a common edge to form V_2O_8 dimers of two distinct types in equal number. In one dimer (V_3 , V_4 ; Figure 2) the two $\text{V}=\text{O}$ groups are in a *cis* orientation and point outwards from the center of the cluster. In the other dimer (V_1 , V_2) the $\text{V}=\text{O}$ groups are in a *trans* orientation. Two oxygen atoms related by a center

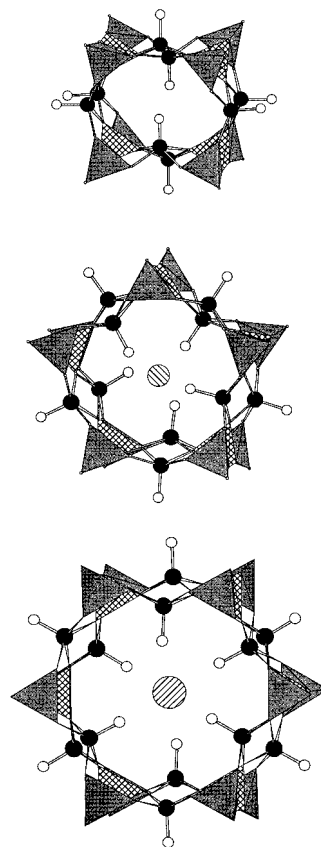


Figure 1. The cyclic cluster anions present in **1** (top), **2** (middle), and **3** (bottom). The PO_4 and BO_4 units are shown as filled and crosshatched tetrahedra, vanadium and oxygen atoms as filled and open circles, and sodium and nitrogen atoms as hatched circles.

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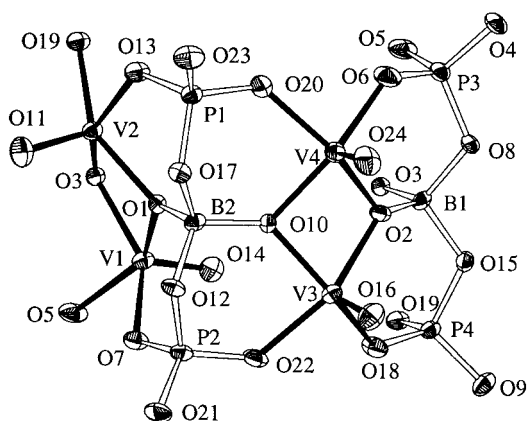


Figure 2. The coordination environments of the vanadium, phosphorus, and boron atoms in **1**. The thermal ellipsoids are shown at the 50% probability level.

of symmetry, one from each of the two *trans* dimers, point into the center of the anion. The *trans* orientation minimizes nonbonded oxygen–oxygen repulsions and is usually observed for V_2O_8 dimers. For example, in the recently reported compound **1** the $V=O$ groups also adopt a *trans* orientation in closely similar structural units.^[10] In the present case, insufficient space is available inside the cluster anion to accommodate more than two oxygen atoms, and consequently the $V=O$ groups in two of the dimers are forced into a *cis* orientation. The basal planes of the VO_5 square pyramids in the *cis*-oriented V_2O_8 dimers are tilted at an angle of 169.5° to give a reasonable nonbonded O–O contact of 3.175 Å. Bond valence calculations give values of 4.04, 4.08, 4.10, and 4.15 for the four nonequivalent vanadium atoms.

Two PO_4 tetrahedra and a BO_4 tetrahedron are connected through common corners to form BP_2O_{10} trimers (Figure 2). In each trimer, two phosphorus-bound oxygen atoms are unshared. The BP_2O_{10} trimers and V_2O_8 dimers are connected by sharing oxygen atoms to give a building unit of composition $(VO)_2BP_2O_{10}$. Two phosphorus oxygen atoms are also bound to vanadium atoms in P–O–V connections, and one boron-bound oxygen atom adopts a μ^3 -O arrangement with bonds to two vanadium atoms. In **1**, four of these $(VO)_2BP_2O_{10}$ units are connected in a cyclic fashion to form a four-ring of composition $[(VO)_2BP_2O_{10}]_4^{12-}$. In the three-dimensional structure, the four-rings of the cluster anion are oriented perpendicular to each other. The cluster ion charge is balanced by diprotonated piperazine molecules and water molecules occupy the remaining lattice volume.

If piperazine is replaced by NH_4^+ in a similar hydrothermal synthesis reaction, a cluster anion is obtained containing the same $(VO)_2BP_2O_{10}$ building unit found in **1**, but with a larger ring size (Figure 1, bottom). The structure of **3** ($A = NH_4^+$) also contains a cyclic anion which is formed by condensation of six $(VO)_2BP_2O_{10}$ building units and is centered by an ammonium ion. Similar compounds **3** ($n \approx 12$) containing six-rings centered by the corresponding cation A are obtained from reactions with $A = K^+$, Rb^+ , and Cs^+ .

In **3** (Figure 3), the vanadium atoms (V1, V2) and vanadyl oxygen atoms (O3, O2) are disordered equally over two positions, giving two possible orientations for the VO_5 square

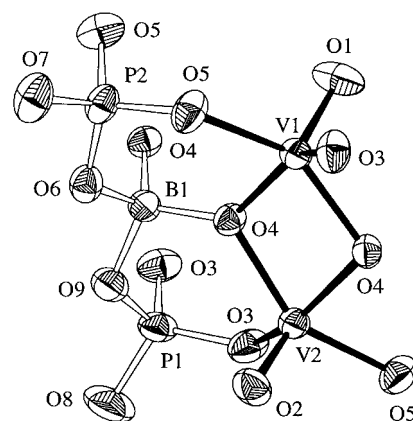


Figure 3. The coordination environments of the vanadium, phosphorus, and boron atom in **3**. The thermal ellipsoids are shown at the 50% probability level.

pyramids in each dimer. As noted above, the *trans* orientation is considered more likely (Figure 1, bottom; the orientations of adjacent $V=O$ units are also assumed to alternate around the anion ring). The other possible arrangement results in unfavorable O–O nonbonded contacts (2.371 Å). In contrast to **1**, the cluster anions in **3** are oriented with the rings parallel to each other in the three-dimensional structure. Bond valence calculations give values of 4.08 and 4.12 for V1 and V2, respectively.

The presence of a cation centering the cluster anion in **3** is an important difference between the anions in **1** and **3**. The anion in **3**, formed from six $(VO)_2BP_2O_{10}$ building units, has enough space in the center to include a cation and enough flexibility to accommodate cations over a limited size range (K^+ (1.38 Å) to Cs^+ (1.67 Å)). The cluster anion in **1** is formed in the absence of any small cation and is not centered. The (piperazine H_2) $^{2+}$ counteranions in **1** apparently cannot stabilize a still larger cluster anion. A comparison of the anions in **1** and **3** suggests that the included cation acts as a template in the formation of the six-ring. To confirm this, we have investigated the use of Na^+ (1.02 Å) as the templating cation in the synthesis of the five-ring structure previously reported.^[12] To confirm this, we have investigated the use of Na^+ (1.02 Å) as the templating cation.

Using reaction conditions identical to those for the synthesis of **3**, but substituting Na_2CO_3 for NH_4OH , we obtained in high yield a pure phase of **2**, as was shown by single-crystal X-ray diffraction. In **2** the five building $(VO)_2BP_2O_{10}$ units are arranged in a cyclic cluster anion centered by a Na^+ cation (Figure 1, middle). Due to the odd number of V_2O_8 dimers, two pairs of neighboring vanadyl oxygen atoms necessarily appear in a *cis* configuration. The anion is closely similar to the anion previously reported in $(C_2N_2H_{10})_2[(Na(VO)_{10}(BP_2O_{10}H_2)]^{12-}$ and is not described in detail here.

The thermogravimetric studies showed that all samples lose their water molecules between room temperature and 200 °C. The structures collapse on loss of water and the samples become amorphous, as indicated by powder X-ray diffraction. Between 200 and 400 °C all the organic and/or NH_3 molecules are lost, and a glassy matrix composed of VO_2 , P_2O_5 , B_2O_3 , and M_2O ($M = Na, K, Rb, Cs$) is formed.

The variations in the magnetic susceptibility and effective magnetic moment for **3** are shown in Figure 4. At higher temperatures ($120 < T < 280$ K) the sample is paramagnetic and obeys the Curie law with $\mu_{\text{eff}} = 1.74 \mu_{\text{B}}$, which is close to the value of $1.73 \mu_{\text{B}}$ expected for V^{4+} ($3d^1$, $S = 1/2$). Below 120 K, the magnetic properties are dominated by antiferro-

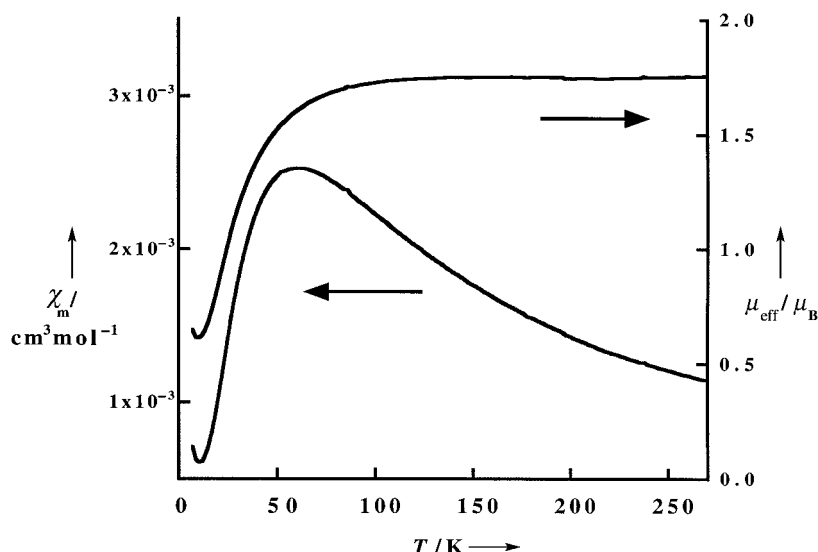


Figure 4. The molar magnetic susceptibility χ_m and the effective magnetic moment μ_{eff} of **3** as a function of temperature.

magnetic interactions within isolated V^{4+} – V^{4+} dimers, and the susceptibility goes through a maximum at about 60 K. The width of the maximum, however, is broader than expected from a dimer with a single exchange constant. The small upturn in the magnetic susceptibility at low temperature is most likely due to the presence of a small number of isolated V^{IV} defects. The magnetic susceptibility data for **1** also indicate antiferromagnetic interactions, but the maximum in the susceptibility occurs at even higher temperature. The present data suggest additional interactions between adjacent dimers. A more detailed analysis of the susceptibility data for the three clusters and correlation of the results with the structural features will be reported elsewhere.

In conclusion, we have demonstrated a template-directed synthesis of vanadium borophosphate cluster anions of different sizes. Synthesis of larger vanadium borophosphate cluster anions by careful selection of templates of appropriate size is currently being investigated.

Experimental Section

General: The hydrothermal synthesis were carried out in Teflon-lined steel autoclaves at 160 °C for three days. Elemental analyses were performed at Galbraith Laboratories, Knoxville, TN. The IR spectra were measured in the range 400–4000 cm^{-1} with KBr pellets on a Galaxy FTIR 5000 spectrometer. Thermogravimetric analyses were carried out at a heating rate of 2 K min^{-1} in air with a TA Instruments TGA 2950 Thermogravimetric Analyzer. Magnetic data were measured in the range $4 < T < 290$ K, $H = 0.1$ T, on a Quantum Design MPMS SQUID XL Magnetometer.

1: V_2O_5 (0.125 g, 0.8 mmol), H_3BO_3 (0.103 g, 1.7 mmol), H_3PO_4 (0.11 mL, 1.6 mmol, 80 wt % solution in H_2O), piperazine (0.1436 g, 1.7 mmol), and H_2O (2 mL) were allowed to react. The solution pH values before and after the reaction were 7.42 and 8.07, respectively. Reaction products: rod-shaped deep blue crystals of **1** ($n = 2$), average size 0.1–0.3 mm, yield approx-

imately 30 % based on vanadium, together with blue crystals of **1** ($n = 6$) in about 40 % yield (elemental analyses calcd: N 7.99, V 19.37, P 11.78, B 2.06; found: N 8.24, V 20.51, P 11.05, B 1.94) and pale blue plates of **1** ($n = 14$) in about 5 % yield. **1:** IR: $\tilde{\nu} = 466$ (w), 604 (m), 690 (w), 717 (m), 888 (sh), 940 (s), 978 (sh), 1047 (s), 1156 cm^{-1} (m).

2: V_2O_5 (0.2248 g, 1.5 mmol), H_3BO_3 (0.3090 g, 5 mmol), H_3PO_4 (0.342 mL, 5 mmol, 85 wt % solution in H_2O), Na_2CO_3 (0.5299 g, 10 mmol), and H_2O (2 mL) were allowed to react. The solution pH values before and after the reaction were 8.25 and 8.90, respectively. The product was filtered, and the dark blue filtrate was left to crystallize at room temperature for one week. Transparent blue crystals of **2** were obtained as a single phase. **2:** IR: $\tilde{\nu} = 543$ (m), 608 (m), 731 (w), 896 (sh), 955 (s), 991 (s), 1043 (s), 1164 cm^{-1} (sh).

3 ($A = \text{NH}_4^+$): V_2O_5 (0.2248 g, 1.5 mmol), H_3BO_3 (0.3090 g, 5 mmol), H_3PO_4 (0.342 mL, 5 mmol, 85 wt % solution in H_2O), NH_4OH (2 mL, 15.4 mmol, 29.6 % solution in H_2O), and H_2O (2 mL) were allowed to react. The solution pH values before and after the reaction were 7.53 and 8.80, respectively. Reaction product: transparent blue crystals of **3** ($A = \text{NH}_4^+$), average size 0.1–0.3 mm, yield 66.3 % based on vanadium (elemental analyses calcd ($n = 12$): N 9.20, V 22.30, P 13.56, B 2.37; found: N 9.03, V 20.36, P 12.20, B 2.20). The K, Rb, and Cs analogues were synthesized using the same reaction conditions and reactant molar ratios with the corresponding alkali metal carbonates. All compounds were obtained as single-phase products. **3:** IR: $\tilde{\nu} = 463$ (m), 546 (w), 615 (m), 683 (w), 729 (w), 901 (w), 960 (s), 995 (s), 1032 (s), 1169 cm^{-1} (s).

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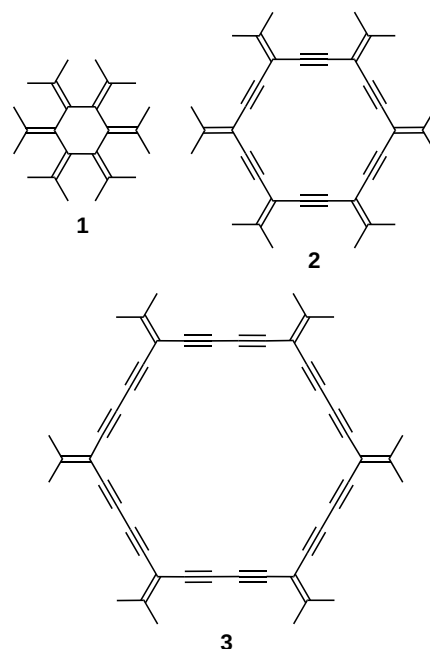
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- [13] Crystal structure analyses: a) **1** ($n=6$) at $T=293$ K: monoclinic, space group $P2_1/n$ (no. 14), $a=12.9822(9)$, $b=16.2608(12)$, $c=17.0246(11)$ Å, $\beta=103.412(2)^\circ$, $V=3495(9)$ Å³, $Z=2$, $\rho_{\text{calcd}}=1.998$ g cm⁻³; diffractometer: Siemens SMART 1 K CCD (MoK α radiation, graphite monochromator); $\mu=1.329$ mm⁻¹; a hemisphere of data (1271 frames at 5-cm detector distance), narrow-frame method with scan widths of 0.30° in ω and exposure time of 30 s per frame, $2\theta_{\text{max}}=53.96^\circ$, 9372 measured reflections, 5582 independent reflections; corrections for Lorentz factor, polarization, air absorption, and absorption due to variations in the path length through the detector faceplate. The program SADABS^[14a] was used for the absorption correction; structure solved by direct methods (SHELXTL^[14b]) and refined by least-squares methods (all atoms anisotropic, SHELXTL^[14c]); 502 free parameters; GOF=1.126, R values ($I>2\sigma(I)$): $R1=0.057$, $wR2=0.106$; max./min. residual electron density: $0.586/-0.436$ e⁻¹ Å⁻³.^[15] b) **1** ($n=2$): deep blue crystal, space group $P2_1/c$, $a=10.9147(7)$, $b=11.9186(7)$, $c=25.3828(15)$ Å, $\beta=95.011(1)^\circ$, $V=3289(9)$ Å³. c) **1** ($n=14$): pale blue crystal, space group $P2_1/c$, $a=13.2229(7)$, $b=28.7520(16)$, $c=21.2716(11)$ Å, $\beta=98.916(1)^\circ$, $V=7989(13)$ Å³. d) **3** ($A=\text{NH}_4^+$, $n=12$) at $T=293$ K: rhombohedral, space group $R\bar{3}m$ (no. 166), $a=14.116(2)$ Å, $\alpha=71.96(2)^\circ$, $V=2470.7(5)$ Å³, $Z=1$, $\rho_{\text{calcd}}=1.651$ g cm⁻³; data collection, corrections and structure solution as above for **1** ($n=6$); $\mu=1.376$ mm⁻¹, 2689 measured reflections, 1487 independent reflections; 147 free parameters; GOF=1.037, R values ($I>2\sigma(I)$): $R1=0.056$, $wR2=0.151$; max./min. residual electron density: $0.415/-0.370$ e⁻¹ Å⁻³.^[15] e) **2** at $T=293$ K: monoclinic, space group $P2_1/n$ (no. 14), $a=22.198(1)$, $b=15.3947(8)$, $c=26.118(1)$ Å, $\beta=115.113(1)^\circ$, $V=8081.8(8)$ Å³. f) Crystallographic data (excluding structure factors) for the structures of **1** and **3** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-115529 and -115530. 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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- [15] Selected bond lengths [Å] and angles [°] for **1** ($n=6$): V–O 1.932–2.001, V=O 1.588–1.613, P–O 1.497–1.579, B–O 1.452–1.497; O–P–O 105.5–114.0, O–B–O 104.5–112.4; for **3** ($A=\text{NH}_4^+$, $n=12$): V–O 1.932–2.022, V=O 1.590, P–O 1.496–1.569, B–O 1.461–1.480; O–P–O 107.5–112.0, O–B–O 105.1–111.0.

Expanded Radialenes: Modular Synthesis and Characterization of Cross-Conjugated Enyne Macrocycles**

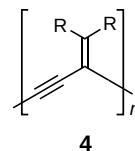
Sara Eisler and Rik R. Tykwinski*

Structurally intermediate between the radialenes **1**^[1] and expanded radialenes **3**^[2] are a class of hitherto unknown enyne macrocycles **2** constructed of 3-alkylidene-1-propynyl



[$-\text{C}\equiv\text{C}-\text{C}(\text{=CR}_2)-$] building blocks.^[3] Our interest in the electronic effects of cross-conjugation^[4] in iso-polydiacetylenes (iso-PDAs, **4**), the acyclic analogues of **2**, led us to explore the synthesis of macrocycles such as **2** with a view to extending our understanding of π -electron delocalization in this intriguing class of molecules.

Our initial studies of the electronic absorption behavior of iso-PDAs showed that π -electron delocalization in these compounds is not limited to the longest linearly conjugated segment; instead, it increases as a function of oligomer length and reaches saturation around the length of the nonamer (**4**; $R=\text{Me}$, $n=9$).^[5] This analysis suggested a subtle contribution from cross-conjugation to the overall electronic communication along the enyne framework of iso-PDAs. It was anticipated that the more structurally rigid enyne framework of **2** would allow evaluation of electronic effects from cross- or



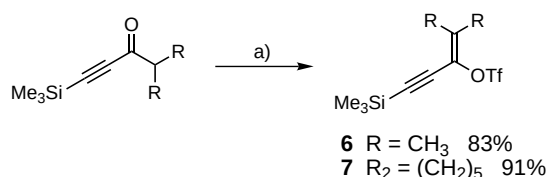
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homoconjugation that may not be observable in the rotationally flexible iso-PDAs.

An iterative method of chain elongation was used to assemble the cross-conjugated precursors to the radialenes **2**^[6] by utilizing palladium-catalyzed cross-coupling of terminal alkynes with vinyl triflates.^[7] Our coupling sequence is sufficiently mild to tolerate the unsaturated iso-PDA framework, and its versatility allows modular incorporation of the different substituents required to maintain solubility or vary electronic effects. The target compound **5** (see Scheme 2) demonstrates our ability to selectively append different substituents to the same radialene framework.

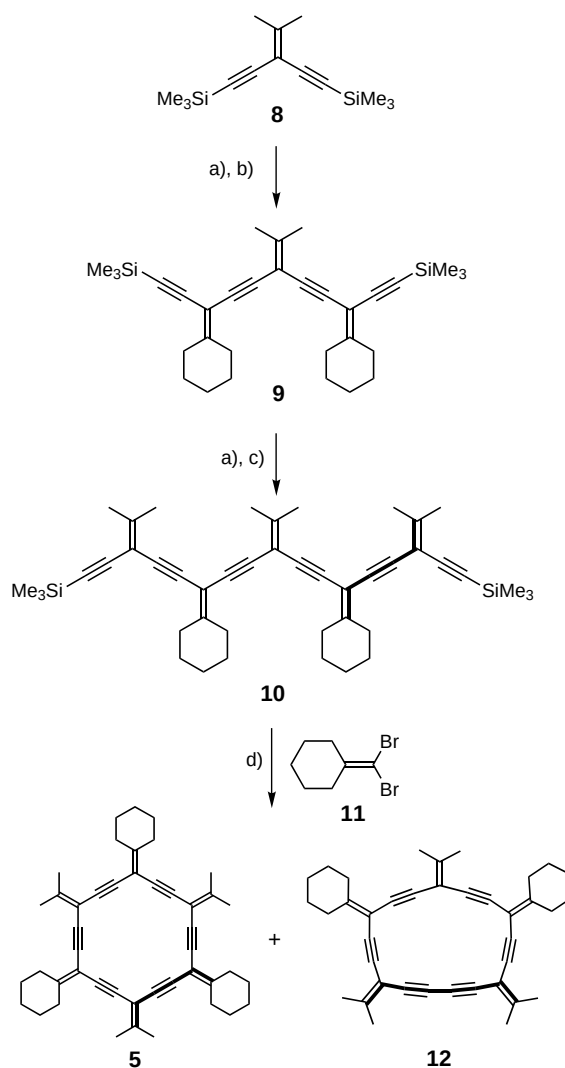
The vinyl triflate building block **6** was synthesized from the corresponding ketone as described by Stang and Fisk,^[8] and an analogous method gave vinyl triflate **7** (Scheme 1). The



Scheme 1. Synthesis of vinyl triflates **6** and **7**. a) 2,6-*t*Bu₂-4-CH₃-C₅NH₂, (CF₃SO₂)₂O, CH₂Cl₂, room temperature.

stepwise synthesis of radialene **5** is outlined in Scheme 2. Cross-conjugated enediyne **8**^[5] was desilylated with K₂CO₃ in wet THF/MeOH (1/1) to afford the deprotected diyne, which, after work-up, was cross-coupled with vinyl triflate **7** to give the mixed trimer **9**. Cross-coupling to form **9** proceeds rapidly at room temperature in DMF with [Pd(PPh₃)₄] as catalyst, CuI as co-catalyst, and a slight excess of the vinyl triflate **7**. Trimer **9** was subjected to a second iteration of the protodesilylation and cross-coupling procedure, in this case with vinyl triflate **6**, to give pentamer **10** in a modest yield of 28%.^[9]

The assembly of radialene **5** required protodesilylation of **10** followed by cross-coupling with dibromoolefin **11** (Scheme 2). To minimize the intramolecular Cu^{II}-catalyzed oxidative coupling^[10] of deprotected **10** to give the hybrid radialene **12**, precursor **10** was desilylated *in situ*.^[11] Tetrabutylammonium fluoride (TBAF, 1M in THF) was added to a dilute degassed solution (ca. 0.006M) of **10** and **11**^[12] in THF in the presence of [Pd(PPh₃)₄], CuI, and piperidine. The reaction mixture was stirred at room temperature and the reaction monitored by TLC, which showed sequentially the slow desilylation of **10**, cross-coupling with **11**, and finally cyclization to **5** (oxidative coupling to give **12** was observed as well). Although the dilute reaction conditions ultimately favored cyclization to **5** rather than the formation of linear oligomers, the reaction reached completion only after seven days. Work-up and column chromatography gave the pure macrocycles **5** and **12** in modest yields as a result of decomposition during purification. As pure solids, radialenes **5** and **12** are stable at room temperature under argon for only short periods of time (hours). They can, however, be stored in dilute, degassed solutions for days at room temperature or months under refrigeration with minimal loss. In spite of their modest environmental stability, both radialenes were completely characterized (Table 1).



Scheme 2. Modular synthesis of radialenes **5** and **12**. a) K₂CO₃, wet MeOH/THF (1/1), room temperature, 2 h; b) **7**, [Pd(PPh₃)₄], CuI, Et₃NH, DMF, room temperature, 2 h, 41% (from **8**); c) **6**, [Pd(PPh₃)₄], CuI, Et₃NH, DMF, room temperature, 2.5 h, 28% (from **9**); d) **11**, TBAF, [Pd(PPh₃)₄], CuI, piperidine, THF, 9% **5**, 5% **12** (from **10**).

Table 1. Selected spectroscopic data for **5**, **12**, and **15**.

5: White solid; m.p. 150 °C (decomp); IR (solid): $\tilde{\nu}$ = 2927, 2854, 2198, 1577 cm⁻¹; ¹H NMR (300 MHz, CD₂Cl₂): δ = 2.80 (m, 12H), 2.12 (s, 18H), 1.70 (m, 18H); ¹³C NMR (125 MHz, CD₂Cl₂): δ = 160.7, 153.3, 102.1, 98.6, 88.3, 88.2, 33.4, 28.1, 26.5, 23.0; UV/Vis (CHCl₃): λ_{max} (ϵ) = 286 nm (53 600); HR-MS calcd for C₄₅H₄₈: m/z: 588.3756; found (EI, 70 eV): 588.3750. 12: White solid; m.p. 140 °C (decomp); IR (solid): $\tilde{\nu}$ = 2928, 2854, 2201, 2134, 1577 cm⁻¹; ¹H NMR (300 MHz, CD₂Cl₂): δ = 2.60 (m, 8H), 2.14 (s, 6H), 2.08 (s, 6H), 2.07 (s, 6H), 1.66 (m, 12H); ¹³C NMR (125 MHz, CD₂Cl₂): δ = 160.7, 154.7, 153.1, 102.3, 102.1, 98.8, 89.4, 89.0, 88.6, 88.4, 83.0, 77.2, 33.6, 33.4, 28.1, 28.1, 26.6, 23.3, 23.2, 22.9; UV/Vis (CHCl₃): λ_{max} (ϵ) = 336 (13 200), 293 nm (47 000); HR-MS calcd for C₃₈H₃₈: m/z: 494.2974; found (EI, 70 eV): 494.2967. 15: White solid; m.p. 136–138 °C; IR (solid): $\tilde{\nu}$ = 2931, 2227, 2133, 1604 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ = 2.35 (m, 4H), 1.96 (s, 6H), 1.95 (s, 6H), 1.55 (m, 8H), 1.29 (m, 2H); ¹³C NMR (125 MHz, CDCl₃): δ = 151.3, 101.8, 93.8, 81.7, 79.1, 75.9, 30.3, 29.6, 28.9, 22.6, 22.5, 19.6; UV/Vis (CHCl₃): λ_{max} (ϵ) = 328 (24 500), 309 (26 500), 290 (15 700), 276 (8 400), 260 nm (25 000); HR-MS calcd for C₂₃H₂₆: m/z: 302.2035; found (EI, 70 eV): 302.2035.

A comparison of the UV/Vis spectrum of radialene **5** with that of iso-PDA oligomer **10** (Figure 1) distinguishes the electronic absorptions resulting from the *cis* and *trans* orientations of the ene–yne–ene segments that represent the longest linearly conjugated paths in these molecules

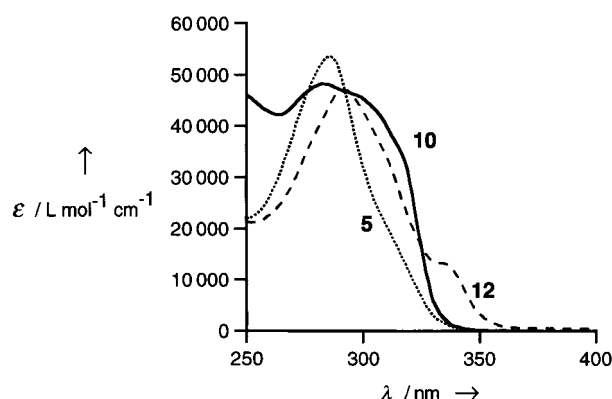


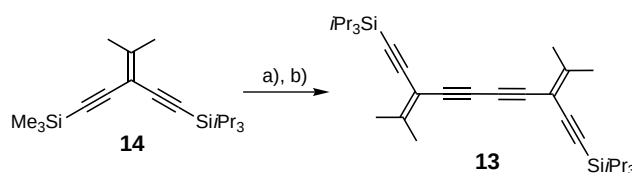
Figure 1. Electronic absorption spectra of **5**, **10**, and **12** in CHCl_3 at room temperature.

(Scheme 2, shown in bold for **5** and **10**). Rotationally unencumbered acyclic **10** shows two low-energy absorptions at 283 nm and about 305 nm for *cisoid* and *transoid* ene–yne–ene orientations, respectively.^[13] Structurally rigid radialene **5** shows only one major low-energy absorption, at 286 nm, which is ascribed to the *cisoid* ene–yne–ene configuration of this molecule.

Molecular modeling studies predict a somewhat flattened chair conformation for the hexameric radialene **5**.^[14] Despite this nonplanar structure, the rigid macrocycle is constrained to an orientation that is nearly optimal for overlap of the in-plane sp orbitals of the acetylenic units. The UV/Vis spectra suggest, however, that homoconjugation contributes little to the overall π -electron delocalization in **5**, since the radialene **5** has similar absorption characteristics to acyclic **10** (Figure 1).^[15] The similar absorption energies of **5** and **10** predict virtually the same extent of overall π -delocalization in these two molecules as a sum of linear and cross-conjugation.^[5]

Comparing the UV/Vis spectrum of **5** with that of the hybrid radialene **12** (Figure 1) shows that major electronic absorption of **12** is slightly red-shifted by 10 nm to 293 nm, possibly the result of increased planarity in **12**, as was observed for other radialenes.^[16] The absorption spectrum of **12** has an additional low-energy band around 336 nm. This new band was initially attributed to the longer linearly conjugated segment present in **12** (Scheme 2, ene–yne–ene, shown in bold). To confirm the assignment of this electronic transition, the synthesis of two model compounds, each containing the linearly conjugated C_8 segment of **12**, was undertaken.

The first model compound, dimer **13**, was readily derived from the enediyne **14** (Scheme 3).^[5, 17] The UV/Vis spectrum of **13** (Figure 2) showed absorption maxima at 262 and 269 nm and lower energy absorptions at 288, 307, and 328 nm. The lowest energy absorption of **13** (328 nm) corresponds approximately to that of radialene **12**, although it is slightly blue-



Scheme 3. Synthesis of **13**. a) K_2CO_3 , wet MeOH/THF (1/1), room temperature, 2 h; b) CuI , TMEDA , O_2 , CH_2Cl_2 , room temperature, 2 h, 95 % (from **14**). $\text{TMEDA} = N,N,N',N'$ -tetramethylethylenediamine.

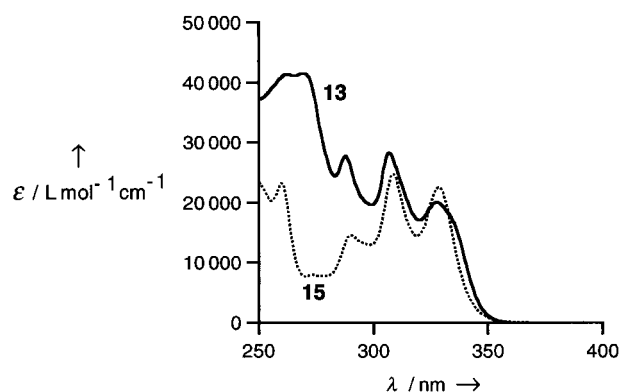
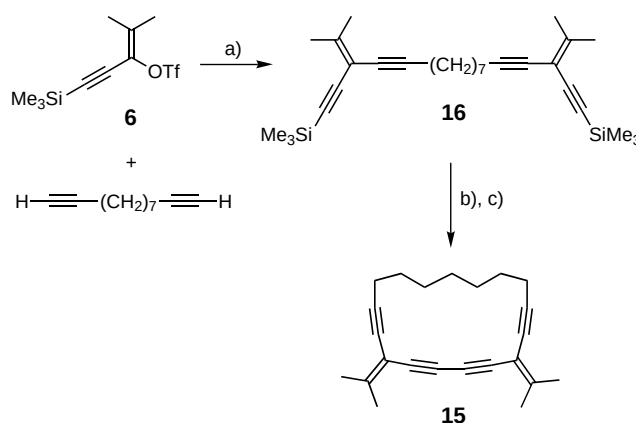


Figure 2. Electronic absorption spectra of **13** and **15** in CHCl_3 at room temperature.

shifted by about 8 nm. The rotational freedom of **13**, however, is expected to result in individual absorptions for the *cis* and *trans* rotamers (vide supra), which were not clearly assignable in the spectrum of **13**. Hence, the origin of the low-energy absorption in **12** is still uncertain.

To provide more definitive information, the conformationally restricted analogue of **13**, cyclic **15** (Table 1), was assembled as outlined in Scheme 4.^[18] The electronic absorp-



Scheme 4. Synthesis of **15**. a) $[\text{Pd}(\text{PPh}_3)_4]$, CuI , Et_3NH , DMF , room temperature, 5 h, 26 %; b) K_2CO_3 , wet MeOH/THF , room temperature, 3 h; c) CuI , TMEDA , O_2 , CH_2Cl_2 , 4 h, 22 % (from **16**).

tion spectrum of **15** (Figure 2) shows major low-energy absorptions similar to those of **13** at 290, 309, and 328 nm. A direct comparison of the absorption spectra of **13** and **15** suggests that the bands of the *cis* and *trans* forms of **13** are similar in energy; the latter is barely visible as a shoulder at about 336 nm for **13** and absent from the spectrum of **15**. As observed for **13**, the lowest energy absorption of **15** at 328 nm

is also blue-shifted by about 8 nm versus that of radialene **12**. There is little doubt, however, that in all three molecules this absorption band is due to the linearly conjugated ene–yne–yne–ene segment.

The slight bathochromic shift of the lowest energy absorption of radialene **12** relative to **13** or **15** is interesting as it could signify an increased contribution from cross- or homoconjugation to the overall electronic communication that results from the constricted interior yne–ene–yne bond angles of **12** relative to those of **15**.^[15] At this point, however, it can not be ruled out that this red shift results from inductive effects or the increased planarity and/or ring strain of **12** versus **15**. However, these explanations seem less likely, given the similar absorption spectra of **13** and **15** (Figure 2). Additional model compounds are now being synthesized in an effort to resolve this question.

The synthesis and characterization of radialenes **5** and **12** provides preliminary details of the electronic properties of these cross-conjugated enyne macrocycles. The hexameric radialene **5**, with nearly optimal sp² interior bond angles, shows no enhanced π -electron delocalization attributable to homoconjugation, as shown by comparison with acyclic iso-PDA analogues. The absorption spectrum of hybrid radialene **12**, however, suggests that a reduction of the interior bond angles could lead to additional electronic effects in these molecules that ultimately result in a red shift of the lowest energy absorption. The syntheses of the tri-, tetra-, and pentameric members of this new class of expanded radialenes represented by hexamer **5** are currently being conducted. The more rigorously planar structure of these smaller rings and the smaller interior bond angles promise to provide a more detailed view of the π -electron delocalization in expanded radialenes.

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- [13] The absorption band for the *transoid* form at 305 nm, visible as a shoulder in the spectrum of **10**, is more clearly discernible in **9** and other shorter iso-PDAs.^[5]
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- [15] For a similar discussion of homoconjugation in peryclines, see L. T. Scott, M. J. Cooney in *Modern Acetylene Chemistry* (Eds.: P. J. Stang, F. Diederich), VCH, Weinheim, **1995**, pp. 321–351.
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- [17] The trimethylsilyl group of **14** was selectively removed with methanolic K₂CO₃ to give the terminal alkyne, which was then oxidatively coupled^[10] to give **13** as a colorless solid.
- [18] Vinyl triflate **6** was coupled with 1,10-undecadiyne to give the tethered bis-enediyne **16**. Protodesilylation of **16** gave the terminal alkynes, which were then oxidatively coupled^[10] to give **15** as a white solid.

A New Radical Vinylation Reaction of Iodides and Dithiocarbonates**

Frédérique Bertrand, Béatrice Quiclet-Sire, and Samir Z. Zard*

Aryl and vinyl halides can be coupled to vinylic or acetylenic groups by a number of reactions mediated by transition metals, particularly palladium.^[1] In contrast, the analogous direct reaction with aliphatic halides is much more limited and remains a challenge in organic synthesis.^[1e] Recently, an attempt to fill this gap was reported by Bräse, Waegell, and de Meijere,^[2] but the scope of their study remains limited to bromoadamantane so far. Radical arylations and vinylations are more useful,^[3] especially when such groups are delivered internally. Intermolecular addition–elimination is more sensitive to competing side reactions but can

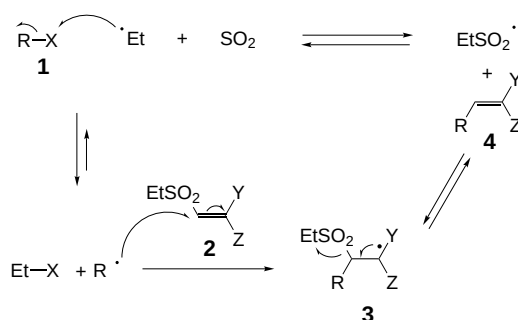
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be made efficient by the use of activated olefinic partners.^[4] Almost all of these approaches hinge upon organotin derivatives. We now report a new method for the alkenylation and, indirectly, the alkynylation of aliphatic iodides and dithiocarbonates (xanthates) by a radical mechanism which is based on sulfone chemistry and does not involve heavy metals.

Our concept is based on the generation of radicals under conditions where the main side reactions are made either degenerate or strongly reversible. This gives the intermediate radical a sufficient lifetime to react with hindered sulfones. The system, outlined in Scheme 1, is designed in such a way that a chain reaction can nevertheless be propagated. The coproducts are sulfur dioxide and ethyl iodide or ethyl xanthate, depending on the starting material used.

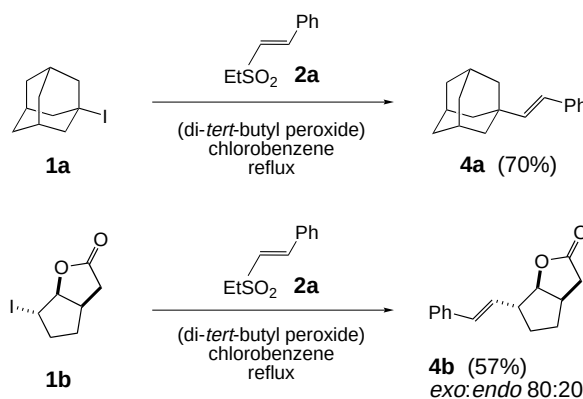


Scheme 1. Radical reaction of an aliphatic iodide or xanthate **1** with an ethyl vinyl sulfone **2**, X = I, SCSOEt.

We have previously found that allyl ethyl sulfone can act by a similar mechanism as an allylating reagent for aliphatic and alicyclic iodides and xanthates;^[5] however, whereas transfer of an allyl group requires an addition–fragmentation sequence on an unhindered terminal carbon, a similar introduction of a vinyl group is significantly more difficult as it implies radical addition on the same terminus of the alkene bearing the sulfone group. Additions to unsubstituted phenyl vinyl sulfone are known to occur from the least hindered and electronically more activated carbon, located β to the sulfone.^[6] However, a few scattered reports suggest that if the β carbon is substituted by, for example an aryl group, then radical addition can take place at the desired α site, followed by expulsion of the sulfonyl radical. Early work by Russell et al.^[7] with an organomercury derivative as the radical source was followed recently, and concurrently with our own efforts, by an elegant and more extensive study of unsaturated trifluoromethyl sulfones (triflones) by Fuchs and his collaborators.^[8] The highly electron withdrawing triflone group appears to activate strongly the desired α position but, because the radical is mostly created by hydrogen abstraction by the highly electrophilic trifluoromethyl radicals, the method remains limited to relatively simple substrates (usually the solvent). The difficult problem of regioselectivity must thus be addressed if the reaction is to be applied to more complex, multifunctionalized molecules.

To establish the feasibility of the radical vinyl coupling to iodides, we prepared ethyl 2-phenylvinyl sulfone (**2a**) by radical addition of ethylsulfonyl chloride to styrene followed by elimination of HCl according to the procedure of Truce

and Goralski.^[9] Upon heating a chlorobenzene solution of **2a** and 1-iodoadamantane (**1a**) in the presence of di-*tert*-butyl peroxide as the initiator, a smooth reaction occurred to give the desired benzylidene derivative **4a** in 70% yield (Scheme 2). In the same way, iodolactone **1b** provided **4b** in 57% yield (72% yield based on recovered starting material) as an 80:20 mixture of *exo* and *endo* epimers.

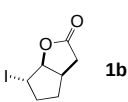
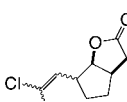
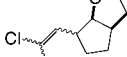
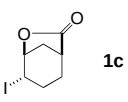
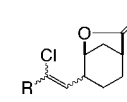
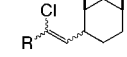
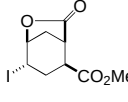
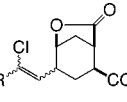
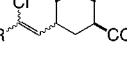
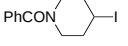
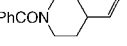
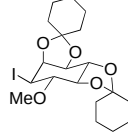
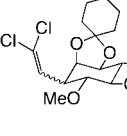
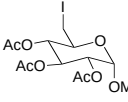
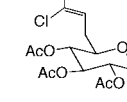


Scheme 2. Phenylvinylation of iodides with ethyl 2-phenylvinyl sulfone (**2a**).

For this approach to have some generality, groups other than aryl must be tolerated. In the first instance, we considered the introduction of a 2,2-dichlorovinyl unit with 2,2-dichlorovinyl ethyl sulfone (**2b**),^[10] where the two chlorine atoms would hinder attack from the unwanted β site. We were initially dismayed to find that when **1b** was subjected to the same reaction conditions in the presence of **2b**, the desired reaction proceeded poorly and the reaction mixture darkened ominously. However, upon repeating the reaction in a refluxing heptane/chlorobenzene mixture (6/1) with lauroyl peroxide as the initiator (more suitable at this lower reaction temperature), the iodine atom was replaced by a dichlorovinyl group to give **4c** in 64% yield (76% yield based on recovered starting material) and high selectivity (*exo:endo* 85:15).^[11] A number of other substrates were subjected to the same transformation (Table 1). We were also pleasantly surprised to find that even a chlorovinyl group can be introduced with relative ease (**4c'**, **4d'**, **4e'**, **4f**). Why, in the case of the chlorovinyl sulfone **2c**, the regioselectivity of the radical attack is still mostly on the α carbon (with respect to the sulfone) is not clear and raises the question of the extent (and mode) of activation of the α and β positions by a sulfone group. It is possible that an addition–elimination process with formation of highly reactive chlorine atoms took place on the β terminus in the case of **2c** because the reaction mixture darkened even under these milder conditions.

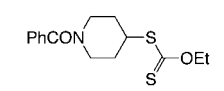
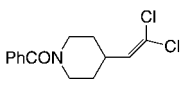
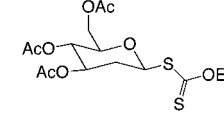
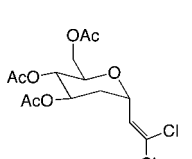
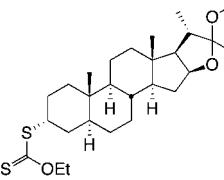
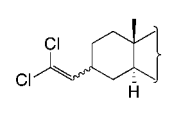
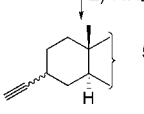
Transfer of dithiocarbonates (xanthates)^[12] is akin to that of iodides, and the same vinylation reaction can be performed with such substrates (Table 2). Xanthates can be made by substitution of halides with potassium or sodium *O*-alkylxanthate or, more importantly, by a radical xanthate transfer reaction which is itself a carbon–carbon bond forming process.^[12] The latter possibility is exemplified by the synthesis of **11** from xanthate **1k** and allyl acetate; this reaction is

Table 1. Radical chlorovinylation and dichlorovinylation of iodides.

Iodide 1	Y in vinyl sulfone 2 with Z = Cl	Product 4	Yield [%] ^[a] isomers
 1b	Cl (2b) H (2c)	 4c , R = Cl  4c' , R = H	64 (76) <i>exo:endo</i> 85:15 75 three isomers 55:37:8
 1c	Cl (2b) H (2c)	 4d , R = Cl  4d' , R = H	69 <i>exo:endo</i> 20:80 79 four isomers 35:25:25:15
 1d	Cl (2b) H (2c)	 4e , R = Cl  4e' , R = H	88 <i>exo:endo</i> 30:70 67 four isomers 55:35:5:5
 1e	H (2c)	 4f	49 (64) <i>trans:cis</i> 55:45
 1f	Cl (2b)	 4g	39 $\alpha:\beta$ 35:65
 1g	Cl (2b)	 4h	19 (46)

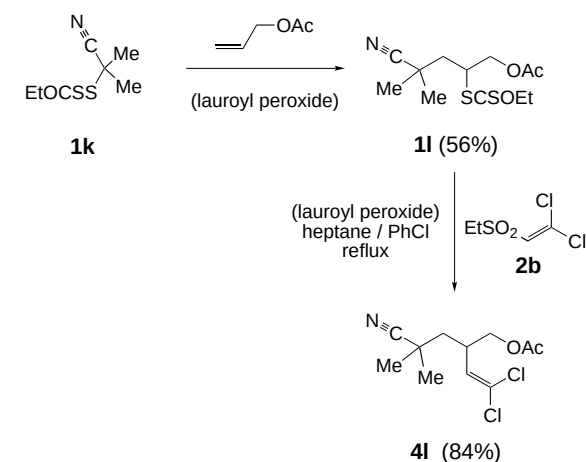
[a] The yields based on recovered starting material are given in parentheses.

Table 2. Radical dichlorovinylation of xanthates.

Xanthate 1	Product 4	Yield [%] isomers
 1h	 4i	76
 1i	 4j	68 < 5 % β -Isomer
 1j	 4k	57 $\alpha:\beta$ 2:1
	 5	100 $\alpha:\beta$ 2:1

followed by the efficient (84 %) exchange of the xanthate group by a dichlorovinyl group (Scheme 3).

The possibility of introducing a monochloro, dichloro-, and other substituted vinyl unit into aliphatic or alicyclic iodides or xanthates with cheap, readily available ethyl vinyl sulfone reagents opens up tremen-



dous opportunities for synthetic planning. The reaction works well with secondary and tertiary derivatives, but is less efficient with primary substrates (for example, **1g**) because the corresponding primary radical has a similar stability to the ethyl radical used to generate it. Both the monochloro and dichlorovinyl groups are immediate precursors of an acetylide anion by exposure to a simple base or butyllithium, respectively, in the Corey–Fuchs reaction.^[13] This is illustrated by the quantitative conversion of **4k** into alkyne **5** (Table 2). The dichlorovinyl motif also serves as a source of chloroalkynes by exposure to base.^[14] Chloroalkynes and both the monochloro- and dichlorovinyl derivatives are substrates for various transition metal induced couplings.^[1] Moreover, ongoing preliminary studies seem to indicate that the vinyl sulfone reagent can be substituted in various other ways to provide a convergent, flexible strategy for the rapid assembly of complex structures.

Experimental Section

Typical procedures are as follows:

Procedure A: A solution of iodide **1a**, **b** (1 mmol) and ethyl vinyl sulfone **2a** (3 mmol) in degassed chlorobenzene (2 mL) was heated under reflux and treated portionwise with di-*tert*-butyl peroxide (3 to 4 drops) over 12–16 h. The solvent was evaporated under reduced pressure and the crude residue was purified by column chromatography on silica gel to furnish the desired products **4a**, **b**.

Procedure B: A solution of iodide or xanthate **1b–j** (1 mmol) and ethyl vinyl sulfone **2b**, **c** (3 mmol) in degassed heptane/chlorobenzene (6/1, 2 mL) was heated under reflux and treated portionwise by lauroyl peroxide (0.12–0.3 mmol) over 5–12 h. The solvent was evaporated under reduced pressure, and the crude residue was purified by column chromatography on silica gel to give the desired products **4c–k**. With **2b** as sulfone, prior treatment for a few minutes with aqueous ammonia (to destroy excess sulfone) can sometimes simplify purification.

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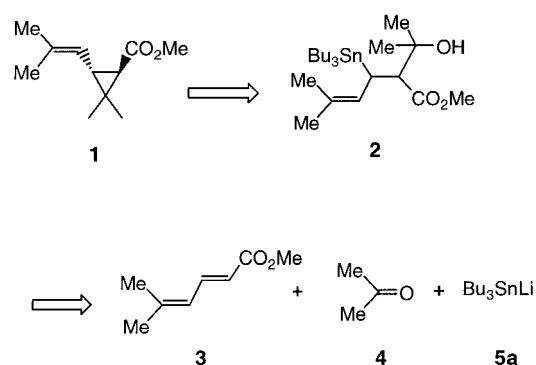
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Metal-Mediated, Completely Diastereofacial Conjugate Addition of Trialkylstannylmetal Reagents to γ -Alkoxy- α,β -Unsaturated Esters**

Alain Krief,* Laurent Provins, and Willy Dumont

Dedicated to Professor L. Ghosez
on the occasion of his 65th birthday

A few years ago, we disclosed an efficient “three-component” synthesis of *trans*-methyl chrysanthemate **1** which allows the construction of the cyclopropane ring by insertion of the isopropylidene moiety arising from acetone **4** (Scheme 1).^[1] We wanted to extend this strategy to the

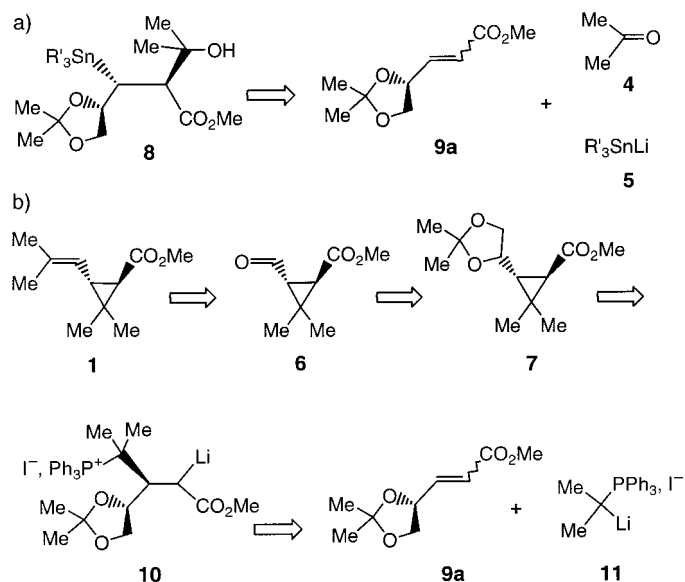


Scheme 1. Synthesis of (1R)-*trans*-methyl chrysanthemate from a dienoic ester, acetone, and tributylstannyl lithium.

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synthesis of the optically active (1*R*)-*trans* enantiomer, which is part of pyrethrin I, a natural product extracted from *Chrysanthemum cinerariaefolium* and a valuable insecticide.^[2] We chose the α,β -unsaturated ester **9a** derived from D-glyceraldehyde as the starting material and a synthetic strategy (Scheme 2a) completely different from that described by Mulzer and Kappert for the synthesis of the same product (Scheme 2b) from the same unsaturated ester **9a**.^[3]



Scheme 2. Synthetic strategies for (1*R*)-*trans*-methyl chrysanthemate.

Conjugate addition of the stannyl moiety to the α,β -unsaturated esters **9** was identified as one of the crucial steps of the process, since the absolute stereochemistry of chrysanthemic acid is fixed at this stage by the face of attack of the γ -alkoxy- α,β -unsaturated ester. From the mechanism of the 1,3-elimination reaction we knew that the tributyltin moiety should be introduced at the *Re* face.^[1]

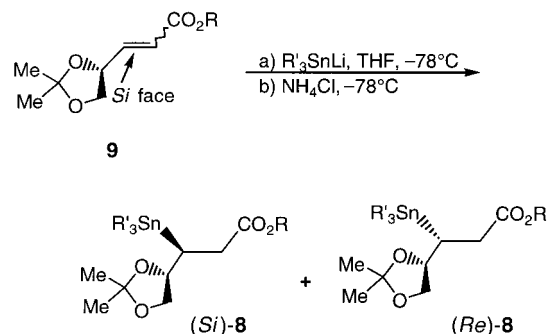
To our knowledge, only tributyltin hydride has been treated with (*E*)-**9** under radical conditions (nBu_3SnH , AIBN cat., hexane, reflux).^[4] This reaction does not suit our needs well i) since it requires a further metalation/hydroxyalkylation step to produce **8**, ii) due to the quite poor *de* previously reported for the resulting mixture of stereoisomers (58% yield, *Re/Si* 58/42, *de* 16%),^[4] and iii) because we found that application of the same reaction to the *Z* stereoisomer did not lead to a much better diastereoselectivity (70% yield, *Re/Si* 63/37, *de* 23%). We therefore planned to introduce the stannyl moiety by means of its organometallic derivatives and to trap the resulting enolate with acetone.

Conjugate addition of various organometallic compounds to γ -alkoxy- α,β -unsaturated esters bearing an asymmetric center in the γ position was extensively studied in the past decade. This is the case for alkyl- and aryllithium compounds,^[5] organocuprates,^[6] phosphorus^[3,7] and sulfur ylides,^[7] α -metallonitroalkanes,^[8a] α -lithiosulfones,^[8b] and lithium dialkylamides.^[9] However, in most cases, the face of attack cannot be predicted accurately and depends not only

on the reagent^[5,10,11] but also upon the *E* or *Z* stereochemistry of the double bond.^[7,11]

We report here our preliminary results on the addition of metal stannyl compounds, especially the tri-*n*-butylstannyl derivative **5a**, to the esters (*Z*)- and (*E*)-**9**. We quenched the resulting enolates with water to enable us to compare our results with those reported for tri-*n*-butyltin hydride.^[4]

We found that tri-*n*-butylstannyllithium^[12] reacts quite efficiently with methyl esters (*Z*)- and (*E*)-**9a** to provide, in both cases after hydrolysis, compounds that arise from attack on the *Si* face (Scheme 3; Table 1, entries 1 and 2). The reaction is completely face selective for (*Z*)-**9a** (entry 1) but



Scheme 3. Addition of stannyllithium reagents to **9**.

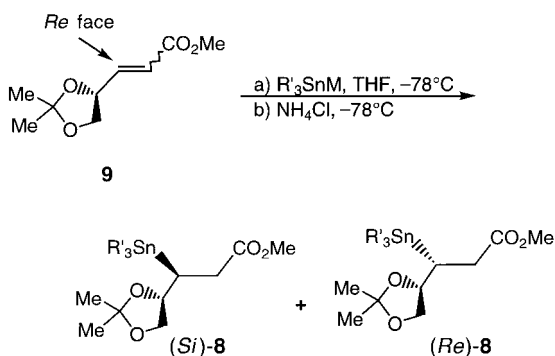
Table 1. Addition of stannyllithium reagents to **9**.

Entry	9	R	R'	Yield of 8 [%]	(<i>Si</i>)- 8 /(<i>Re</i>)- 8
1	(<i>Z</i>)- 9a	Me	<i>n</i> Bu	58	100/0
2	(<i>E</i>)- 9a	Me	<i>n</i> Bu	50	74/26
3	(<i>E</i>)- 9b	<i>t</i> Bu	<i>n</i> Bu	64	58/42
4	(<i>Z</i>)- 9b	<i>t</i> Bu	<i>n</i> Bu	64	100/0
5	(<i>Z</i>)- 9a	Me	Me	74	100/0

occurs with poorer diastereoselectivity for (*E*)-**9a** (entry 2). An even lower diastereoselectivity was observed for the *tert*-butyl ester (*E*)-**9b** (entry 3). For the *Z* stereoisomer, the reaction is completely diastereoselective regardless of the nature of the groups attached to the ester and of the tin reagent (entries d and e).

These results are surprising since stannyllithium compounds react at the opposite face to that attacked by alkylolithium reagents.^[5] Furthermore, although these results are quite good, the required (*Re*)-**8** stereoisomer cannot be obtained as a single stereoisomer by this reaction. One solution would have been to use the enantiomeric ester (*Z*)-**9a**, but this is not readily obtained. Given the known difference in selectivity between alkylolithium reagents and related cuprates, we decided to study the reactivity of stannylcuprates.

Stannylcuprates were efficiently prepared by transmetalation of tin hydrides with higher order alkylcuprates.^[13] They reacted mainly at the *Re* face of the γ -alkoxyester **9a**, that is, the opposite face to that attacked by the related lithium compounds (Scheme 4; Table 2, entries 1 and 2). Again, there was no difference between the trimethyl- and the tri-*n*-butylstannyl derivatives (cf. entries 3 and 1), and much higher



Scheme 4. Addition of stannylcuprate and stannylzincate reagents to **9**.

Table 2. Addition of stannylcuprate and stannylzincate reagents to **9**.

Entry	9a	R_3SnM	Yield of 8 [%]	(<i>Si</i>)- 8 /(<i>Re</i>)- 8
1	<i>Z</i>	$n\text{Bu}_3\text{Sn}(\text{Bu})\text{Cu}(\text{CN})\text{Li}_2$	37	10/90
2	<i>E</i>	$n\text{Bu}_3\text{Sn}(\text{Bu})\text{Cu}(\text{CN})\text{Li}_2$	84	43/57
3	<i>Z</i>	$\text{Me}_3\text{Sn}(\text{Bu})\text{Cu}(\text{CN})\text{Li}_2$	71	11/89
4	<i>Z</i>	$n\text{Bu}_3\text{Sn}(\text{Et}_2)\text{ZnLi}$	84	0/100
5	<i>E</i>	$n\text{Bu}_3\text{Sn}(\text{Et}_2)\text{ZnLi}$	73	10/90
6	<i>Z</i>	$\text{Me}_3\text{Sn}(\text{Et}_2)\text{ZnLi}$	77	0/100

diastereoselectivity was obtained with the *Z* stereoisomer (entries 1 and 2).

The diastereoselection in favor of (*Re*)-**8**, although very good, did not reach the standard attained for the synthesis of its stereoisomer (*Si*)-**8** from the same starting material and stannyl lithium compounds, but since the counterion proved to have a dramatic effect on the stereochemical outcome of this reaction, we decided to replace lithium by zinc. To our knowledge, stannylzincates are unknown. However, their silyl analogues^[14] were prepared from silyllithium compounds and diethylzinc and offered advantages over the related silyllithium and silylcuprate reagents.

We prepared stannylzincates by a procedure analogous to that reported for the synthesis of their silyl analogues^[14] and found that they add to ester (*Z*)-**9a** like stannylcuprates but with complete stereocontrol in favor of the *Re* adducts (*Re*)-**8** (Scheme 4; Table 2, entry 4). These reagents also reacted at the *Re* face of (*E*)-**9a** and, although the stereoselection was not as high as with (*Z*)-**9a**, it provides an unusually high stereocontrol (Table 2, entry 5; cf. Table 2, entry 2 and Table 1, entry 2).

We have shown that the synthesis of the β -stannylesters (*Si*)-**8** and (*Re*)-**8** can be achieved in a completely stereoselective fashion from (trialkyltin)metal reagents and the γ -alkoxy- α,β -unsaturated ester **9a** given the appropriate choice of stereoisomer (*Z*) and counterion (Li or LiZnEt_2 , respectively). The stannylmetal reagents give far superior stereocontrol than radical-induced reactions of tin hydride.

These results could be explained in terms of an appropriate model such as the degenerate nonchelated Felkin–Anh or chelated Cram model. However, we feel that it is not worthwhile to do so, since there is at present no adequate universal model which allows the stereochemical outcome of the reactions of γ -alkoxy α,β -unsaturated esters bearing a chiral γ -carbon to be predicted.

A configurational dichotomy that depends on reaction conditions, especially the counterion, was previously observed in conjugate addition reactions,^[15] but not with the extremes reported here. Work is in progress to understand the reasons for such behavior and to generalize the reactions reported here.

Experimental Section

Lithium tri-*n*-butylstannyl diethylzincate was prepared by dropwise addition of a solution of tributylstannyl lithium in THF/hexanes to a solution of diethylzinc (1M in hexane) at 0 °C. The yellow solution was then stirred for an additional 0.4 h at this temperature before use. Tributylstannyl lithium was prepared^[12] by addition of *n*-butyllithium (1.6M in hexane) to hexa-*n*-butyldistannane in anhydrous THF at 0 °C.

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Peptide Mini-Vectors for Gene Delivery**

Robert G. Cooper, Richard P. Harbottle, Holm Schneider, Charles Coutelle, and Andrew D. Miller*

It is widely anticipated that gene therapy will represent one of the key medical technologies of the next century. However, a great deal of progress still needs to be achieved for this to be possible. In general terms, gene therapy may be defined as “the delivery of nucleic acids (with a vector) to patients for some therapeutic purpose”.^[1a] Hence a major preoccupation of current gene therapy research has been the design of appropriate vectors for nucleic acid delivery. This is a sphere in which the chemist has a clear role to play,^[1] and indeed we ourselves have been working on the development of synthetic cationic liposome vector systems for the delivery of nucleic acids in vivo.^[2] Here we describe an alternative, peptide-based system which represents one of the smallest and simplest vector systems yet reported for the delivery of nucleic acids.^[1]

The peptide-based system described here originated from our initial discovery that synthesized peptide **1**, containing a cyclic N-terminal moiety and a hexadeca(L-lysine) DNA-binding moiety, could mediate gene delivery in vitro.^[3] The cyclic N-terminal moiety of **1** contains an Arg-Gly-Asp (RGD) peptide motif of a type shown to interact with high avidity to integrins such as $\alpha_{IIb}\beta_3$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$,^[4, 5] proteins which are found routinely at many cell-surface membranes.^[5, 6] We had anticipated that **1** would act by binding to nucleic acids by means of the hexadeca(L-lysine) moiety and then enter cells by a two-step process involving cell-surface integrin binding followed by receptor-mediated endocytosis. Such an integrin-mediated cell entry process is widely used by several pathogens including the adenovirus,^[7] which has itself been the subject of numerous gene delivery experiments in recent times.^[1] Therefore, we were in effect anticipating that **1** would operate like a minimalist adenovirus gene delivery vector.

Gratifyingly, peptide **1** did indeed behave in the anticipated way,^[3] but it proved very difficult to synthesize. A second-generation RGD peptide vector was then prepared in the

form of peptide **2** in which the hexadeca(L-lysine) moiety was relocated to the N terminus. Whilst **2** outperformed **1** as a gene delivery vector, it also proved difficult to prepare.^[8] There are known to be “difficult sequences” in solid-phase peptide synthesis which form secondary structures (such as α helix and β sheet) on the solid-phase resin as they are being synthesized. These secondary structures are believed to reduce amino acid coupling efficiencies and lower the recovered yields of peptide products.^[9, 10] Such difficult sequences may be identified by the equation of Milton et al.^[11] [Eq. (1)], where $\langle P_c^* \rangle$ is the average coil parameter

$$\langle P_c^* \rangle = \sum P_{c^*}^*_{i-n} / n \quad (1)$$

for a developing protected peptide chain of total length n residues, and $P_{c^*}^*_{i-n}$ are the individual refined coil parameters for each amino acid in the protected peptide chain at each individual position i . Typically, when the sequence of a given protected peptide chain is analyzed with Equation (1), a high $\langle P_c^* \rangle$ value (1.0–1.4) suggests that the chain will have a tendency to form random coil. This implies that the next amino acid residue to be coupled will be incorporated with ease (amino acid coupling efficiency >99.8%). A low $\langle P_c^* \rangle$ value (0.7–0.9) suggests the opposite. When a complete analysis was made of the protected peptide chains leading to the synthesis of **1**, $\langle P_c^* \rangle$ values of approximately 0.9 were obtained throughout. Similar results were also obtained for **2**. Therefore, both **1** and **2** appeared to be uniformly comprised of difficult sequences.

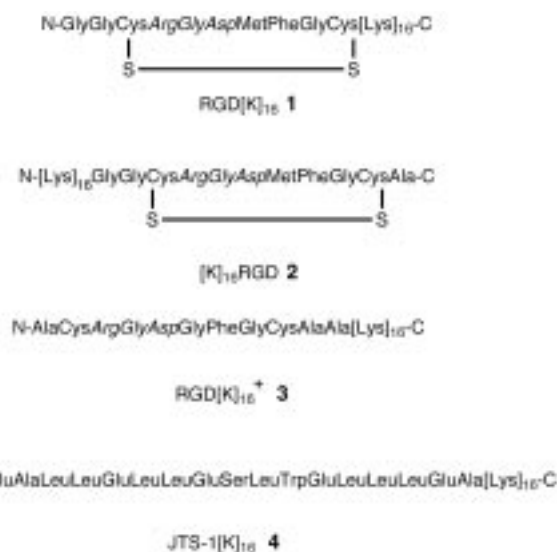
Given the potential importance of **1** and **2** for nucleic acid delivery, we were concerned to develop an alternative solid-phase peptide synthesis strategy which would overcome the problems presented by difficult sequences and also allow for alternative functional peptide moieties to be attached to the hexadeca(L-lysine) moiety. This would widen the utility and potential applicability of peptide-based vector systems for the delivery of nucleic acids. In developing this alternative strategy, we chose to prepare two new nucleic acid delivery peptides **3** and **4**. Peptide **3** is a variant of **1** and represents our third generation of RGD peptide vectors. In contrast, peptide **4** is made up of an alternative fusogenic peptide moiety

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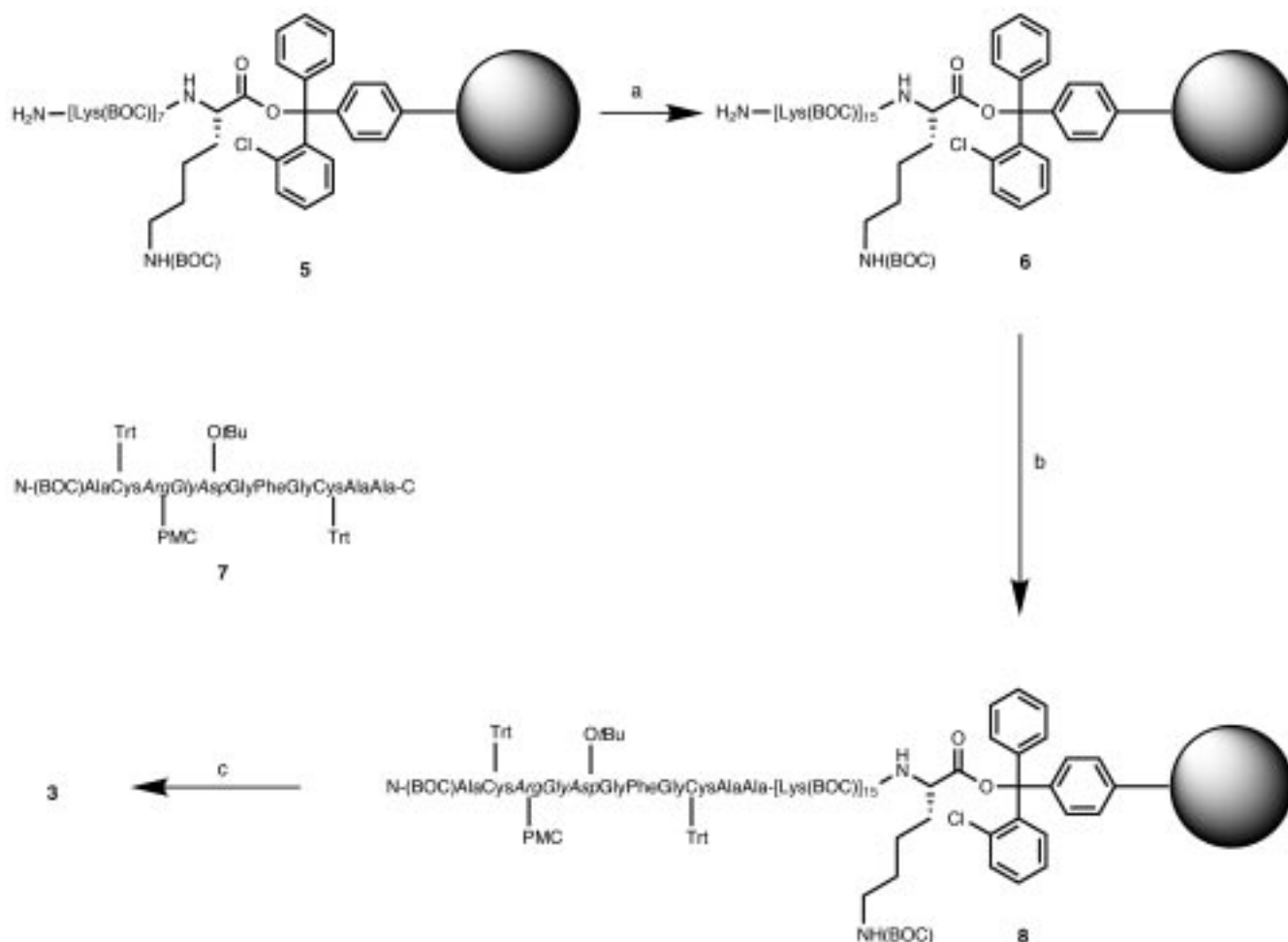


attached at the N terminus of the hexadeca(L-lysine) DNA-binding moiety. This fusogenic peptide moiety, christened JTS-1, had been previously shown to have potent endosomolytic properties.^[12] Therefore, we anticipated that if **3** and **4** were used in combination, the resulting peptide-based vector system would be able to bind electrostatically to nucleic acids, enter cells by integrin-binding mediated endocytosis by virtue of the RGD peptide motif of **3**, and then promote the escape of electrostatically associated nucleic acids from endosome compartments through the endosomolytic activity of the N-terminal fusogenic peptide moiety of **4**. Endosome escape is well known to be a serious problem for non-viral nucleic acid delivery systems.^[1] Hence the action of **4** was expected to synergize with the action of **3** and result in a more efficacious peptide-based vector system for the delivery of nucleic acids.

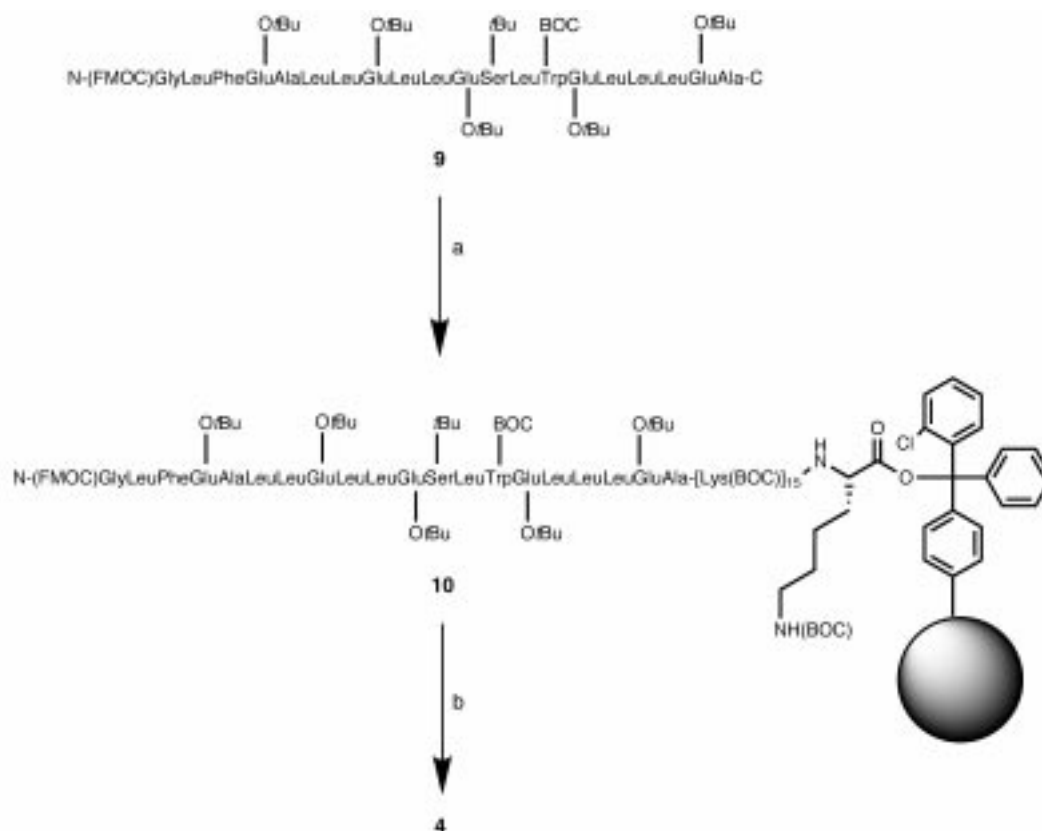
The solid-phase synthesis strategy for the preparation of **3** and **4** was as follows. A convergent segment condensation approach was adopted in which resin-bound octa(L-lysine) **5** was initially prepared on a super acid-labile 2-chlorotrityl resin and then coupled to Fmoc-[L-Lys(BOC)]₈-OH (**5a**) with benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) to give the resin-bound hexade-

ca(L-lysine) **6** (Scheme 1).^[13] Oligo(L-lysine) peptides such as **6** would normally be difficult to synthesize in a direct linear manner, especially when more than eight amino acid residues are involved ($\langle P_C^* \rangle$ values 0.8–0.9). Therefore, this particular segment condensation was carried out to avert this complication and optimize the yield of full-length **6**. One portion of **6** was then coupled to a fully protected RGD motif peptide **7** with PyBOP to give **8**, which was then cleaved from the resin and deprotected to give **3** in an overall yield of 3% (Scheme 1).

Another portion of **6** was coupled to fully protected fusogenic peptide **9** with dicyclohexylcarbodiimide/ hydroxybenzotriazole (DCC/HOBt) to give **10**. This too was released from the resin and deprotected to give **4**, also in an overall yield of 3% (Scheme 2). The synthesis of the intermediate RGD motif peptide **7** was performed by linear solid-phase peptide synthesis without incident. By contrast, the synthesis of intermediate fusogenic peptide **9** proved more difficult ($\langle P_C^* \rangle$ values approx. 0.8 throughout synthesis), and was only accomplished with the extensive use of extended coupling times and double coupling throughout. Overall, the yields of purified **3** and **4** were judged to be satisfactory, especially given the fact that the syntheses of both by normal linear



Scheme 1. Reagents and conditions: a) 1. Fmoc-[L-Lys(BOC)]₈-OH (**5a**), PyBOP (2 equiv), *i*Pr₂EtN (3 equiv), DMSO/DMF (1/3), RT, 72 h; 2. piperidine in DMF (20% v/v); b) **7**, PyBOP (10 equiv), *i*Pr₂EtN (10 equiv), DMSO/DMF (1/3), RT, 24 h; c) 1. phenol (7% w/v), ethanedithiol (2% v/v), thioanisole (4% v/v), water (4% v/v) in TFA, RT, 5 h; 2. MTBE (overall yield 3%). BOC = *tert*-butoxycarbonyl, *t*Bu = *tert*-butyl, Fmoc = (9-fluorenyl)methoxycarbonyl, MTBE = methyl *tert*-butyl ether, PMC = 2,2,5,7,8-pentamethylchroman-6-sulfonyl, TFA = trifluoroacetic acid, Trt = trityl.



Scheme 2. Reagents and conditions: a) **6**, DCC (40 equiv), HOBt (40 equiv), DMSO/DMF (1/4), RT, 24 h; b) 1. piperidine in DMF (20 % v/v), RT, 45 min; 2. ethanedithiol (2.5 % v/v), water (2.5 % v/v) in TFA, RT, 3 h; 3. MTBE (overall yield 3 %).

synthetic strategies were judged to be essentially impossible according to an analysis made with Equation (1).

Initially, the efficacy of **3** was evaluated in comparison to **2** as a vector for the *in vitro* delivery of the pGL3 plasmid into CaCo2 (human colonic epithelial) cells. The pGL3 plasmid expresses the American firefly (*Photinus pyralis*) luciferase reporter gene, leading to an accumulation of luciferase enzyme activity in CaCo2 cells following plasmid gene delivery. The level of enzyme activity then found in cells is directly proportional to the efficacy of the gene delivery process,^[8] so that the higher the enzyme activity is, the more effective is the gene delivery process. With the pGL3 delivery assay, peptides **2** and **3** were found to be indistinguishable as plasmid gene delivery vectors (results not shown). In contrast, our third-generation RGD-peptide vector **3** was able to deliver the pGL3 plasmid between four and five times more effectively than **4**, the endosomolytic vector (Figure 1). Gratifyingly, when a combination of **3** and **4** was used, the gene delivery efficiency of the combination was found to be almost ten times the efficiency of **3** alone (Figure 1). This showed that **3** and **4** were indeed functioning synergistically as we had hoped, most likely by the mechanism suggested above. The combined gene delivery effects of **3** and **4** is beginning to rival the *in vitro* gene delivery efficiency of LipofectAMINE cationic liposomes (Figure 1). Therefore, we are confident that the combination peptide system described here could supersede the use of cationic liposomes *in vitro*, given further optimization studies. However, a more fruitful route forward

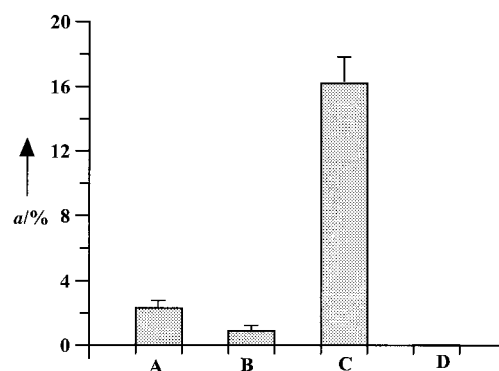


Figure 1. Luciferase enzyme activity *a* in cell-free extracts of CaCo2 cells 48 h after peptide mini-vector mediated delivery of the pGL3 plasmid which expresses the luciferase gene. Plasmid gene delivery was effected by treating cells with plasmid pGL3 condensed with either **3** and/or **4**, as indicated. In each transfection performed, the optimal plasmid/total peptide ratio was 1 µg/2.5 µg (1/2.5 w/w). The plasmid/peptide mixtures were pGL3 + **3** (A), pGL3 + **4** (B), pGL3 + **3** and **4** (C), and pGL3 alone (D). Luciferase enzyme activity is expressed as a percentage (%) of the activity found in the cell-free extracts of CaCo2 cells following control cationic liposome mediated plasmid gene delivery experiments, performed concurrently with LipofectAMINE (GIBCO BRL) cationic liposomes and pGL3 plasmid (24 µg/1 µg, 24/1 w/w).

may be to adopt an additional combination strategy where both peptides and cationic liposomes are used together to effect nucleic acid delivery. Indeed, we have just recently discovered that our second-generation RGD peptide vector **2** will enhance the *in vitro* gene delivery efficacy of Lipofect-

AMINE cationic liposomes,^[1] by at least an order of magnitude.^[14] Such an enhancement of cationic liposome gene delivery efficacy could have important implications for in vivo cationic liposome-mediated gene delivery.

In conclusion, we have devised a convergent synthesis for peptide mini-vectors, which were demonstrated to be able to mediate plasmid gene delivery in vitro. To the best of our knowledge, these are some of the smallest delivery vectors for nucleic acids yet reported and could be very useful tools for future gene therapy studies.

Experimental Section

All peptides were synthesized on an ABI 431A solid-phase batch peptide synthesizer. Unless otherwise stated above, syntheses were conducted on an 0.1-mmol scale with a fivefold excess of Fmoc-protected L-amino acids (Novabiochem, Nottingham, UK) and FastMoc reagents 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/hydroxybenzotriazole (HBTU/HOBt) (Alexis Corporation, Laufelfingen, Switzerland and SMPE Ltd, Croydon, UK) as the amide coupling agent. Coupling steps were carried out in N-methylpyrrolidinone (NMP) and dichloromethane (Rathburn, Walkerburn, UK) on super acid-labile 2-chlorotriptyl resins (200–400 mesh; Novabiochem, Nottingham, UK) as the solid support. After synthesis, peptides were cleaved from the resin by trifluoroacetic acid (TFA) in dichloromethane (1% v/v), followed by treatment with pyridine (2.5 equiv) in methanol. Desalting was performed on a P2 biogel column (2 × 28 cm; Bio-Rad Laboratories, Herts., UK) attached to an FPLC system (Amersham Pharmacia Biotech UK, Bucks., UK) eluting with 0.1% aqueous TFA (0.75 ml min⁻¹; monitoring at 214 or 280 nm). Reverse-phase HPLC purification was usually carried out with a Vydac column (C18, 5 μm, 2 × 25 cm; Hichrom Ltd, Berks., UK) attached to a Gilson HPLC system (Anachem, Beds., UK). Peptides were eluted with a gradient of acetonitrile in 0.1% aqueous TFA (5 ml min⁻¹; monitoring at 220–230 nm). Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry was performed on a LaserMat 2000 (ThermoBioanalysis Ltd, Herts., UK) with a matrix of α-cyano-4-hydroxycinnamic acid (α-CMC) (33 mM) in acetonitrile/methanol (Hewlett-Packard, Cheshire, UK).

Peptide **3** was synthesized as described above (Scheme 1). After final desalting, HPLC purification (eluting with acetonitrile at 53.5%, v/v) and lyophilization, **3** was obtained as a white powder. Overall yield: 8 mg (2.6 μmol, 3%); MS (MALDI-TOF) *m/z* calcd for C₁₃₆H₂₅₅N₄₆O₃₀S₂: 3078.9 [*M* + H]⁺; found 3078.1. The sequence was further confirmed by amino acid composition and sequence analyses; homogeneity was judged to be greater than 95% by HPLC analysis.

Peptide **4** was synthesized as described above (Scheme 2). After final HPLC purification (eluting with acetonitrile at 96.5% v/v) and lyophilization, **4** was obtained as a white powder. Overall yield: 12 mg (2.7 μmol, 3%); MS (MALDI-TOF) *m/z* calcd for C₂₀₆H₃₆₆N₅₃O₄₈: 4353.5 [*M* + H]⁺; found 4353.8. The sequence was further confirmed by amino acid composition and sequence analyses; homogeneity was judged to be greater than 95% by HPLC analysis.

Peptides **3** and **4** (100 μg mL⁻¹) were dissolved in 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid, pH 7.3, containing 150 mM NaCl (HEPES buffered saline). The solution of **3** was then stirred for 16 h at room temperature in order to form an intramolecular disulfide bond. Bond formation was monitored by Ellman assay.^[15] Finally, the gene delivery efficacies of oxidized **3** and/or **4** were tested as described previously.^[8]

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Metathesis of Alkanes: Evidence for Degenerate Metathesis of Ethane over a Silica-Supported Tantalum Hydride Prepared by Surface Organometallic Chemistry**

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Here we report on evidence for a catalytic process in which ethane molecules mutually exchange a methyl group with one another on the silica-supported tantalum(III) hydride catalyst^[1] (≡SiO)₂Ta-H ([Ta]_s-H) (**1**) under mild conditions. We recently reported that the surface complex **1** catalyzes the metathesis of linear and branched alkanes and effectively

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converts a given alkane into a mixture of higher and lower molecular weight alkanes. For example, ethane can be converted to methane, propane, and to a lesser extent, heavier alkanes [Eq. (1)].^[2]



This unprecedented catalytic reaction involves both cleavage and formation of C–C bonds of alkanes,^[3] which may occur simultaneously or stepwise at the metal center. In the case of olefins, the metathesis reaction was discovered in the late 1960s and was the subject of numerous studies since then.^[4] In particular, it was shown that a degenerate process that scrambles the reagent occurs together with the productive process. For example, the production of bideuterated ethylene from a mixture of tetra- and undeuterated ethylene over various catalysts constituted a proof for the existence of a degenerated process in the case of unlabeled olefins. We have now discovered the occurrence of a similar process in alkane metathesis by using ¹³C-monolabeled ethane (¹³CH₃–CH₃).

The metathesis of ¹³C-monolabeled ethane in the presence of **1** was carried out in a batch reactor at 150 °C; the gas phase was analyzed by GC and the isotopic distribution of ethane was monitored by GC/MS. After 1 h the expected metathesis products methane, propane, isobutane, butane, and traces of pentane and isopentane were evolved. After 100 h, the conversion of ethane into metathesis products reached 11 %, which correspond to about 13 turnovers (Figure 1a). Under these reaction conditions, the activity and the selectivity for the products (see Experimental Section) were the same regardless of whether labeled or unlabeled ethane was used.

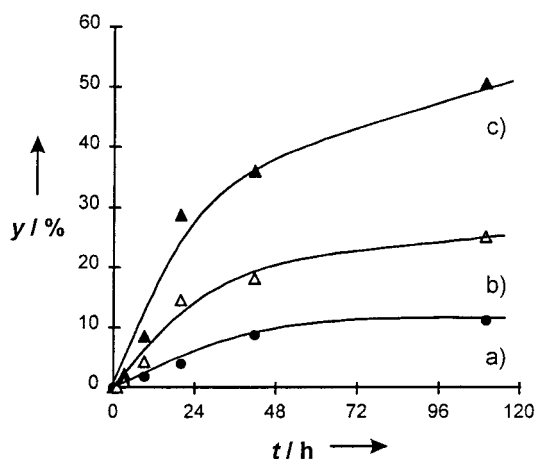


Figure 1. Amounts *y* of monolabeled ethane converted into a) other alkanes (methane, propane, butanes), b) unlabeled and dilabeled ethane (detectable degenerate metathesis), and c) unlabeled, monolabeled, and dilabeled ethane (calculated degenerate metathesis) versus time.

Analysis of the gas phase by GC/MS reveals an additional process that occurs in parallel to the formation of higher alkanes: monolabeled ethane is converted to its unlabeled and dilabeled isotopomers in similar amounts (Figure 2). The initial rate of this new process (Figure 1b) is about 2.5 times higher than that of the metathesis reaction (Figure 1a). This ratio does not change during the reaction, and after 100 h, 25% of the monolabeled ethane was converted to a 1/1

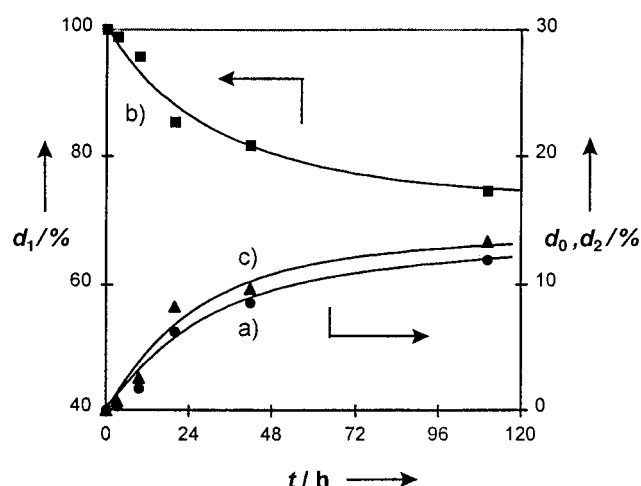
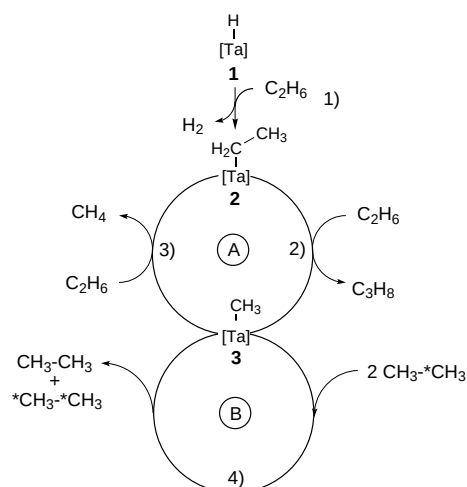


Figure 2. Degenerate metathesis of ethane, studied using ¹³CH₃–CH₃. Contribution of a) unlabeled (*d*₀), b) monolabeled (*d*₁), and c) dilabeled (*d*₂) ethane to the isotopomer distribution versus time.

mixture of ethane and dilabeled ethane, whereas only about 11 % was transformed into other alkanes.

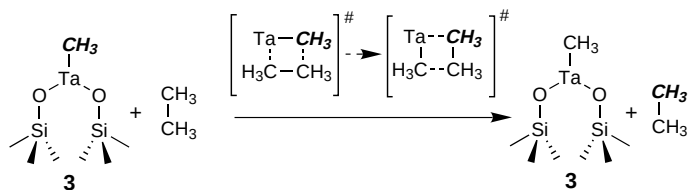
The metathesis of alkanes over **1** has some similarities with olefin metathesis. Indeed, in most cases, the latter reaction involves two processes: one that converts the substrate to its lower and higher homologues, and one that leads to its scrambling. By analogy, we refer to the conversion of ethane into other alkanes as *productive* metathesis, and the conversion of monolabeled ethane to its isotopomers as *degenerate* metathesis. In contrast to ethylene metathesis, which is an exclusively degenerate process, ethane metathesis involves both productive and degenerate processes.

Productive and degenerate metatheses both require C–C bond cleavage of monolabeled ethane and formation of a new C–C bond. In the proposed mechanism for productive metathesis (Scheme 1 A), two intermediates were proposed: (≡SiO)₂Ta–Et (**2**) and (≡SiO)₂Ta–Me (**3**).^[2] The surface complex **2** reacts with ethane in a C–C bond-activation process (2) to form C₃H₈ and **3**. The surface complex **3** is implicated in a C–H bond-activation process (3) which



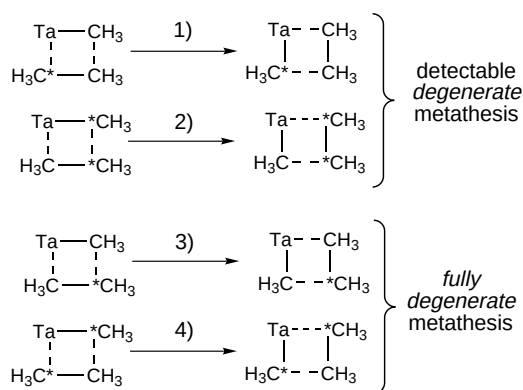
Scheme 1. Catalytic cycle for ethane metathesis. A) Productive metathesis, B) degenerate metathesis.

regenerates **2**. This simple catalytic scheme can be extended to explain the degenerate metathesis of ethane. Since the surface complexes **2** and **3** are very similar, **3** should be able to perform C–C bond activation of ethane, which would lead to exchange of a methyl group with an incoming molecule of ethane (Scheme 2).



Scheme 2. σ -Bond metathesis: C–C bond activation of ethane by the surface complex **3**.

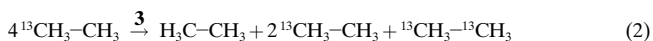
Since the methyl group of **3** can be labeled or unlabeled^[5] and monolabeled ethane is an unsymmetrical molecule, four reaction pathways can be envisaged (Scheme 3). The formation of unlabeled and dilabeled ethanes is the only detectable



Scheme 3. Degenerate (1, 2) and fully degenerate (3, 4) ethane metathesis.

part of the reaction [detectable degenerate metathesis, reactions 1 and 2]; it should also be accompanied by the undetectable production of monolabeled ethane from monolabeled ethane [fully degenerate metathesis, reactions 3 and 4]. Experimental measurements show that unlabeled and dilabeled ethane are produced in a 1:1 ratio (Figure 2). No measurable isotopic effects occur in these processes, and all the reaction pathways (1)–(4) have the same probability. Consequently, the rate of fully degenerate metathesis should be equal to the rate of detectable degenerate metathesis, which in turn is equal to the sum of the rates of formation of unlabeled and dilabeled ethane. After 100 h, ethane and dilabeled ethane are formed in equal amounts and correspond to 12.5% of the initial quantity of monolabeled ethane. We can then calculate that about 50% of the monolabeled ethane underwent a degenerate metathesis reaction (Figure 1c), while the degree of conversion to productive metathesis products was about 11%. Under these experimental conditions degenerate metathesis is on average five times faster^[6] than productive metathesis, and this is consistent with the lower steric hindrance of $(\equiv\text{SiO})_2\text{Ta-Me}$ (**3**) relative to $(\equiv\text{SiO})_2\text{Ta-Et}$ (**2**).

We have discovered a new catalytic process, that can be referred to as degenerate metathesis, that leads to the isotopomeric redistribution of labeled ethane [Eq. (2)]. This



process is about five times faster than productive metathesis. These results allow us to complete the catalytic cycle proposed for alkane metathesis (Scheme 1 (B)) and demonstrate again the high activity of our tantalum catalyst^[7] in the cleavage and formation of C–C bonds of alkanes.

Experimental Section

The reactions were carried out in the absence of solvent under inert atmosphere or vacuum. The product mixture and the isotopomer distribution were monitored at different stages of the reaction: the reaction vessel was cooled to room temperature, an aliquot was allowed to expand in a small volume, brought to atmospheric pressure with hydrogen, and analyzed by gas chromatography (HP 5890 apparatus, $\text{Al}_2\text{O}_3/\text{KCl}$ on fused silica column (50 m $\times$ 0.32 mm)) and GC/MS (HPG 1800A apparatus). Elemental analyses were performed at the CNRS Central Analysis Service of Solaize. Ethane (Air liquide) and monolabeled ethane (Cambridge Isotope Laboratories) were dried over freshly regenerated molecular sieves (3 Å) and deoxo traps immediately before addition. Silica (Degussa, 200 m² g^{−1}) was dehydroxylated under vacuum at 500 °C for 15 h.

An IR cell, in which the supported tantalum hydride was previously synthesized^[1] (60 mg, 6.77 wt % Ta) was charged with 100% monolabeled ethane. ($p = 16$ kPa, substrate/catalyst ratio $\approx 120:1$). The reaction was carried out at 150 °C. After 100 h, the conversion of ethane was about 11% (calculated from the consumption of ethane), and the selectivities for the products were as follows: methane 70, propane 27, isobutane 1.6, butane 1.2, and pentanes 0.2%.

Determination of the isotopomer distribution of ethane: The mass spectrum of a mixture of variously labeled ethanes was evaluated for the peak distribution between m/z 24 and 33. The spectrum of unlabeled ethane has a peak distribution between m/z 24 and 31. It is assumed that the same distribution of peaks is shifted by one unit mass to a higher value for each ^{13}C atom in the molecule. A theoretical spectrum was calculated in which the parameters represent the relative amount of unlabeled, monolabeled, and dilabeled ethane, and the sums of the squares of the differences between corresponding peaks of the theoretical and experimental spectra were minimized with the computer program Excel solver.

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A Giant Carceplex Permanently Entraps Three Organic Molecules**

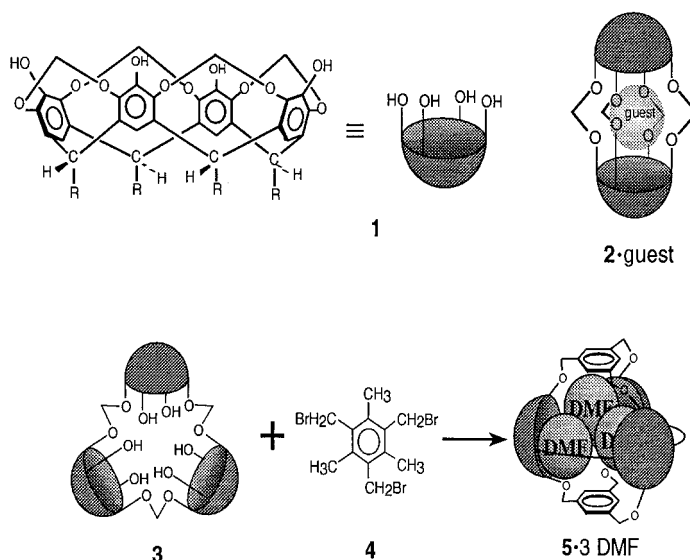
Naveen Chopra and John C. Sherman*

A current trend in supramolecular chemistry is the creation of large hosts that can accommodate several guests or one large guest. Particularly exciting is the possibility of encapsulating several molecules within molecular vessels and thus facilitating the study of a "microsolvent". The formation of such a species could allow a sophisticated study of templation versus solvent effects, as several molecules may have to be displaced by several others. To date, carceplexes, which permanently entrap molecules within their confines,^[1] were only shown to entrap single small guest molecules.^[2, 3] We report here the preparation of a carceplex roughly triple the size of any reported previously. We demonstrated the clean and selective permanent entrapment of three molecules of DMF and probed the properties of the entrapped microfluid medium. Such a system should provide a novel opportunity to study complex template effects.

Many large host systems have been reported^[4] that could potentially be used to bind several guests: The holand by

Reinhoudt et al. is a very large and rigid macrocycle of four concave host units.^[3b] It has a huge cavity with correspondingly large holes; although it has not been shown to retain or complex guests, related species have been shown to bind steroids.^[4a] The resorcinarene hexamer of Atwood and MacGillivray is a noncovalent assembly that may contain several solvent molecules according to electron density found in the crystal structure; no definitive characterization of encapsulated guests has been reported, nor has the assembly been shown to retain guests in solution.^[5] Rebek and Conn encapsulated single large guests in their capsules, and in one case two guests were complexed; the reversible complex formation indicates rapid guest exchange.^[6] Cram et al. reported several large hemicarceplexes, in which large portals allow guests to escape and preclude the retention of small molecules.^[7] In contrast, the host reported here is unique in that it irreversibly retains several guest molecules.

In designing a large carceplex, certain criteria must be met, including structural rigidity and an effectively closed surface. Cavitands such as tetrol **1** (Scheme 1) are rigid bowl-shaped



Scheme 1. Schematic representation of the synthesis of carceplexes containing guest molecules. R = $\text{CH}_2\text{CH}_2\text{Ph}$. For clarity, methyl groups are omitted from the caps of **5·3 DMF**.

molecules with an enforced cavity and are hence attractive building blocks for the construction of carceplexes. Indeed, two molecules of tetrol **1** were linked to create the small carceplex **2**.^[3a] We recently reported the synthesis of a cyclic trimer of bowls (**3**),^[8] which is a rigid barrel-shaped molecule with an enforced cavity. Trimer **3** seems ideally suited as a precursor for a large carceplex, and hence it was combined with cap **4** in DMF with K_2CO_3 as base in the presence of KI at ambient temperature for 24 h. The product was identified as carceplex complex **5·3 DMF** (36% yield). The complex **5·3 DMF** is readily soluble in CHCl_3 and was easily isolated from polymeric side products by chromatography on silica gel. The matrix-assisted laser desorption/ionization (MALDI) mass spectrum of **5·3 DMF** showed a single predominant peak (m/z : 3641; calcd for **5·3 DMF**· Na^+ : 3642), which

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suggests that **5** selectively entraps three DMF molecules; peaks for two or four entrapped DMF molecules were not observed. No DMF was lost from **5**·3DMF on heating to 160 °C for 6 h in nitrobenzene. Figure 1 shows a space-filling drawing of **5**·3DMF; it is apparent that the vessel is effectively sealed.

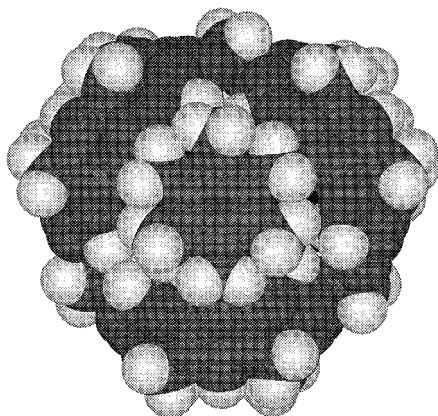


Figure 1. Space-filling depiction of **5**·3DMF. The view is downwards onto the top of a cap. The structure was drawn with Chem 3D Pro (Cambridge Software) and energy-minimized by MM2 calculations. Pendent phenethyl groups are omitted for clarity.

The ^1H NMR spectrum of **5**·3DMF in CDCl_3 is quite simple, and the signals for the entrapped DMF molecules are easily assigned (Figure 2). The integration is consistent with the composition of three DMF molecules per carceplex. The

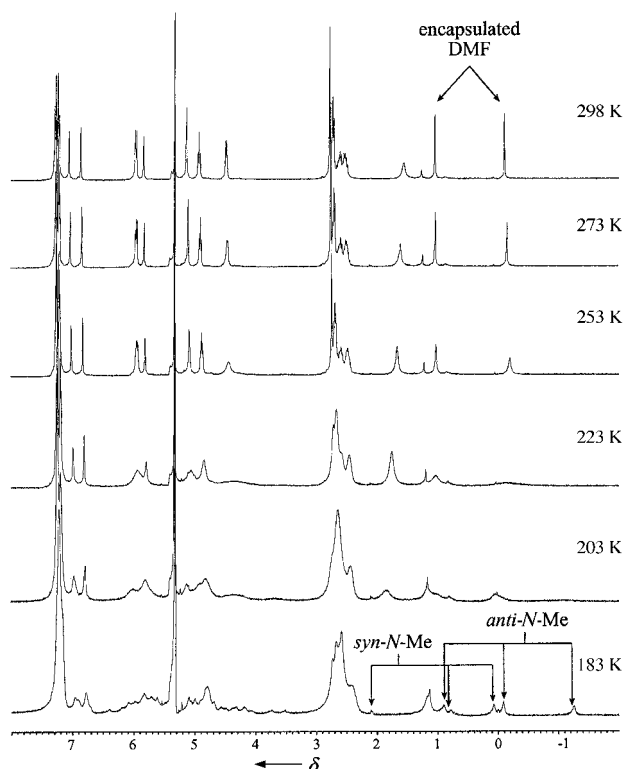


Figure 2. ^1H NMR (400 MHz) spectra of **5**·3DMF in CD_2Cl_2 (*syn*- and *anti*-*N*-methyl groups assigned arbitrarily).

signals of the entrapped DMF molecules are shifted upfield from those of free DMF in solution; the degree of shielding varies significantly with the position of the guests in the interior of the carceplex and thus provides a "magnetic map" of guest orientation. The $\Delta\delta$ values in CDCl_3 at ambient temperature are 2.97 and 1.94 for the *N*-methyl groups (*syn* and *anti* assigned arbitrarily) and 2.08 for the formyl protons. These $\Delta\delta$ values are somewhat smaller than those of **2**·DMF,^[3a] and this implies a more spacious interior for **5**. Indeed, a CPK model of **5**·3DMF reveals that the DMF molecules have ample room to move around inside.

Not surprisingly, there is only one set of ^1H NMR signals for the entrapped DMF molecules; this suggests that all three move rapidly within the carceplex on the ^1H NMR timescale. Variable-temperature ^1H NMR has been used to probe the mobility of guests entrapped in carceplexes.^[3a, 9] For example, the energy barrier to rotation about amide bonds is sensitive to the phase and to solvent polarity in solution: The barrier generally decreases on going from a polar solvent to a nonpolar solvent to the gas phase.^[10] In ^1H NMR spectra of **5**·3DMF in $[\text{D}_5]\text{nitrobenzene}$ from 298 K to 423 K, the *N*-methyl signals broaden as the temperature is increased; at 423 K they were indistinguishable from the baseline. The coalescence temperature T_c was estimated to lie between 423 and 428 K, which corresponds to a $\Delta G^\ddagger$ value^[3a, 9] of 19.3–19.5 kcal mol^{-1} for rotation about the C–N bond. This energy barrier is lower than that of free DMF in nitrobenzene (20.2 kcal mol^{-1}),^[3a] but slightly higher than that of the single DMF molecule in **2**·DMF (18.9 kcal mol^{-1}).^[3a] These data imply that the interior of **5**·3DMF resembles the gas phase less than that of the smaller carceplex or that it is more like a polar solvent. Since CPK models suggest that the three DMF molecules have much more freedom in **5**·3DMF than the single DMF molecule in **2**·DMF, it seems that the three DMF molecules create a polar "microsolvent" environment within the cavity of **5**.

Low-temperature ^1H NMR experiments in CD_2Cl_2 (Figure 2) showed that at 223 K the signals for *N*-methyl groups broadened out entirely into the baseline. At 183 K, new signals appeared: One set of *N*-methyl signals at $\delta = -1.2$, -0.1 , and $+0.9$, which coalesce at $\delta = -0.1$, and another at $\delta = +0.1$, 0.8 , and 2.1 , which coalesce at $\delta = +1.0$. Hence, the three DMF molecules appear to reside in unique and dramatically different environments, for which the $\Delta\delta$ values relative to the *N*-methyl signals of free DMF range from 0.8 to 4.1.^[11] The IR spectra show two carbonyl bands (1676 and 1682 cm^{-1}), and this suggests that at least one DMF molecule is in a different environment than the other two on the IR time scale. This may indeed be the same nonequivalence that is observed by ^1H NMR spectroscopy at low temperature, as it is likely that two of the three carbonyl bands are simply coincident (the bands at 1676 and 1682 cm^{-1} are themselves only just resolvable).

The entrapment of several molecules offers new opportunities to explore the chemical and physical properties of confined media. One of our interests in container compounds is the mechanism of their formation. In particular, what is the role of the guest molecule as a template in the assembly process? We studied this process in detail for a small

carceplex with a single small molecule as template.^[12] A current challenge is the exploration of larger containers, for which a large molecule or several small molecules can act as templates. Such a system would add the dimension of guest molecularity to the template question. Moreover, the effect of solvation would be far more complex because excluding solvent by simply using a large molecule would no longer be practical. We have demonstrated here the first step toward such a program, the creation of a large robust container that permanently entraps several molecules, the number, identity, "effective phase," mobility, and orientation of which have been clearly characterized. Efforts are currently underway to optimize the synthesis of **5**·guest complexes so as to analyze the role of templation.

Experimental Section

5·3DMF: Trimer **3** (25.4 mg, 0.00823 mmol), K₂CO₃ (70 mg, 0.506 mmol, 61 equiv), KI (85 mg, 0.512, 62 equiv), and 1,3,5-(bromomethyl)mesitylene (20.5 mg, 0.0511 mmol, 6 equiv) were added to DMF (10 mL). The reaction mixture was stirred under N₂ for 24 h at 25 °C. The solvent was removed in vacuo, and HCl (2 M, 30 mL) was added to the crude mixture. The aqueous layer was extracted with CHCl₃ (3 × 10 mL), and the combined CHCl₃ extracts were dried with anhydrous MgSO₄, filtered, and evaporated to give a brown residue, which was eluted through a pad of silica gel with CHCl₃. A purple band indicating the presence of I₂ was coeluted with the desired product. This purple band was collected, solvent was removed and the residue was recrystallized from CHCl₃/hexanes to give 10.7 mg of **5**·3DMF as a white solid (0.00295 mmol, 36%). M.p. > 250 °C; ¹H NMR (CDCl₃, 400 MHz): δ = 7.10–7.30 (brm, CH₂CH₂C₆H₅, CHCl₃), 6.99 (s, 6H, *p*-H_{OAr}), 6.77 (s, 6H, *p*-H_{acetal}), 5.97 (s, 3H, (CH₃)₂NCHO), 5.96 (d, *J* = 7.1 Hz, 12H, H_{out}), 5.85 (s, 6H, H_{acetal}), 5.12 (s, 12H, OCH₂Ar), 4.94 (t, *J* = 7.7 Hz, 12H, H_{methine}), 4.47 (d, *J* = 7.1 Hz, 12H, H_{in}), 2.77 (s, 18H, ArCH₃), 2.71 (t, 24H, *J* = 7.6 Hz, CH₂CH₂C₆H₅), 2.52 (m, 24H, CH₂CH₂C₆H₅), 1.04 (s, 9H, (CH₃)₂NCHO), –0.11 (s, 9H, (CH₃)₂NCHO); IR (KBr): $\tilde{\nu}$ = 1682, 1676 cm^{–1} ($\nu_{C=O}$); MS (MALDI): *m/z* (%): 3640 [*M*+Na⁺] (100); calcd for C₂₁₉H₁₉₂O₃₆·3C₃H₇ON+Na⁺: 3642; elemental analysis calcd for C₂₁₉H₁₉₂O₃₆·3C₃H₇ON: C 75.67, H 5.93, N 1.16; found: C 75.27, H 5.95, N 1.00.

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Porphomethenes and Porphodimethenes Synthesized by the Two- and Four-Electron Oxidation of the *meso*-Octaethylporphyrinogen**

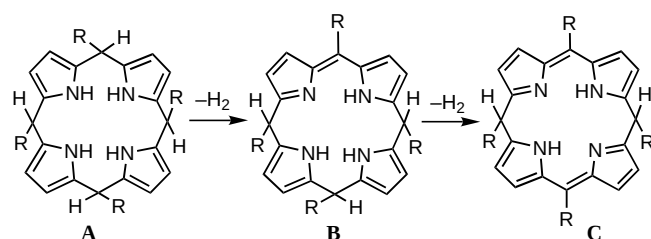
Jean-Marc Benech, Lucia Bonomo, Euro Solari, Rosario Scopelliti, and Carlo Floriani*

Porphomethene **B** and porphodimethene **C** are supposed to pave the way from porphyrinogen **A** to porphyrin (Scheme 1).^[1,2] However, these intermediates have never been synthesized from porphyrinogen but rather from metalloporphyrins by reductive protonation or reductive alkylation.^[3] The yield and scale of such synthetic methods are severely limited due to the compulsory use of octaalkylmetalloporphyrins, whose steric bulk is necessary to protect the porphomethene and porphodimethene from the easy oxidation to porphyrins.^[3] We report here on the stepwise deal-

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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. The two- and four-electron oxidation of porphyrinogen **A** to porphomethene **B** and porphodimethene **C**.

kylation of the *meso*-octaethylporphyrinogen [**A** ($R = Et$)]^[4, 5] to porphomethene [5,10,15-triethyl-22-hydroporphyrin, **B** ($R = Et$)] and porphodimethene [5,15-diethylporphyrin, **C** ($R = Et$)], which accounts for an overall two- and four-electron oxidation of the porphyrinogen skeleton.^[6]

The synthetic sequence, which provides porphomethene and porphodimethene on a large scale in their metalated or metal-free forms, is shown in Scheme 2. The tin(IV) derivative

variety of metals. For example, we prepared porphodimethene complexes with nickel(II), **9**, and zirconium(IV), **10**. In the case of **10**, the conformation of the ligand explains the *cis* arrangement of the chloride ions. Complexes **2–10** have all been fully characterized;^[7] the structures of all except compound **4** were also determined by X-ray crystallography. Some details are reported here for **7**.^[8]

The structure of **7** is shown in Figure 1 with the most relevant structural parameters. The porphodimethene skeleton is essentially planar with only a slight folding at the two

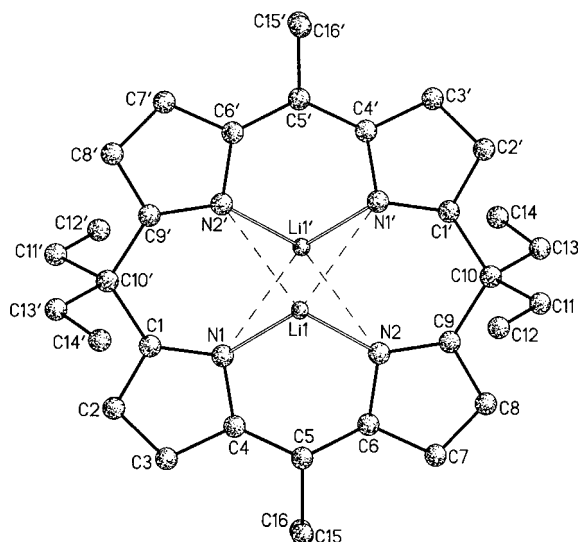
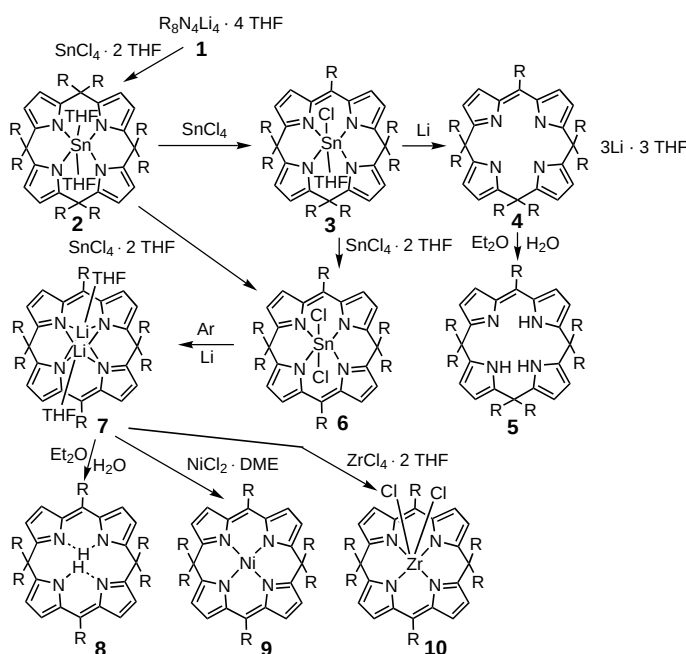


Figure 1. X-ray crystal structure of complex **7** (XP representation). Selected distances [Å]: Li1–N1 2.113(5), Li1–N2 2.156(5), Li1...N1' 2.523(5), Li1...N2' 2.465(5), Li1...Li1' 2.142(8). THF molecules are omitted for clarity. Primed atoms are related to the unprimed by the following symmetry transformation: $-x, -y, -z$.



Scheme 2. The tin-assisted dealkylation of the *meso*-octaethylporphyrinogen to give porphomethene **3** and porphodimethene **6**, and subsequent complexation with other metal ions. $R = Et$; DME = 1,2-dimethoxyethane.

2, synthesized following a conventional procedure, underwent a dealkylation in the presence of $SnCl_4$ (ca. 2 equiv) to yield the porphomethene(tin chloride) complex **3**, which was converted into the corresponding lithium derivative **4** and subsequently hydrolyzed to afford the free acid form **5**. Such a sequence provides access to large quantities of **4** and **5**, which are very useful for synthetic purposes. A large excess of $SnCl_4 \cdot 2 THF$ both with **3** or, better, with **2** led to the dealkylation of both *trans meso* carbon atoms with the formation of the porphodimethene(tin dichloride) complex **6**. The reductive demetalation of **6** with lithium metal was a very clean reaction leading to **7**, which can be hydrolyzed to the free acid form, **8**, or can serve for the complexation of a

saturated carbon atoms,^[3c-f,i,j] as evident from the dihedral angles $(N1-C1-C10')-(N2'-C9'-C10') = 12.5(4)$ and $(N1-C4-C5)-(C5-C6-N2) = 2.7(4)^\circ$. The two lithium cations lie on opposite faces of the N_4 average plane ($\pm 1.031(4)$ Å), and they bind two nitrogen atoms at close distances (Li1–N1 2.113(5), Li–N2 2.156(5) Å), whereas a weak interaction at longer distance occurs with the other two nitrogen atoms (Li1–N1' 2.523(5), Li1–N2' 2.465(5) Å). The conformation of the ligand is essentially imposed by the two lithium cations, which complete their coordination sphere with a THF molecule and are quite close together (Li1...Li1' 2.142(8) Å).^[9] The C–C and C–N distances support the proposed bonding scheme.

Some major features of the present report must be emphasized: 1) This synthetic methodology makes available important, but previously poorly accessible molecular skeletons on a large scale and starting from quite simple compounds. 2) The synthetic sequence (Scheme 2) illustrates the controlled two- and four-electron oxidation of porphyrinogen by means of the porphyrinogen→porphyrin transformation (**A**→**B**→**C** in Scheme 1). 3) The porphomethene and porphodimethene derivatives have never been obtained with unsubstituted pyrroles and as oxidatively stable *meso* fully alkylated forms.^[3]

Experimental Section

2: In portions, $\text{SnCl}_4 \cdot 2\text{THF}$ (28.5 g, 70.0 mmol) was added to a solution of **1** (56.0 g, 70.0 mmol) in toluene (700 mL). The reaction mixture was heated at reflux for about 12 h. The undissolved white solid, LiCl, was filtered off, and the resulting red-violet solution was concentrated to dryness. *n*-Hexane (300 mL) was added to the residue to give a pink powder, which was collected and dried in vacuo (yield: 40.0 g, 71 %). Crystals suitable for X-ray structure analysis were grown in a mixture of THF and *n*-hexane. ^1H NMR (200 MHz, C_6D_6 , 298 K): δ = 6.37 (s, 8H; $\text{C}_4\text{H}_2\text{N}$), 3.13 (m, 8H; THF), 1.96 (q, $J(\text{H,H})$ = 7.4 Hz, 16H; CH_2), 1.08 (m, 8H; THF), 0.90 (t, $J(\text{H,H})$ = 7.4 Hz, 24H; CH_3); elemental analysis calcd for **2** ($\text{C}_{44}\text{H}_{64}\text{N}_4\text{SnO}_2$) (%): C 66.08, H 8.07, N 7.01; found: C 66.12, H 8.51, N 7.04.

3: A solution of SnCl_4 (1.0 g, 3.8 mmol) in toluene (50 mL) was added dropwise at -40°C to a solution of **2** (12.2 g, 15.2 mmol) in toluene (200 mL). The reaction mixture was warmed to room temperature and stirred for about 12 h. To complete the reaction, this red solution was heated for 2 h at 80°C . It was concentrated to dryness, and *n*-hexane (70 mL) was added to give a red powder which was collected and dried in vacuo (yield: 9.3 g, 61 %). Crystals suitable for X-ray structure analysis were grown in toluene. ^1H NMR (200 MHz, C_6D_6 , 298 K): δ = 7.10 (d, $J(\text{H,H})$ = 4.4 Hz, 2H; $\text{C}_4\text{H}_2\text{N}$), 6.43 (d, $J(\text{H,H})$ = 4.0 Hz, 2H; $\text{C}_4\text{H}_2\text{N}$), 6.38 (d, $J(\text{H,H})$ = 4.4 Hz, 2H; $\text{C}_4\text{H}_2\text{N}$), 6.31 (d, $J(\text{H,H})$ = 4.0 Hz, 2H; $\text{C}_4\text{H}_2\text{N}$), 2.85 (m, 4H; THF), 2.53 (m, 4H; CH_2), 2.29 (m, 6H; CH_2), 1.89 (m, 4H; CH_2), 1.33 (t, $J(\text{H,H})$ = 7.3 Hz, 3H; CH_3), 1.10 (t, $J(\text{H,H})$ = 7.8 Hz, 3H; CH_3), 0.91–0.86 (m, 15H; CH_3 , overlapping with m, 4H; THF).

6 (one-pot reaction): $\text{SnCl}_4 \cdot 2\text{THF}$ (72.8 g, 179.0 mmol) was added to a solution of **2** (47.8 g, 60.0 mmol) in toluene (400 mL). The reaction mixture was heated at reflux for about 12 h, and after cooling a red crystalline solid precipitated. This product was collected, washed by extraction with Et_2O (500 mL), and then dried in vacuo (yield: 21.3 g, 53 %). Crystals suitable for X-ray structure analysis were grown in toluene. ^1H NMR (400 MHz, $\text{C}_5\text{D}_5\text{N}$, 298 K): δ = 7.73 (d, $J(\text{H,H})$ = 4.4 Hz, 4H; $\text{C}_4\text{H}_2\text{N}$), 6.83 (d, $J(\text{H,H})$ = 4.4 Hz, 4H; $\text{C}_4\text{H}_2\text{N}$), 2.98 (q, $J(\text{H,H})$ = 7.32 Hz, 8H; CH_2), 2.29 (q, $J(\text{H,H})$ = 7.32 Hz, 4H; CH_2), 1.25 (t, $J(\text{H,H})$ = 7.32 Hz, 6H; CH_3), 0.93 (t, $J(\text{H,H})$ = 7.32 Hz, 12H; CH_3); UV/Vis (THF): λ_{max} (ϵ) = 240 (34957), 304 (13026), 434 sh (33696), 454 nm (187122); elemental analysis calcd for **6** ($\text{C}_{32}\text{H}_{38}\text{N}_4\text{SnCl}_2$) (%): C 57.51, H 5.73, N 8.38; found: C 57.41, H 5.84, N 7.80.

7: Lithium metal (0.37 g, 52.8 mmol) and naphthalene (1.0 g, 7.8 mmol) were added under argon atmosphere to a solution of **6** (8.5 g, 13.2 mmol) in THF (200 mL). The reaction mixture was stirred for about 12 h, and the metallic residue was filtered off. The resulting orange solution was concentrated to dryness, and benzene (200 mL) was added to remove the undissolved LiCl. The solution was concentrated to dryness and the residue washed with *n*-pentane (100 mL). An orange powder was collected and dried in vacuo (yield: 4.2 g, 50 %). Crystals suitable for X-ray structure analysis were grown in a mixture of THF and *n*-hexane. ^1H NMR (200 MHz, $\text{C}_5\text{D}_5\text{N}$, 298 K): δ = 7.41 (d, $J(\text{H,H})$ = 3.9 Hz, 4H; $\text{C}_4\text{H}_2\text{N}$), 6.67 (d, $J(\text{H,H})$ = 3.9 Hz, 4H; $\text{C}_4\text{H}_2\text{N}$), 3.63 (m, 8H; THF); 3.14 (q, $J(\text{H,H})$ = 7.32 Hz, 4H; CH_2), 2.42 (q, $J(\text{H,H})$ = 7.32 Hz, 8H; CH_2); 1.59 (m, 8H; THF), 1.44 (t, $J(\text{H,H})$ = 7.32 Hz, 6H; CH_3), 1.05 (t, $J(\text{H,H})$ = 7.32 Hz, 12H; CH_3); UV/Vis (THF): λ_{max} (ϵ) = 296 (6147), 426 (43608), 452 (125892), 506 nm (8980); elemental analysis calcd for **7** ($\text{C}_{40}\text{H}_{54}\text{N}_4\text{Li}_2\text{O}_2$) (%): C 75.45, H 8.55, N 8.76; found: C 74.8, H 8.48, N 8.60.

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[8] Crystal data for **7** ($\text{C}_{40}\text{H}_{54}\text{Li}_2\text{N}_4\text{O}_2$): M_r = 636.75, monoclinic, space group $P2_1/n$, a = 9.8247(11), b = 10.9196(12), c = 16.940(2) Å, β = 90.346(10)°, V = 1817.3(4) Å³, Z = 2, ρ_{calcd} = 1.164 g cm^{−3}, $F(000)$ = 688, $\text{MoK}\alpha$ radiation (λ = 0.71073 Å), $\mu(\text{MoK}\alpha)$ = 0.71 cm^{−1}; crystal dimensions 0.25 × 0.20 × 0.18. Diffraction data were collected on a KUMA CCD at 143 K. For 2616 observed reflections [$I > 2\sigma(I)$] the conventional R was 0.0658 ($wR2$ = 0.1974 for 3418 independent reflections). 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-116587. 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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Novel and Efficient Isomerization of Allylic Alcohols Promoted by a Tetrapropylammonium Perruthenate Catalyst**

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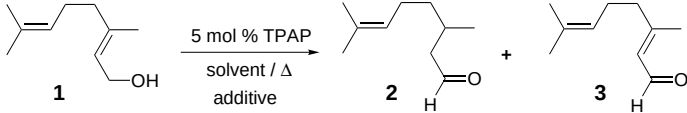
Dedicated to Professor Barry M. Trost

The oxidation of alcohols to aldehydes and ketones is a ubiquitous functional group transformation in organic chemistry.^[1] Although stoichiometric amounts of generally noxious reagents are typically employed to effect this conversion, a number of efficient and chemoselective catalytic oxidations of alcohols is slowly emerging.^[2] From economic and environmental viewpoints, catalytic processes employing oxygen or H₂O₂ as the ultimate stoichiometric oxidant are the most interesting.^[3] In this context, we have recently reported the results of our studies on the ecologically friendly oxidation of alcohols catalyzed either by a copper(I) phenanthroline complex^[4] or by tetrapropylammonium perruthenate (TPAP).^[5, 6]

During the course of the optimization of the TPAP-catalyzed aerobic oxidation of geraniol (**1**) into geranial (**3**), we sometimes observed the formation of a minor impurity (5–7%) which was identified as citronellal (**2**). Since no citronellol could be detected in the starting material, we concluded that under the aerobic oxidation conditions **1** was somehow transformed into **2**. This intriguing observation prompted us to investigate in greater detail the origin of **2**. Some of our results are collected in Table 1.

Whilst presaturation of the reaction mixture with O₂ (or air) before addition of the ruthenium catalyst completely suppressed the formation of **2** (entry 1), careful degassing led to significantly increased yields of **2** (entry 2). It was also found that running the reaction in the absence of molecular sieves (MS, 4 Å) resulted in improved conversion of **1** into **2**, but did not alter the ratio of **2** versus **3** (entry 3). Furthermore,

Table 1. TPAP-catalyzed isomerization of geraniol.



Entry	Solvent	4-Å MS	Additive	Conversion [%] ^[a]	Ratio 2:3 ^[b]
1	CH ₃ C ₆ H ₅	yes	O ₂	100	0:100 ^[c]
2	CH ₃ C ₆ H ₅	yes	–	62	47:53 ^[d]
3	CH ₃ C ₆ H ₅	no	–	100	42:58 ^[e]
4	FC ₆ H ₅	no	–	100	72:28 ^[e]
5	FC ₆ H ₅	no	<i>i</i> PrOH	72	56:44 ^[e]
6	FC ₆ H ₅	no	2-BuOH	70	73:27 ^[e]
7	FC ₆ H ₅	no	C ₁₀ H ₂₁ OH	100	100:0 ^[e]
8	FC ₆ H ₅	no	2-undecanol	100	100:0 ^[e]

[a] The conversions were monitored by ¹H NMR spectroscopy. [b] The ratios of **2:3** were measured by ¹H NMR spectroscopy. [c] The reaction mixture was presaturated with O₂ before addition of TPAP. [d] Careful degassing was performed before addition of TPAP. [e] The reaction was effected under argon. Careful degassing is not necessary.

fluorobenzene proved to be a better solvent than toluene, affording for the first time **2** as the major product (entry 4). To suppress the competitive oxidation of **1** to **3**, we investigated the effect of sacrificial alcohol additives. We were gratified to find that, whilst low molecular weight alcohols led to incomplete transformations and mixtures of **2** and **3** (entries 5 and 6), the addition of a stoichiometric amount of either 1-decanol or 2-undecanol resulted in the quantitative and exclusive formation of **2** (entries 7 and 8).

This novel allylic isomerization protocol was then applied to a range of differently substituted allylic alcohols. For practical reasons, 2-undecanol was selected as the preferred sacrificial alcohol. Some pertinent results are displayed in Table 2.

As can be seen from Table 2, monosubstituted (entries 1–3), *cis*-disubstituted (entry 4), *trans*-disubstituted (entries 5–7), and trisubstituted allylic alcohols (entry 8) are all smoothly and quantitatively converted into the corresponding saturated aldehydes and ketones. The reaction is also successful if the C=C bond is enclosed within a ring system (entry 4) or is conjugated with an aromatic substituent (entries 6 and 7).

Although numerous catalysts are known to perform allylic transpositions,^[7] they are all restricted to certain substitution patterns in the alkene substrates.^[8] To the best of our knowledge, the TPAP/2-undecanol system is the first ruthenium catalyst that tolerates such a broad range of allylic alcohols.

A plausible mechanism that accounts for our current observations is shown in Scheme 1. We believe that the initial steps of the catalytic cycle involve the sequential reduction of the Ru^{VII} complex to a Ru^{III} species. This reduction is accompanied by the concomitant and chemoselective oxidation of two equivalents (per ruthenium) of 2-undecanol (**4**) into the corresponding ketone **5**.^[9] The allylic alcohol **6** then interacts with the Ru^{III} catalyst, generating the ruthenium alkoxide **7**.^[10] Subsequent β-hydride elimination affords the ketone–ruthenium species **8**, which undergoes a [1,4]-hydride addition to form the ruthenium enolate **9**.^[11] At this stage,

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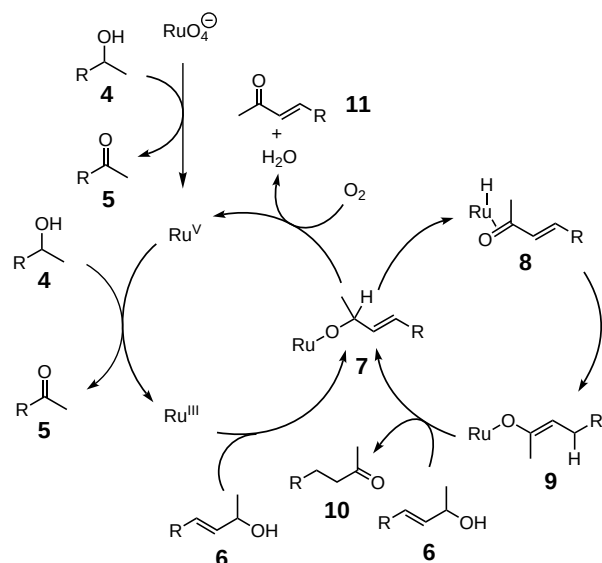
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Table 2. TPAP-catalyzed isomerization of allylic alcohols.

Entry	Substrate	Product	Yield [%] ^[a]
1			90
2			92
3			87
4			89
5			41
6			71
7			48
8			52

[a] All yields refer to pure, isolated products. Conversions were quantitative in all cases. The discrepancy between the yields of the isolated products and the conversions reflects the mechanical losses in isolating some of the products.



Scheme 1. Proposed mechanism for the isomerization of allylic alcohols. R = (CH₂)₈CH₃.

ligand exchange between enolate **9** and allylic alcohol **6** takes place, releasing ketone **10**^[12] and regenerating the ruthenium alkoxide **7**, which reenters a new catalytic cycle. The key roles played by the saturated alcohol additive can now be fully appreciated. Not only does **4** generate the active ruthenium catalyst by double reduction of TPAP, but its faster rate of oxidation suppresses the unwanted oxidation of the allylic

alcohol substrate. Unless the isomerization is conducted in a glove box, unavoidable traces of oxygen always permeate the reaction vessel and rapidly oxidize the Ru^{III} alkoxide **7** into the Ru^V species with concomitant formation of H₂O and enone **11**. The saturated alcohol **4** then performs another important task. By immediately reducing the ruthenium(v) oxidant into the active Ru^{III} species, **4** restores the allylic isomerization cycle. This mechanistic scheme also unites our previously reported TPAP-catalyzed aerobic oxidation of alcohols with the presently observed allylic shift.^[9] Indeed, whilst the isomerization involves a Ru^{III} species, the aerobic oxidation of alcohols takes place through the Ru^{III}/Ru^V manifold through rapid reoxidation of the low-valent ruthenium alkoxide catalyst **7** by oxygen.

In summary, we have shown for the first time that TPAP, an excellent oxidant, is transformed in situ into a low-valent species which is able to catalyze efficiently the transformation of allylic alcohols into the corresponding saturated carbonyl derivatives. Further work is now directed towards delineating the scope of this reaction and understanding the intimate mechanistic details of this unique catalytic system.

Experimental Section

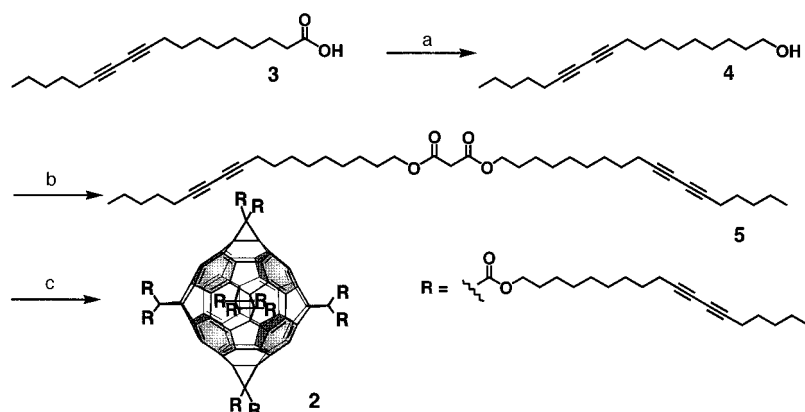
Typical procedure for the isomerization of 1-phenylprop-2-enol to propiophenone (Table 2, entry 1): A solution of 1-phenylprop-2-enol (214 mg, 1.6 mmol) and 2-undecanol (252 mg, 1.6 mmol) in fluorobenzene (20 mL) was degassed for 3 min by sonication in an ultrasound bath under a gentle stream of argon. Solid TPAP (34 mg, 0.096 mmols) was then added, and the reaction mixture was heated at reflux under an argon atmosphere. The progress of the reaction was monitored by thin layer chromatography and/or gas chromatography. Upon completion of the isomerization, the reaction mixture was cooled to room temperature and filtered through a celite pad. After the pad was washed with chloroform (30 mL), the combined organic solvents were removed under reduced pressure and the product was purified by column chromatography (silica gel, ethyl acetate:hexane, 1:9). Analytically pure propiophenone was obtained as a colorless liquid (193 mg, 90 %).

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Scheme 1. Synthesis of the polymerizable lipofullerene hexakisadduct **2**. a) LiAlH_4 , diethyl ether; b) $\text{ClCOCH}_2\text{COCl}$ /pyridine, CH_2Cl_2 ; c) C_{60} , DMA, CBr_4 , DBU, toluene.

size **2** (Scheme 1), 10,12-octadecadiynoic acid (**3**) was reduced and the corresponding alcohol **4** formed. The latter was then treated with malonyl dichloride to give dioctadecadiynyl malonate (**5**). The T_h symmetrical hexakisadduct **2** was obtained from ester **5** by template activation^[9] with dimethylantracene (DMA) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and subsequent exhaustive cyclopropanation^[10, 11] of **2** (Scheme 1), and characterized (Table 1).

To investigate the photochemical networkability of **2** a film coating of the neat monomer on a glass plate was irradiated (300 W lamp) for two days. While the yellow monomer **2** melts at 42°C , the shiny red-brownish and insoluble photolysis product **6** did

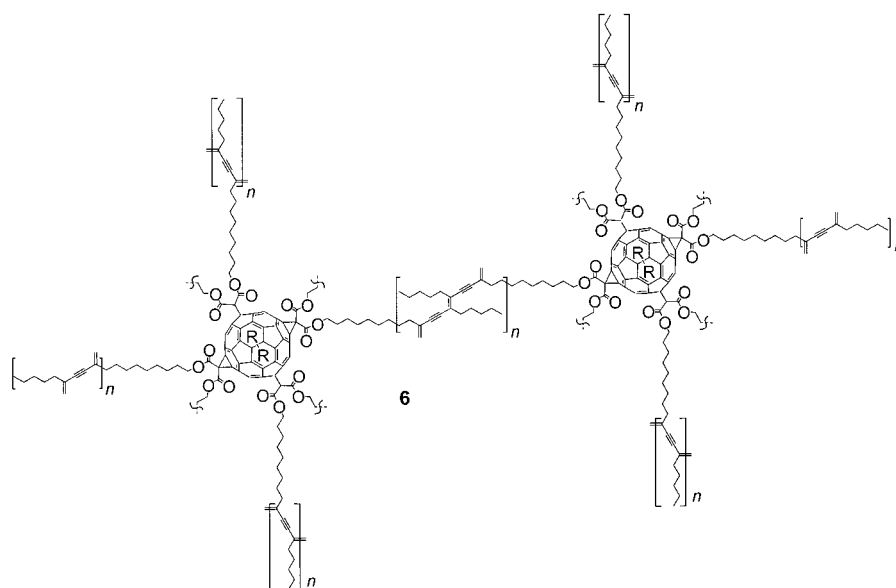


Table 1. ^1H NMR, ^{13}C NMR, IR, UV, and MS data of **2**.

^1H NMR (400 MHz, CDCl_3): δ = 0.90 (t, 36H, J = 7 Hz, CH_3), 1.35–1.55 (m, 216H, CH_2), 1.68 (tt, 24H, $J_1 = J_2$ = 7 Hz, CH_2), 2.24 (t, 48H, J = 7 Hz, $\text{CH}_2\text{C}\equiv\text{C}$), 4.24 (t, 24H, J = 7 Hz, CH_2O); ^{13}C NMR (100.5 MHz, CDCl_3): δ = 13.84 (CH_3), 19.09, 19.13, 22.09, 25.74, 27.98, 28.31, 28.36, 28.78, 28.99, 29.13, 29.31, 30.92 (CH_2), 45.33 (methanobridge), 65.23, 65.26 (sp-C), 66.92 (CH_2O), 69.06 (C_{60} sp³-C), 77.43, 77.48, (sp C), 141.15, 145.81 (C_{60} sp²-C), 163.92 (C=O); IR (KBr): $\tilde{\nu}$ = 2930.8, 2856.4, 2256.1, 2155.5, 1746.3, 1465.1, 1264.4, 1215.8, 1080.2, 715.7, 529.3 cm^{-1} ; UV (CH_2Cl_2): λ_{max} = 270.5, 280.5, 318.0, 336.0, 383.0 nm; MS (α -cyano-*p*-hydroxycinnamic acid (CCA), MALDI): m/z : 4267 [M^+]

not exhibit any phase transition up to 280°C . The ^{13}C solid-state NMR analysis (MAS) as well as the IR spectra of **6** revealed newly formed endiynes units (δ = 90–140; $\tilde{\nu}$ = 2230 ($\text{C}\equiv\text{C}$), 1600 cm^{-1} ($\text{C}=\text{C}$)) as well as intact butadiyne functions (δ = 65, 77). This means, that for steric reasons only two, or very few, monomeric elements might cross-link together by formation of short endiynes units.

To achieve a matrix controlled polymerization **2** was intercalated into multilamellar phospholipid vesicles. Subsequent deuterium solid-state NMR (^2H NMR) and freeze

fracture electron microscopy analyses revealed that **2** exhibited the same tendency for self aggregation as was observed with the analogous saturated and nonpolymerizable, lipofullerenes.^[1, 2] An aqueous dispersion of multilamellar DPPC vesicles containing 15 mol % of **2** was subjected to UV irradiation (8 W, 256 nm) for 12 h at 50°C (fluid lipid phase^[1, 2]) in a quartz-glass cuvette. In control experiments with neat DPPC–MLVs no indication of thermal degradation or irradiation damage of the DPPC was observed by differential scanning calorimetry (DSC) under the experimental conditions applied. In contrast the polymerizable lipofullerene–DPPC system showed significant changes

after the UV treatment with respect to the dynamic properties of the lipids. Figure 1 shows the deuterium NMR spectra of the lipofullerene–DPPC system (chain perdeuterated DPPC) prior and after polymerization under fluid bilayer conditions. The change of the signal line shape is explained by an

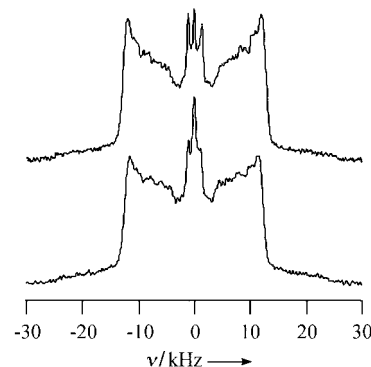


Figure 1. ^2H NMR spectra of perdeuterated multilamellar DPPC vesicles [D_{62}]DPPC containing 15 mol % lipofullerene **2** prior (top) and after UV irradiation (bottom) at 50°C .

enhancement of the membrane bending modulus.^[1] Furthermore, the polymerization is manifested in a significant reduction of the ^2H NMR transverse relaxation time t_2 of the perdeuterated DPPC chains by more than 10%. This reduction in t_2 of the lipid chains can be explained by the presence of rigid structures in their immediate vicinity. Relaxation control experiments revealed that the polymerization was finished after an irradiation period of 12 h and that analogously treated neat DPPC–MLVs did not exhibit any reduction in t_2 .

An investigation of the fluid phase samples by freeze fracture electron microscopy showed that the rodlike structures at the MLV surface observed prior to polymerization (Figure 2a) had vanished. Instead, perfectly spherical objects made up from polymerized lipofullerenes with diameters ranging from 100 nm up to several μm were obtained (Figure 2b). The smaller spheres with diameters of less than 150 nm were hollow and transparent, and the replica background below them was visible through the objects. The larger beads were filled and their centers impenetrable to the electron beam. The DPPC was removed after polymerization by extraction with organic solvent. As a consequence vacuum dried nanospheres were accessible to inspection by transmission electron microscopy (TEM) in the absence of the freeze fracture replica (Figure 2c). Atomic force microscopy (AFM) analysis of single C_{60} balls sticking out from the bead surface revealed a medium C_{60} – C_{60} distance of 16 nm (Figure 3). An average surface coarseness of 0.9 nm with maximum height amplitudes of 1.5 nm was determined after a computational smoothing of the surfaces. The filled beads seem to exhibit an unusually high strength and did not reveal any mechanical damage in spite of the considerable forces they were subjected to (one to two orders of magnitudes greater than the 1 nN chosen for the AFM in Figure 3). The fact that no edge darkening was observed in the transmission electron micrographs of the transparent small hollow spheres indicates that the wall thickness of the beads is below the resolution limit of the microscope used (namely, below 10 nm).

The polymerization presented here can be seen as a special kind of emulsion polymerization, where the monomer in an aqueous solution is polymerized in the presence of a detergent.^[12] From the fact that the formation of round and three-dimensional spheres is observed from the fluid MLV phase, where only a quasi-two-dimensional lateral mobility of the monomeric elements should be allowed, it can be concluded that the vesicles serve an essential template function during polymerization.

Interestingly, **2** can also be polymerized to spheric polymers in the absence of DPPC, however, only in very low yields and in addition to amorphous material. Originally performed as a control experiment, a solution of 5 mg mL^{-1} of **2** in decane was irradiated (80 W, 256 nm) at room temperature. The formation of a shiny brownish precipitate at the vessel walls and additionally a colloidal suspension of small spherical objects within the solution was observed after only 30 min. In this case, however, no hollow spheres were found. To our knowledge, this is the first case reported for an isotropic polymerization, and is likely made possible by the shape and the hyperfunctionality of the highly symmetrical monomer **2**.

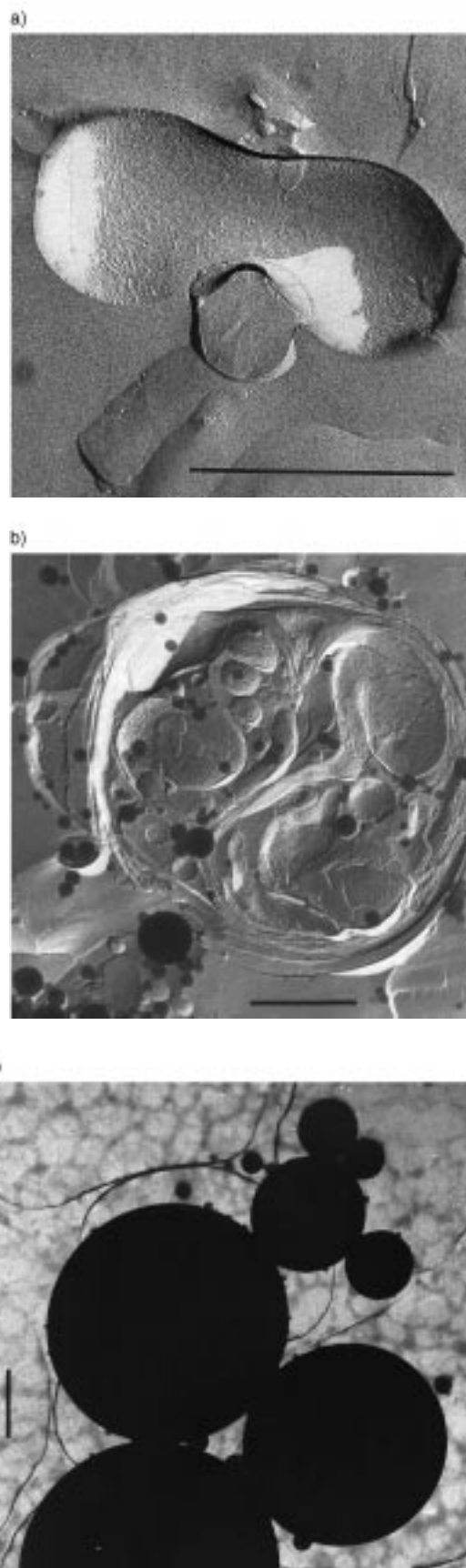


Figure 2. TEM pictures of 15 mol % lipofullerenes **2** in DPPC multi-lamellar vesicles a) prior and b) after UV-initiated polymerization. c) Lipofullerene polymerspheres after extraction of DPPC and subsequent vacuum drying. The bar corresponds to 1 μm .

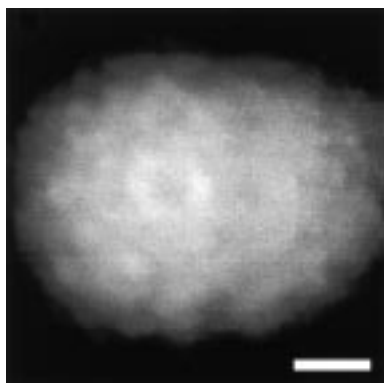


Figure 3. AFM picture of a lipofullerene polymersphere after extraction of the DPPC. The bar corresponds to 50 nm.

Only a small part of the twelve groups available for networking is used up for polymerization because of steric reasons. The remaining unreacted endiynes functions at the beads surface cause a dendritic isotropic polymer growth to an isotropic 3D network by diffusion control of the monomers in solution. Thus, it acts like a “living polymer” filling the space available. The polymerization in solution can to some extent be compared with that in the MLVs. In both cases a medium is available for the diffusion control: the solvent decane or the fluid lipid bilayer. The reason for the formation of the small hollow spheres upon vesicle polymerization can be found in the stability of small vesicles. Only lateral diffusion is allowed as a consequence of their shape and small volumes, and hence an anisotropic and quasi-two-dimensional polymerization to hollow spheres is enabled. With the same arguments, the formation of concentrically filled “onionlike” balls could be expected from the bigger multilamellar vesicles. However, it seems more reasonable that the single lamellae do not withstand the polymerization pressure and a rupture of the membrane might occur. Diffusion of the monomers is now enabled from outside (and inside) of the membrane layer and results in the formation of compactly filled three-dimensional polymer spheres.

Experimental Section

Synthesis of lipofullerene monomeric element **2**: 10,12-Octadecadiyn-1-ol (**4**) is obtained quantitatively from LiAlH_4 reduction of commercially available 10,12-octadecadiynoic acid (**3**) in diethyl ether. The subsequent acylation with malonyl dichloride/pyridine in CH_2Cl_2 gives rise to the formation of di-10,12-octadecadiynyl malonate (**5**, yield 63%). The following template-assisted cyclopropanation^[9–11] of C_{60} (108 mg, 0.15 mmol), DMA (310 mg, 1.50 mmol), **4** (894 mg, 1.50 mmol), CBr_4 (497 mg, 1.50 mmol), and DBU (0.448 mL, 3 mmol) in 70 mL toluene gave, after column (SiO_2 ; hexane/ CH_2Cl_2 1/1) and preparative HPLC chromatography (grom-Sil 100Si, NPI, 5 μL , 20 mL min^{-1} CH_2Cl_2 /heptane 7/3), 1,2:18,36:22,23:27,45:31,32:55,56-hexakis[[di(10,12-octadecadiynyl-oxycarbonyl)]-methano]-1,2,18,36,22,23,27,45,31,32,55,56-dodecahydro-[60]fullerene **2** (166 mg, 26%).

The preparation of the MLV samples for freeze fracture electron microscopy as well as the TEM studies were carried out according to the technique described in reference [1]. Triafol hole foils were used after removal of the lipids and directly inserted into the electron beam for the TEM recordings of the polymer spheres. The dried polymer spheres were deposited in the holes of the foils.

The AFM measurements (Nanoscope IIIa, Digital Instruments, Mannheim, Germany) were carried out in the contact mode on mica plates in

1-propanol at 15 °C after removal of the lipids with CH_2Cl_2 . Recording parameters: constant force (<1 nN), silicon nitride cantilever (30 mN m^{-1}), 6.8 lines per sec, 90° scanning angle.

The ^2H NMR experiments were carried out as described in reference [1]. For the determination of the transverse relaxation time t_2 the pulse distance in the quadrupole echo sequence was varied between 30 and 120 μs , a half logarithmical recording of the echo intensity over the double pulse distance gave $(t_2)^{-1}$ as the slope of a straight line.

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Surface Modification of Porous Silicon by Electrochemical Reduction of Organo Halides**

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Porous silicon is a nano- to macroporous high surface area material that can be fabricated from n- and p-type silicon by chemical or electrochemical etching processes involving hydrofluoric acid. Originally it was studied for its application as an insulating material in microelectronics.^[1] Later on it was discovered that many forms of porous silicon show intense photoluminescence under UV excitation,^[2] which opened the possibility for applications in optoelectronics, display technologies,^[3] and chemical sensing.^[4] Most recently, its passive optical properties have been exploited for antireflective coatings,^[5] wave guides,^[6] interference filters,^[7] and biosensors.^[8]

Pristine porous silicon has a hydride-terminated surface that is prone to oxidation and corrosion. In recent years there has been much progress in the surface modification of porous silicon and hydride-terminated crystalline silicon with organic moieties. Covalently attached (Si-C) stable organic monolayers have been formed by cleavage of Si-Si bonds with Grignard^[9] and alkyllithium reagents.^[10] Similar results were obtained by a series of thermal,^[11] photochemical,^[12] and Lewis acid catalyzed^[13] hydrosilylation reactions. Electrochemical oxidation of methyl Grignard reagents^[9b] on porous silicon and electrochemical reduction of phenyldiazonium salts^[14] on single-crystal silicon have been shown to yield dense monolayers of methyl and phenyl groups, respectively. In many cases the organic functionalization dramatically improves the chemical stability of porous silicon, which has spurred renewed interest in this material for sensor and display applications.

Herein we report on a fast and highly efficient electrochemical method for surface functionalization of n- and p-type porous silicon based on reductive electrolysis of alkyl iodide, alkyl bromide, and benzyl bromide species. This new method provides high surface coverage, while requiring only very short (<2 min) reaction times. Widely available mono- and bifunctional organo halide reagents with low to moderate reactivity make this method convenient to use and generally applicable.

The reductions are performed in 0.2 M to 0.4 M solutions of the organo halides in dry, deoxygenated acetonitrile or mixtures of acetonitrile and tetrahydrofuran containing 0.2 M LiBF₄. Alkyl bromides, which do not react as well as alkyl iodides or benzyl bromides, can be converted into the iodides

in situ by replacing LiBF₄ with LiI as the electrolyte. Passing a cathodic current (2–10 mA cm⁻²) for short periods of time (30–120 s) leads to high coverage of the porous silicon surface with organic species, as determined by FT-IR (Figure 1). The

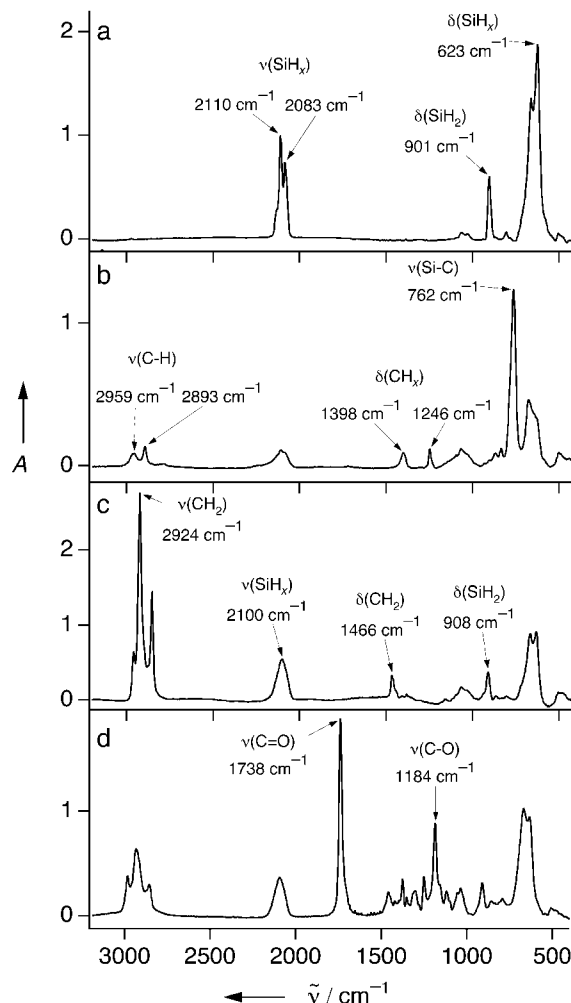


Figure 1. Transmission FT-IR spectra of p-type porous silicon a) before and after derivatization with: b) methyl iodide, c) dodecyl iodide, and d) ethyl 6-bromohexanoate. Absorbance intensities *A* are normalized to the $\tilde{\nu}(\text{SiH})$ band at 2116 cm⁻¹ measured before derivatization. Thin film optical interference effects that contribute to a sinusoidal baseline have been subtracted mathematically. Reaction conditions were: b) 0.4 M CH₃I, 0.2 M LiBF₄, acetonitrile, 2 min; c) 0.4 M C₁₂H₂₅I, 0.2 M LiBF₄, 1:1 acetonitrile/THF, 30 s; d) 0.25 M C₈H₁₅O₂Br, 0.2 M LiI, acetonitrile, 45 s. Cathodic current density was 10 mA cm⁻² for all samples.

alkyl layers formed are stable in organic solvents and ethanolic HF, which indicates that the grafting proceeds through formation of covalent Si-C bonds.^[10, 13] In the case of methyl iodide this is further established by the appearance of the Si-C stretching mode in the infrared spectrum at 766 cm⁻¹.^[9b] This mode is typically not observed for alkanes with longer chain lengths.^[15]

The integrated intensity of the Si-H stretching vibrations located at approximately 2120 cm⁻¹ decreases substantially with increasing alkyl coverage (Figure 2). This suggests that the reduction produces alkyl or benzyl radical species that

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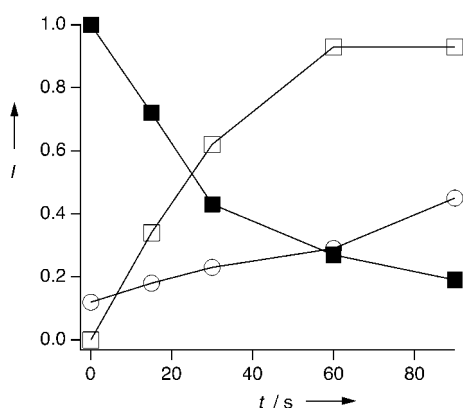
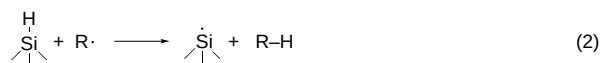
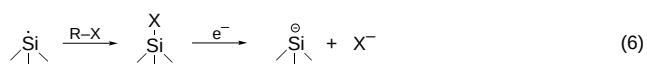
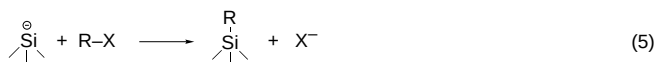


Figure 2. Plot of the integrated absorbance intensities I of the $\tilde{\nu}(\text{Si-H}_x)$ (■, 2300–2000 cm^{-1}), $\tilde{\nu}(\text{Si-C})$ (□, 820–720 cm^{-1}), and $\tilde{\nu}(\text{Si-O})$ (○, 1200–950 cm^{-1}) vibrations as a function of methyl iodide reduction time (the same conditions as in Figure 1 were used). The intensity of the $\tilde{\nu}(\text{Si-C})$ absorbance becomes constant after 60 s, which indicates that the functionalization reaction is complete within this time. The $\tilde{\nu}(\text{Si-H}_x)$ absorption intensity continues to decrease as the $\tilde{\nu}(\text{Si-O})$ absorption continues to increase during the course of the reaction.

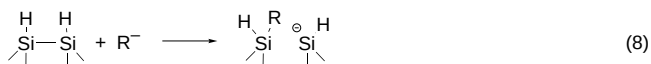
then react with the hydride surface as outlined in Equations (1) and (2).



The derivatization step may occur either by direct reaction of the surface silicon radical with an alkyl- or benzyl radical [Eq. (3)], or by reduction of the silicon radical to the anion followed by nucleophilic attack on the organo halide [Eqs. (4) and (5)]. Solution-based silicon radicals are known to abstract halogen atoms from organo halides, to produce Si-X and the corresponding organic radical.^[16] If this reaction occurs on the porous Si surface it is expected that under the conditions of the electrochemical experiment the Si-X species would be reduced to a silicon anion [Eq. (6)].

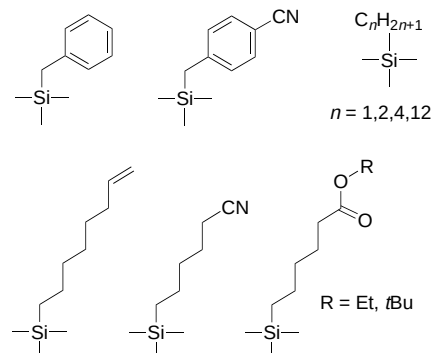


It is also plausible that a minor fraction of the carbon radicals are further reduced to carbanions, which then react with the porous silicon surface by cleavage of Si-Si bonds (or Si-X bonds), similar to Grignard or alkyl lithium reagents ([Eqs. (7) and (8)] followed by equation (5)).^[9a, 9c, 10]



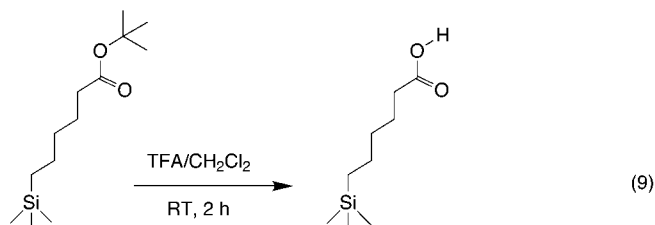
The grafting process is complete within 60–90 seconds (at a cathodic current density of 10 mA cm^{-2}) for all the organo halides studied. Longer reduction times do not lead to increased coverage, but they do lead to a higher degree of oxidation as reflected by the growth of a $\tilde{\nu}(\text{SiO})$ vibrational band at 1100–1050 cm^{-1} (Figure 2).

Even though the reduction of organo halide species requires strongly negative potentials in the range of –1.2 V to –1.8 V (versus the saturated calomel electrode (SCE)), the electrochemical procedure described here is compatible with a surprising number of functional groups (Scheme 1). Particularly useful for further derivatizations are *tert*-butyl esters.



Scheme 1. Functional groups that have been grafted onto porous silicon by electrochemical reduction of organo halides.

The ester functionalized surfaces were converted into the free acids at room temperature by addition of a mixture of trifluoroacetic acid (TFA) and dichloromethane [Eq. (9)]. Interestingly, we were unable to graft phenyl groups onto the surface of porous silicon. Reduction of phenyl iodide causes extensive oxidation and leads to very little grafting.



The photoluminescence (PL) of porous silicon samples, which is readily observable before derivatization, is eliminated entirely upon reductive derivatization with all of the alkyl iodides studied. This is presumably a consequence of residual iodo species (as detected by energy dispersive X-ray analysis, EDX), which can effectively quench the photoluminescence of porous silicon.^[17] Attempts to remove these species or to recover the PL were unsuccessful. Derivatizations with alkyl bromides and benzyl bromide lead to only partial loss of the PL (with LiBF_4 as electrolyte). Approximately 30% of the initial PL is retained after functionalization with the bromide reagents; subsequent rinsing of the samples with ethanolic HF results in restoration of the PL to 70–90% of the original intensity.

The surface derivatization method described here is not limited to porous silicon, but is also applicable to hydride-terminated crystalline silicon. Reduction of butyl iodide on n-type crystalline silicon leads to an organic layer that is stable in boiling chloroform, aqueous ethanolic HF and 0.1M KOH as demonstrated by the difference between contact angles measured on derivatized and nonderivatized surfaces, respectively.

Experimental Section

All reagents were obtained from commercial sources and used as received. *tert*-butyl 6-bromo-hexanoate was prepared from 6-bromohexanoic acid and *tert*-butanol according to the literature.^[18] Acetonitrile and THF were dried and distilled prior to use. Solutions of organo halides containing electrolyte were freeze-pump-thaw degassed three times to remove traces of oxygen and stored under nitrogen.

Porous silicon samples were prepared from n-type (P-doped) and p-type (B-doped) silicon wafers with a (100) orientation and with a resistivity of approximately 1 Ω cm. Porous silicon samples with areas of 0.25 cm² were etched in 48% aqueous HF/EtOH (1/1) for 2 min at a current density of 40 mA cm⁻². Both n- and p-type wafers were illuminated during the etching procedure^[19] with a 300-W tungsten lamp adjusted to an intensity of approximately 100 mW cm⁻² to produce luminescent samples. The porous layers were approximately 3–5 μ m thick (determined by scanning electron microscopy). After the etching process the samples were rinsed with ethanol and dichloromethane, dried under a stream of nitrogen, and evacuated in a one- or two-compartment electrochemical cell equipped with a platinum auxiliary electrode and in some cases a Ag/AgBr pseudo-reference electrode. Crystalline silicon samples (n-type) were degassed in boiling chloroform. The native silicon oxide layer was removed immediately before the derivatization step by soaking in 2% aqueous HF.

Derivatization was performed by passing a cathodic current (usually 10 mA cm⁻²) through 1–2 mL of the organo halide solution that was previously added to the porous silicon sample. The p-type porous silicon samples were illuminated with 200 mW cm⁻² white light to supply sufficient photocurrent. After derivatization the solution was removed and the sample rinsed sequentially with glacial acetic acid, ethanol, and dichloromethane.

Derivatization of single-crystal silicon samples was monitored indirectly by comparing contact angles (water droplets) measured on derivatized and nonderivatized areas, respectively. Prior to the measurement the entire sample was immersed in boiling chloroform (15 min) and in 48% aqueous 1/1 HF/EtOH (2 min) to remove any adsorbed or Si–O bonded species, and then dipped in 0.1M aqueous KOH to render the nonderivatized, silicon hydride terminated areas hydrophilic.

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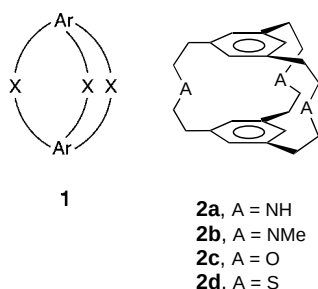
S-Cylindrophanes: From Metal Tweezers to Metal Sandwiches**

Mark Mascal,* Jean-Luc Kerdelhué,
Alexander J. Blake, and Paul A. Cooke

Over the years effort has been directed at attempts to produce cyclophane-based macrobicycles **1** for the purpose of metal ion encapsulation.^[1] In cases where the metal–arene core structure is intrinsically stable, such as in the ferrocenophanes, this has been a straightforward matter of building the cage around the metal. Where weaker interactions would be involved, however, the receptor must be put together first and the metal introduced later. This is the case when the aromatic system is benzene. Although the basic proposition is a simple one—that is, to create a cavity between two benzene rings and involve the π systems in the binding of a metal—its realization

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[**] The use of the EPSRC's Chemical Database Service at Daresbury is acknowledged, and Dr. Richard Wheatley is thanked for helpful discussions.



demands some respect for the conformational preferences of chains which link aromatic rings together.^[2] If the chains are to incorporate heteroatoms for the purpose of coordination to the guest, the first cyclophane which comes into consideration is the *sym*-[5.5.5] system **2**. This is

because with anything less than five atoms in the bridges, no reasonable conformation exists in which both the rings and the chains can participate in metal ion complexation.^[3] Cyclophanes with even numbers of atoms in the bridges are known to be conformationally mobile,^[4] making the [6.6.6] homologue unattractive, and in the [7.7.7] the rings are too far from the midpoint of the cavity for significant interaction with a metal. In [*n.n.n*] systems where *n* is odd, it is a further condition that ligating functionalities be located at odd-numbered positions in the chains (except for the 1- or *n*-position), which converge towards the center of the cavity, unlike those in the even-numbered positions, which are divergent.

With these principles in mind, we recently synthesized a series of aza- and oxacyclophanes **2a–c**.^[5, 6] Structural predictions based on the above conformational arguments could be substantiated by X-ray crystal structure determinations, which revealed the cylindrical nature of these molecules. These “cylindrophanes” functioned as metal tweezers, clasp copper(i) and silver(i) ions between the two aromatic rings as shown in Figure 1 a. However, no inclusion in the

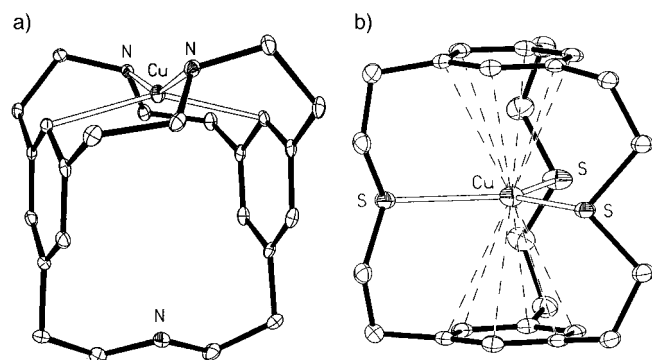
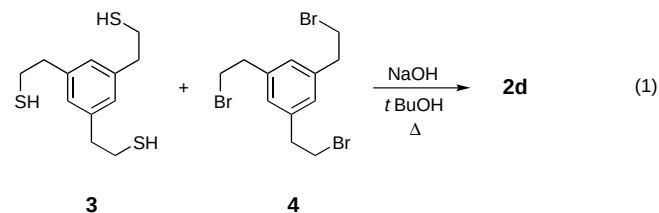


Figure 1. a) A view of the complex cation in the crystal structure of [Cu(**2a**)]BF₄. b) A view of the complex cation in the crystal structure of [Cu(**2d**)]BF₄ · 0.5 CH₃OH.

cavity was possible in these cases due to the prohibitive distance from the heteroatoms to the center of the trigonal plane which they define (≥ 2.5 Å). We now describe the mixed π/η -donor macrobicyclic **2d**, which is capable of trapping Cu^I and Ag^I guests in a trigonal-planar ligand field in the heart of the receptor, sandwiched by the two aromatic rings.

The synthetic approach taken to **2d** was simpler than that required for the preparation of **2a–c**, in which the cage was assembled by a [2+2+2] cyclotrimerization strategy. A hexaoxo analogue of **2d** had been made previously by

reaction of 1,3,5-tris(bromoacetyl)benzene and sodium sulfide in 0.2 % yield.^[7] A modified approach employing the known trithiol **3**^[8] and tribromide **4**,^[9] however, gave the target compound **2d** in 66 % yield [Eq. (1)].



Simple combination of **2d** with AgBF₄ in CH₂Cl₂ or [Cu(MeCN)₄]BF₄ in CH₂Cl₂/MeCN (3/1) gave the stable 1:1 complexes [Ag(**2d**)]BF₄ and [Cu(**2d**)]BF₄. Their ¹H NMR spectra (CDCl₃) showed a substantial interaction between the metals and **2d**, as indicated by the shifts of the SCH₂ signals ($\Delta\delta = +0.6$ – 0.7) relative to those of the free ligand. Most interesting, however, was the marked downfield shift of the aromatic protons ($\Delta\delta = 0.20$ in [Ag(**2d**)]BF₄, 0.12 in [Cu(**2d**)]BF₄), comparable to the deshielding experienced by the aromatic proton in the bis(η^1) complex [Ag(**2a**)]OTf ($\Delta\delta = 0.13$ versus **2a**; Tf = F₃CSO₂).^[6]

Crystals of [Cu(**2d**)]BF₄ could be grown by slow evaporation of a solution in dichloromethane. The X-ray crystal structure^[10a] shows copper occupying the center of the cavity (Figure 1 b), in contrast to the dynamic mode of peripheral complexation it experiences in **2a**. The S–Cu distances are 2.370(2), 2.380(3), and 2.412(3) Å, with S–Cu–S angles of 119.00(9), 119.91(9), and 121.08(9)°. A mirror plane passes through the metal and three sulfur atoms, and the distance from the metal to the centroids of the benzene rings is 2.97 Å with a centroid–Cu–centroid angle of 178.4°.^[11]

[Ag(**2d**)]BF₄ was crystallized by slow diffusion of methanol into a solution of the complex in acetonitrile. Crystallographically, it is isostructural with [Cu(**2d**)]BF₄,^[10b] with respective angles and distances as follows: S–Ag, 2.577(2), 2.578(2), and 2.631(2) Å; S–Ag–S, 117.72(5), 119.98(6) and 122.31(5)°; Ag–centroid 2.92 Å; centroid–Ag–centroid, 177.5°.^[12]

The NMR data had indicated that the phenyl rings in [M(**2d**)]⁺ (M = Cu, Ag) do not simply block the apical positions but actually coordinate the metal. A statistical study of the nature of η^6 -type bonding in the solid state using the Cambridge Structural Database^[13, 14] provides some valuable insights into this mode of complexation. The sampling in the Database of a cylindrical volume 1 Å in diameter and 5 Å in height extending from the centroid of any benzene ring produces 2523 examples of metal atoms occupying this space.^[15] The results are represented graphically by the aryl centroid–metal distance histogram in Figure 2. The plot is clearly bimodal, with the massive peak centered around 1.75 Å, corresponding to classic η^6 complexes of the central transition metals. A conspicuous discontinuity precedes a second maximum around 3.1 Å, thereby establishing a firm statistical grounding for the general description of these longer contacts as real, although certainly weak, interactions. Inspection of the hitset in this region uncovers four examples

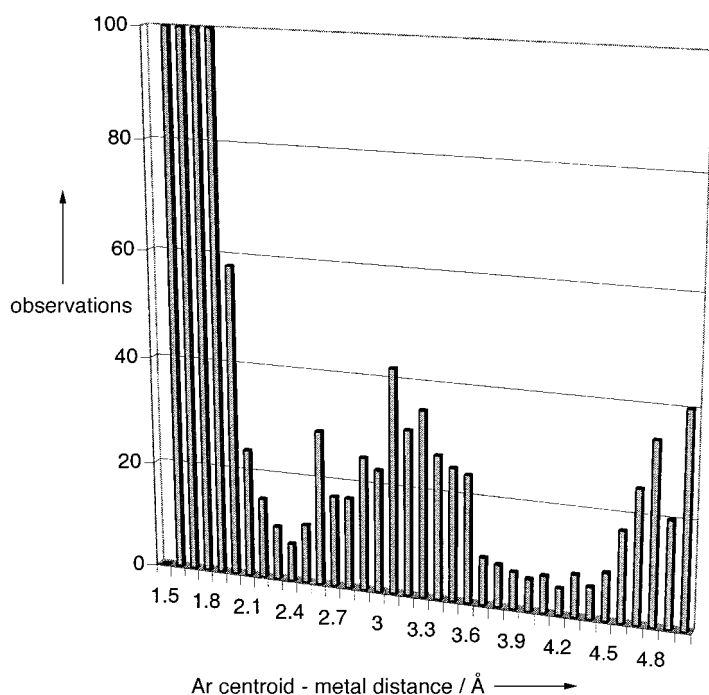


Figure 2. Aryl centroid-metal distance histogram. The bars between 1.4 and 1.9 Å (a total of 1832 observations) are off the scale.

of η^6 bonding for Ag^{I} (aryl centroid-metal 2.89–3.37 Å),^[16] but none for Cu^{I} . Several univalent gallium- and thallium-arene complexes as described by Schmidbaur are also present.^[17] Eight observations of “sandwiched” metals can be found,^[18] for example $[\text{Cs}(p\text{-xylene})_2]^+$, with an aryl centroid-metal distance of 3.4 Å.^[19] Finally, the upward trend in the plot towards 5 Å arises not as a result of direct aryl-metal contact, but of the fixed relationships between arenes and metals in the preponderance of [2.2]paracyclophane complexes with an η^6 -bound metal and μ -bridged bis(η^6) metal species in the Database.

Whilst trigonal-planar coordination of Ag^{I} and Cu^{I} is uncommon,^[20] bis(η^6) complexation of these metals is unprecedented.^[21] This report of the cryptation of metal ions in thiacylindrophane **2d**, with its nearly ideal $\text{Ar}\cdots\text{Ar}$ separation for longer range metal-arene bonding, provides the first example of Ag^{I} and Cu^{I} sandwiches and indeed the only structurally characterized Cu^{I} -aryl centroid interaction. We look forward to investigating the interaction of **2d** with other metals, and the effects of adding an electron to $[\text{M}(\mathbf{2d})]^+$, which should delocalize between the two rings and metal center.

Experimental Section

2d: A solution of trithiol **3**^[8] (0.463 g, 1.79 mmol) and sodium hydroxide (0.22 g, 5.5 mmol) in ethanol (40 mL) and a solution of tribromide **4**^[9] (0.715 g, 1.79 mmol) in toluene (40 mL) were introduced simultaneously over 12 h into refluxing *tert*-butyl alcohol (200 mL) under nitrogen with stirring. The mixture was heated at reflux for a further 15 h and filtered, and the solvent evaporated. The solid residue was purified by chromatography (10% ethyl acetate in hexane) to give **2d** (0.493 g, 66%) as a white solid. M.p. 184–186 °C; ¹H NMR (250 MHz, CDCl_3): δ = 2.52 (12H, t, J = 6.8 Hz), 2.72 (12H, t, J = 6.8 Hz), 6.73 (6H, s); ¹³C NMR (67.8 MHz,

CDCl_3): δ = 35.6, 36.0, 126.9, 140.8; MS (FAB): m/z : 415 $[\text{M}^+ + \text{H}]$; elemental analysis calcd for $\text{C}_{24}\text{H}_{30}\text{S}_3$: C 69.51, H 7.29; found: C 69.38, H 7.35.

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Keywords: coordination modes • cyclophanes • macrocycles • sandwich complexes

- [1] The term “macrocycle” as applied here is used as an analogy to cryptand macrobicycles with aromatic rings counted as bridgeheads, and is not intended to be a formal description of the ring system.
- [2] For a detailed treatment on conformational aspects of larger rings, see J. Dale, *Angew. Chem.* **1966**, *78*, 1070–1093; *Angew. Chem. Int. Ed. Engl.* **1966**, *5*, 1000–1021.
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- [9] W. P. Cochran, P. L. Paulson, T. S. Stevens, *J. Chem. Soc. C* **1968**, 630–632.
- [10] a) X-ray structure analysis of $[\text{Cu}(\mathbf{2d})]\text{BF}_4 \cdot 0.5 \text{CH}_3\text{OH}$: $\text{C}_{24}\text{H}_{30}\text{CuS}_3^+\text{BF}_4^- \cdot 0.5 \text{CH}_3\text{OH}$, M_r = 581.03, colorless rectangular plate $0.60 \times 0.54 \times 0.13$ mm, monoclinic, space group $P2_1/m$, a = 8.391(4), b = 17.971(7), c = 16.329(8) Å, β = 97.09(3)°, V = 2443(2) Å³, Z = 4, ρ_{calcd} = 1.579 g cm⁻³, $\mu(\text{MoK}\alpha)$ = 1.196 mm⁻¹. Stoe Stadi-4 four-circle diffractometer, $\text{MoK}\alpha$ radiation, λ = 0.71073 Å, T = 150(2) K, ω/θ scans. Of 3890 reflections measured ($2\theta_{\text{max}}$ = 45°), 3322 were unique (merging at R = 0.060), giving 2641 with $F \geq 4\sigma(F)$; all 3322 were retained in all calculations. Crystal decay (10.8%) was corrected for, and absorption corrections (T_{min} = 0.529, T_{max} = 0.860) were applied numerically during data processing. Structure solution utilized direct methods^[22] and difference Fourier syntheses. One of the independent half-cations exhibited disorder affecting the copper and the surrounding ligand framework, which was modeled by allowing three alternative sites for the metal. Refinement was by full-matrix least squares^[23] on F^2 , non-solvent H atoms were introduced at geometrically calculated positions and thereafter allowed to ride on their parent C atoms with $U_{\text{iso}}(\text{H}) = 1.2 U_{\text{eq}}(\text{C})$. Final R_1 [$F \geq 4\sigma(F)$] = 0.0576, wR_2 [all data] = 0.154, S [F^2] = 1.17 for 341 refined parameters. The final ΔF synthesis showed no residual feature above 1.35 e Å⁻³.^[10c] b) X-ray structure analysis of $[\text{Ag}(\mathbf{2d})]\text{BF}_4$: $\text{C}_{24}\text{H}_{30}\text{AgS}_3^+\text{BF}_4^-$, M_r = 609.34, colorless rod $0.51 \times 0.16 \times 0.09$ mm, monoclinic, space group $P2_1/m$, a = 8.440(2), b = 17.966(4), c = 16.247(4) Å, β = 96.06(3)°, V = 2449.8(10) Å³, Z = 4, ρ_{calcd} = 1.652 g cm⁻³, $\mu(\text{MoK}\alpha)$ = 1.121 mm⁻¹. Of 6933 reflections measured as described for $[\text{Cu}(\mathbf{2d})]\text{BF}_4$ ($2\theta_{\text{max}}$ = 50°), 4448 were unique (merging at R = 0.064), giving 3655 with $F \geq 4\sigma(F)$; all 4448 were retained in all calculations. Crystal decay (7.2%) was corrected for, and absorption corrections (T_{min} = 0.821, T_{max} = 0.898) were applied numerically during data processing. Structure solution

utilized heavy-atom methods^[22] and difference Fourier syntheses. Refinement was carried out as described for [Cu(**2d**)]BF₄. Final R_1 [$F \geq 4\sigma(F)$] = 0.0416, wR_2 [all data] = 0.100, S [F^2] = 1.06 for 310 refined parameters. The final ΔF synthesis showed no residual feature above 1.47 e Å⁻³.^[10c] c) 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-111910 ([Cu(**2d**)]BF₄) and 111911 ([Ag(**2d**)]BF₄). 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).

- [11] Data represent the ordered half-molecule in the asymmetric unit.
 [12] Data represent one of two very similar half-molecules in the asymmetric unit.
 [13] Cambridge Structural Database Version 5.16 (190307 structures, October 1998 release): F. H. Allen, O. Kennard, *Chem. Des. Automat. News* **1993**, 8, 31–37.
 [14] The United Kingdom Chemical Database Service: D. A. Fletcher, R. F. McMeeking, D. Parkin, *J. Chem. Inf. Comput. Sci.* **1996**, 36, 746–749.
 [15] The query was constructed by defining three points on an aromatic ring: x , one C atom of the ring; o , the centroid of the ring; and y , the centroid of one of the C–C bonds parallel to xo . A metal atom at point m would have x, y coordinates $x_m = om \cos(x\hat{om})$ and $y_m = om \cos(y\hat{om})$. The radius of the cylinder (r) was defined by the relationship $x_m^2 + y_m^2 \leq r^2$, and the 5 Å height of the cylinder was limited by constraining om .
 [16] CSD refcodes: DOCPAM, TEXZUR, YUHDEK, ZODZAT.
 [17] H. Schmidbaur, *Angew. Chem.*, **1985**, 97, 893–904; *Angew. Chem. Int. Ed. Engl.* **1985**, 24, 893–904.
 [18] In all cases the benzene rings are associated independently to the metal. CSD refcodes: BEDBER, DEKMAH, FICXEU, HALVEV, JIVTEN, JIVTIR, SEJKAT, SIRROA.
 [19] J. L. Atwood, W. E. Hunter, R. D. Rogers, J. A. Weeks, *J. Inclusion Phenom.* **1985**, 3, 113–123.
 [20] A total of 9141 crystal structures in the Cambridge Structural Database contain Cu and 1605 contain Ag, of which 104 (1.1%) and 32 (2.0%), respectively, exhibit the metal in a trigonal-planar ligand field (i.e., trivalent with bond angles between 115° and 125°). It must be noted that it is not possible using the CSD's QUEST-3D software to specify metal oxidation states, so the above figures for Cu include both Cu^I and Cu^{II}.
 [21] Two examples are found in the literature where a crown ether incorporates a single benzene ring whose centroid is involved in bonding to Ti^I (metal–centroid, 2.96 Å) and Ag^I (metal–centroid, 2.89 Å): F. Fages, J.-P. Desvergne, H. Bouas-Laurent, J. Hirschberger, P. Marsau, M. Petraud, *Nouv. J. Chim.*, **1988**, 12, 95–106; H. Andrianatoadndro, Y. Barrans, P. Marsau, J. P. Desvergne, F. Fages, H. Bouas-Laurent, *Acta Crystallogr. Sect. B* **1995**, 51, 293–300, respectively.
 [22] SHELXS-97: G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, 46, 467–473.
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N-Oxidation of Epothilone A – C and O-Acyl Rearrangement to C-19- and C-21-Substituted Epothilones**

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Dedicated to Professor Wolfgang Steglich on the occasion of his 65th birthday

Epothilone A (**1a**) and B (**1b**) form the parent compounds of a new family of cytotoxic and antifungal macrolides from the mycobacterium *Sorangium cellulosum*.^[1] According to Bollag et al.,^[2] their effect upon eucaryotic cells is based on the induction of tubulin polymerization and stabilization of microtubules, causing a massive disruption in mitosis and ultimately programmed cell death (apoptosis). This mechanism, which had been extremely rare for a long time, was first observed for taxol (paclitaxel),^[3] and has recently been reported for discodermolide,^[4] a polyketide from marine sponges, and eleutherobin,^[5] a diterpene from soft coral. The high in vitro activity against multiresistant tumor cell lines^[2, 6, 7] as well as other beneficial properties^[1, 8] quickly made the epothilones interesting candidates for cancer chemotherapy and for total synthesis.^[9]

First results of in vivo studies on mouse models were reported by Danishefsky et al. In these studies, not epothilone B (**1b**), which demonstrated the highest in vitro activity, but the significantly less active deoxyepothilone B (=epothilone D, **1d**) showed a promising therapeutic range with good activity against sensitive and multiresistant tumors up to the point of complete remission.^[10]

In the search for possible clinically suitable derivatives, we have concentrated our efforts on the isolation of natural structural variations^[11] and the chemical modification of the epothilones obtained by fermentation.^[12] We report herein the unexpectedly simple functionalization of the thiazol moiety.

During the preparation of epothilones by fermentation from *Sorangium cellulosum*, numerous structural variants from biosynthesis and biotransformation were obtained in addition to the main components A and B. Particularly

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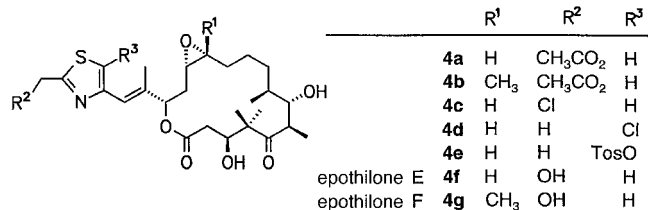
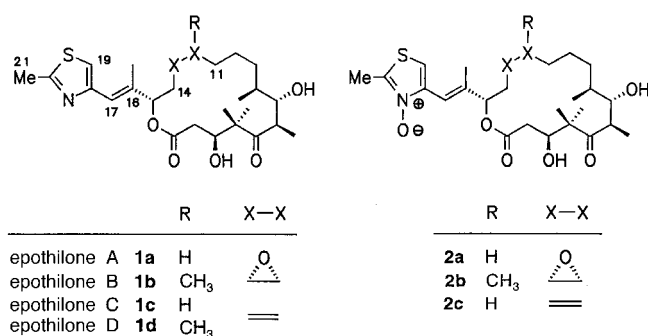
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interesting are the epothilones C (**1c**) and D (**1d**),^[11] as they represent the last intermediates in both the biosynthesis and several total syntheses.^[9] In contrast to the biotransformation of **1c** and **1d**, which leads exclusively to **1a** and **1b**,^[13] chemical epoxidation proceeds with only low chemo- and stereoselectivity. Whereas epoxidation with *m*-chloroperbenzoic acid at -20°C leads to the formation of **1a** and **1b** in at least 40 and 70 % yield,^[14] respectively, new compounds **2a–c** dominate when the same reaction is carried out at room temperature. The HPLC studies of these new compounds attracted attention as they show a band at $\lambda_{\text{max}} = 236 \text{ nm}$ in the UV spectrum, which is uncharacteristic for epothilones. The main components **2a** and **2b** can also be obtained from the reaction of **1a** and **1b** with *m*-chloroperbenzoic acid, thus making them easily accessible in large amounts.



According to HPLC/ESI-MS and elemental analysis, **2a** contains one oxygen atom more than **1a** and differs only in the thiazol side chain, as can be seen in the respective ¹H and ¹³C NMR spectra. As expected for a thiazol-*N*-oxide,^[15] the signals for C-18, C-19, and C-20 are shifted towards higher field by $\Delta\delta = 9.8$ and 20. It could be confirmed by crystal structure analysis^[16] that an *N*-oxide, and not an *S*-oxide, was

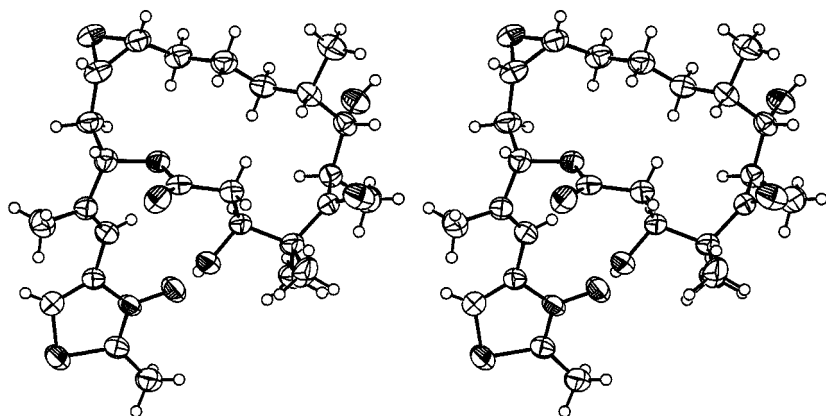
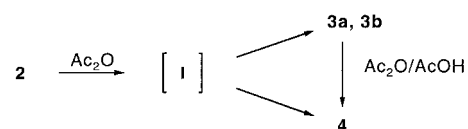


Figure 1. Stereo representation of the structure of **2a** in the crystal. The heteroatoms S, N, and O are indicated by shaded ellipsoids, and two methanol molecules are omitted for clarity.

actually formed (Figure 1).^[17] The oxygen atom of the *N*-oxide is involved in strong hydrogen-bonding interactions to the OH group at C-3 (N–O 274 pm). Based on the structure of **1a**, this arrangement requires rotations around the C-1/C-2, C-2/C-3, and C-3/C-4 bonds by $+148^{\circ}$, $+31^{\circ}$, and -132° ,^[18] respectively, as well as minor changes in the torsion angles of the thiazol side chain. This conformation also exists in solution, as can be seen by the NOE effect between the axial 3-H atom and 17-H, as well as the chemical shift of the 3-OH proton at $\delta = 6.48$ (d, $J_{3\text{-H},3\text{-OH}} = 5.6 \text{ Hz}$).

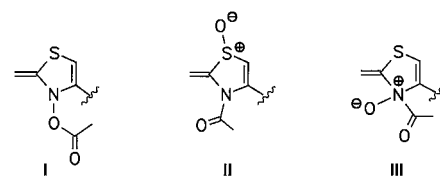
In line with rearrangements reported for α -picoline and quinaldine *N*-oxide with carboxylic acid anhydrides,^[20] we have investigated the behavior of the *N*-oxides **2** towards acetic anhydride. When an excess of acetic anhydride was used, a 1:1:2 mixture of **3a**, **3b**, and **4a** (¹H NMR) was obtained from **2a** within a few minutes at 75°C . After 10–20 min this mixture converted completely into compound **4a** (Scheme 1). Depending on the timing of the workup, either all



Scheme 1. Reaction of the thiazol-*N*-oxide **2** with acetic anhydride.

three compounds or only **4a** can be obtained by silica gel chromatography. The 21-acetoxythiazol structure of **4a** can be concluded from spectroscopic data ($\delta_{\text{H}} = 5.34$ and $\delta_{\text{C}} = 62.5$ (m)), and also from the observation that reaction with ammonia in methanol leads to epothilone E (**4f**).^[11, 19]

The MS and NMR spectra reveal that **3a** and **3b** are very similar isomers of **4a**. Given the specific rotation values of -185.1 and 119.6 , **3a** and **3b** appear to be diastereomers that differ from **4a** only by the presence of a 20-*exo* methylene group (20-CH_2 : $\delta_{\text{H}} = 5.2$ and 5.7 , $\delta_{\text{C}} = 107$). This information leads to the proposed structures **I–III**. Structure **I**, an



expected intermediate of the total reaction,^[20] can certainly be excluded since **3a** and **3b** do not undergo a change in configuration when heated to 140°C in toluene. Since structure **II**, which contains a cross-conjugated chromophore, is not compatible with the observed UV spectrum ($\lambda_{\text{max}} = 267$ and 315 nm), only the structure of an *N*-acetylammonium-*N*-oxide (**III**) remains as a feasible option. However, there appears to be no precedent for this type of structure.^[21]

Important for preparative applications of this *N*-oxide rearrangement is that not only other carboxylic acid anhydrides but also

activated carboxylic acid derivatives such as acid chlorides can be used in the presence of 2,6-lutidine. In contrast to the case of anhydrides, the 19-chloro-, 21-chloro-, and 19-sulfonyloxy-epothilones **4c**, **4d**, and **4e** were obtained with *p*-tosyl chloride and mesyl chloride/1,4-lutidine (tosyl = H₃CC₆H₄-SO₂; mesyl = H₃CSO₂) in dichloromethane.

Biological testing of the easily accessible thiazol derivatives of type **4** confirm earlier results,^[11, 12] which suggest that structure modifications at C-21 but not at C-19 are tolerated (Table 1). The studies also reflect the trend towards higher

Table 1. Comparison of in vitro activities of the epothilone derivatives **2a–c** and **4a–g** with those of paclitaxel and epothilone A–D (**1a–d**).

Compound	Cell line		
	L929 ^[a] IC ₅₀ [nM]	KB-3.1 ^[b] IC ₅₀ [nM]	A-549 ^[c] IC ₅₀ [nM] ^[d]
paclitaxel (taxol)	80	12	4
epothilone A (1a)	8	2	1.4
epothilone B (1b)	1.4	1.2	0.2
epothilone C (1c)	100	40	60
epothilone D (1d)	20	24	20
2a	200	100	160
2b	4	2	1.5
2c	1400	200	600
4a	30	10	5
4c	400	130	20
4d	1100	600	1100
4e	1800	1500	1500
epothilone E (4f)	40	10	6
epothilone F (4g)	3	1.0	0.2

[a] Mouse fibroblasts (ATCC CCL1). [b] Cervix carcinoma (human; DSM ACC158). [c] Lung carcinoma (human; DSM ACC107). [d] The growth was determined by the tetrazolium reduction test.

activities of the epothilones of the B series as compared to those of the A series. For the *N*-oxides **2a** and **2b**, for instance, even a difference by a factor of 50 to 100 can be observed. Epothilone F (**4g**),^[11] in vitro the most effective derivative after epothilone B, can be prepared with particular ease with the procedures presented herein starting from **1b**, which is obtained by fermentation. Furthermore, the route via the *N*-oxides **2** offers access to a variety of other derivatization and degradation reactions at C-21. It is furthermore noteworthy that the *N*-oxide rearrangement^[22] which has hardly been used on thiazoles was employed for the first time on a complex natural product in the presence of sensitive functional groups.

Experimental Section

General procedure for the preparation of the epothilone-*N*-oxides **2**: A solution of *m*-chloroperbenzoic acid (2 mmol) in dichloromethane (10 mL) was added to a stirred solution of **1** (1 mmol) in dichloromethane (0.5 mL). After 3 h the reaction was quenched with an aqueous Na₂SO₃ solution, and the resulting mixture distributed between an aqueous saturated NaHCO₃ solution and ethyl acetate. The combined organic layers were dried over MgSO₄ and concentrated in vacuo. The residue (0.6–0.7 g) was separated by chromatography using RP-18 silica gel with acetonitrile/0.05 M ammonium acetate (pH 7.5, 35/65). Yield **2a**: 40%, **2b**: 48%, **2c**: 20%.

2a: Colorless crystals from methanol; m.p. 115 °C; TLC: *R*_f = 0.15 (silica gel Si60, dichloromethane/methanol (95/5), detection with vanillin/sulfuric acid, bluish gray stain upon heating to 120 °C); HPLC: *R*_t = 2.2 min (HD-Sil100, C-18, 5 μm, 125 × 4-mm column, acetonitrile/water (40/60), 1.5 mL min⁻¹, detection at 254 nm); UV (MeOH): λ_{max} (ε) = 283 sh (1700),

264 sh (4700), 236 nm (17000); [α]_D²⁵ = −114.0 (*c* = 0.9 in methanol); IR (KBr): ν̄ = 3430, 2966, 2933, 2879, 1741, 1688 cm⁻¹; ¹H NMR (CDCl₃): δ = 2.16 (dd, *J* = 13.0, 2.6 Hz, 2-H_a), 2.46 (t, *J* = 12.0 Hz, 2-H_b), 4.54 (brd, *J* = 12.0 Hz, 3-H), 6.65 (d, *J* = 5.6 Hz, 3-OH), 3.22 (dq, *J* = 2.3, 5.0 Hz, 6-H), 3.72 (d, *J* = 5.0 Hz, 7-H), 1.26–1.86 (m, 8-H, 9-H₂, 10-H₂, 11-H₂), 2.91 (m, 12-H), 2.96 (m, 13-H), 1.66 (ddd, *J* = 15.2, 11.2, 9.7 Hz, 14-H_a), 2.23 (brd, *J* = 15.2 Hz, 14-H_b), 5.32 (d, *J* = 11.2 Hz, 15-H), 6.81 (brs, 17-H), 7.08 (s, 19-H), 2.59 (s, 21-H₃), 1.02 (s, 22-H₃), 1.43 (s, 23-H₃), 1.18 (d, *J* = 6.8 Hz, 24-H₃), 0.99 (d, *J* = 7.1 Hz, 25-H₃), 2.07 (s, 27-H₃); ¹³C NMR (150 MHz, CDCl₃): δ = 170.5 (C-1), 40.0 (C-2), 70.9 (C-3), 55.1 (C-4), 221.4 (C-5), 40.7 (C-6), 72.5 (C-7), 37.3 (C-8), 31.5 (C-9), 22.0 (C-10), 33.3 (C-11), 62.3 (C-12), 63.0 (C-13), 33.4 (C-14), 75.6 (C-15), 144.3 (C-16), 111.1 (C-17), 143.3 (C-18), 110.3 (C-19), 144.7 (C-20), 13.4 (C-21), 15.3 (C-22), 23.2 (C-23), 12.5 (C-24), 16.5 (C-25), 22.2 (C-26), 18.2 (C-27); HR-EI-MS (70 eV): *m/z* (%): 509.2483 (45, *M*⁺), calcd (C₂₆H₃₀NO₅S): 509.2447; EI-MS (70 eV): *m/z* (%): 494 (4), 493 (4), 492 (4), 410 (48), 322 (50), 304 (18), 194 (15), 182 (52), 154 (100), 126 (44).

Preparation of **3a**, **3b**, and **4a**: a) Compound **2a** (102 mg, 0.2 mmol) was dissolved in acetic anhydride (2 mL) and heated at 75 °C for 5 min. The solution was subsequently concentrated at 30 °C/1 mbar to a viscous oil, which was separated on silica gel (Si60; hexanes/methyl *tert*-butyl ether/methanol, 66/33/1), during which first 65 mg (41 %) of **4a**, then 17 mg (11 %) each of **3a** and **3b** were eluted. b) Compound **2a** (102 mg, 0.2 mmol) was dissolved in acetic anhydride (1 mL) and heated at 75 °C for 5 min. Acetic acid (0.2 mL) was added, and the mixture was maintained at 75 °C for an additional 80 min. Workup and separation as in a) provided 81 mg (74 %) **4a**.

3a: Colorless oil; TLC: *R*_f = 0.66 (for conditions see **2a**); UV (MeOH): λ_{max} (ε) = 203 (13800), 267 (13200), 315 nm (5000); [α]_D²⁵ = −185.1 (*c* = 0.94 in CHCl₃/MeOH 1:1); IR (KBr): ν̄ = 3446, 2965, 2936, 2877, 1742, 1691 cm⁻¹; ¹H NMR (CDCl₃): δ = 2.43 (dd, *J* = 14.8, 3.7 Hz, H 2_a), 2.53 (dd, *J* = 14.8, 10.2 Hz, H 2_b), 4.13 (m, 3-H), 3.33 (d, *J* = 6.4 Hz, 3-OH), 1.86 (dt, *J* = 15.0, 7.8 Hz, 14-H_a), 2.08 (m, 14-H_b), 5.39 (dd, *J* = 7.8, 2.2 Hz, 15-H), 6.23 (brs, 17-H), 6.95 (s, 19-H), 5.18 (s, 21-H_a), 5.71 (brs, 21-H_b), 2.26 (brs, 27-H₃), 2.12 (s, CH₃CO); ¹³C NMR (CDCl₃): δ = 73.4 (C-3), 52.8 (C-4), 151.5 (C-16), 116.0 (C-17), 158.0 (C-18), 88.7 (C-19), 166.9 (C-20), 107.2 (C-21), 20.7 (C-22), 170.2, 21.2 (acetyl); positive-ion HPLC/ESI-MS (acetonitrile/0.02 M ammonium acetate buffer (pH 7)): *m/z*: 569 [*M*+NH₄⁺]. **3b**: Colorless oil; TLC: *R*_f = 0.69 (for conditions see **2a**); [α]_D²⁵ = 119.6 (*c* = 1.1 in CHCl₃/MeOH, 1/1); ¹H NMR (CDCl₃): 1.90 (m, 14-H_a), 2.09 (m, 14-H_b), 5.42 (dd, *J* = 7.8, 2.2 Hz, 15-H), 6.92 (s, 19-H), 2.23 (s, 27-H₃), 2.10 (s, CH₃CO); ¹³C NMR (CDCl₃): 150.8 (C-16), 116.5 (C-17), 17.2 (C-27), 170.3, 21.0 (acetyl).

4a: Colorless oil; TLC: *R*_f = 0.45 (for conditions see **2a**); UV (MeOH): λ_{max} (ε) = 213 (15800), 246 (13500), 257 sh (9800) 275 sh (2900); [α]_D²⁵ = −34.4 (*c* = 0.6 in methanol); IR (KBr): ν̄ = 3470, 2965, 2936, 2877, 1741, 1690 cm⁻¹; ¹H NMR (CDCl₃): δ = 2.43 (dd, *J* = 14.7 Hz, 3.4, 2-H_a), 2.53 (dd, *J* = 14.7, 10.3 Hz, 2-H_b), 4.13 (m, 3-H), 3.26 (d, *J* = 6.6 Hz, 3-OH), 1.89 (dt, *J* = 15.1, 8.0 Hz, 14-H_a), 2.10 (ddd, *J* = 15.1, 4.6, 2.7 Hz, 14-H_b), 5.43 (dd, *J* = 8.0, 2.7 Hz, 15-H), 6.60 (brs, 17-H), 7.14 (s, 19-H), 5.34 (s, 21-H₃), 1.09 (s, 22-H₃), 1.35 (s, 23-H₃), 2.10 (s, 27-H₃), 2.14 (s, Acetyl); ¹³C NMR (CDCl₃): δ = 38.8 (C-2), 73.5 (C-3), 52.7 (C-4), 43.6 (C-6), 74.8 (C-7), 137.8 (C-16), 119.6 (C-17), 152.5 (C-18), 118 (C-19), 167.7 (C-20), 62.5 (C-21), 20.7 (C-22), 21.3 (C-23), 14.3 (C-24), 15.5 (C-27), 170.2, 20.9 (acetyl); HR-EI-MS (70 eV): *m/z* (%): 551.2519 (26, *M*⁺), calcd (C₂₈H₄₁NO₈S): 551.2553; EI-MS (70 eV): *m/z* (%): 364 (65), 224 (100), 222 (56), 209 (35), 164 (36).

4g: a) Compound **4b** (40 mg, 70 μmol) was dissolved in methanol (1 mL), and a 25% ammonia solution (60 μL, 8 mmol) was added. After 3 min at 40 °C the solution was concentrated to dryness in vacuo, and the residue was separated by preparative HPLC using Nucleosil C-18 with acetonitrile/water (3/7). Yield 25 mg (68 %). b) Compound **2b** (40 mg, 77 μmol) was dissolved in dichloromethane (1 mL), and 2,6-lutidine (80 μL, 690 μmol) and trifluoroacetic acid anhydride (80 μL, 570 μmol) were added with stirring. After heating at 75 °C for 10 min, the solution was concentrated to dryness in vacuo. The residue was treated with 25% ammonia (0.5 mL) in THF (2 mL) and heated at 45 °C for 10 min, then concentrated to dryness in vacuo and separated by chromatography as above. Yield 31 mg (78 %) colorless oil; TLC: *R*_f = 0.15; HPLC: *R*_t = 2.2 min (for conditions see **2a**); UV (MeOH): λ_{max} (ε) = 212 (19000), 247 nm (13800); [α]_D²⁵ = −27.4 (*c* = 0.5 in methanol); IR (KBr): ν̄ = 3437, 2959, 2930, 2874, 1735, 1690 cm⁻¹;

¹H NMR (CDCl₃): δ = 2.80 (dd, *J* = 7.4, 5.0 Hz, 13-H), 4.92 (s, 21-H₂), 1.27 (s, 23-H₃), 1.35 (s, 26-H₃); ¹³C NMR (CDCl₃): δ = 32.2 (C-11), 61.3 (C-12), 61.5 (C-13), 170.0 (C-20), 62.2 (C-21).

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Molecular Beside Ionic: Crystal Structures of a 1/1 and a 1/4 Adduct of Pyridine and Formic Acid**

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*Dedicated to Professor Heinz Dieter Lutz
on the occasion of his 65th birthday*

In the context of longstanding work in this laboratory on binary adducts composed of a neutral (uncharged) selected Brønsted base and acid, two such phases have recently been identified in the system pyridine/formic acid and their crystal structures determined. With rare reported precedence in analogous systems, one is molecular and the other ionic. Phase analysis of the system has been done by differential thermal analysis (DTA) and differential scanning calorimetry (DSC) as well as temperature-dependent X-ray powder diffraction. The adducts have stoichiometries C₅H₅N·HCOOH and C₅H₅N·4HCOOH and melt at 219 and 233 K, respectively. The structures have been determined at 173 and 183 K.^[1]

The results are visualized in Figure 1. In the 1/1 compound, a neutral molecule of the base and one of the acid form an O–H···N hydrogen-bonded heterodimer. The 1/4 compound is a pyridinium salt, [C₅H₅NH][HCOO(HCOOH)₃]. In the complex anion, reported here in a crystal for the first time, a central formate ion is coordinated, through rather strong O–H···O hydrogen bonds, by the three extra molecules of the acid. In these, the conformation of the carboxylic group is twice synplanar and once antiplanar. A hydrogen bond

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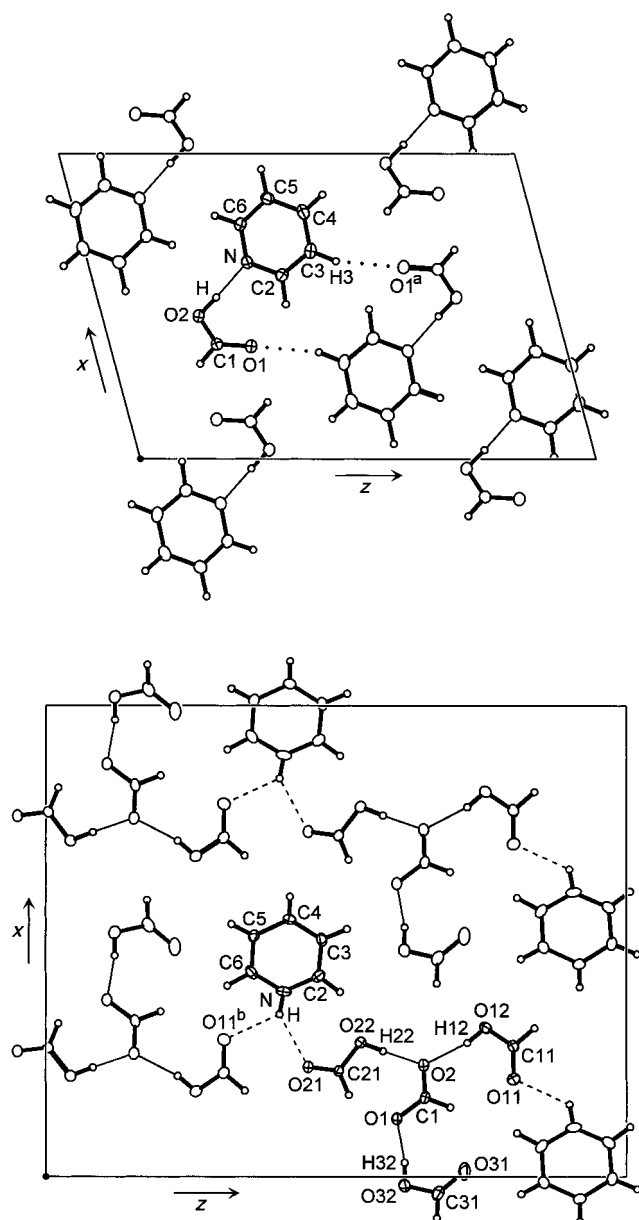


Figure 1. Projections of the molecular 1/1 adduct (top) and ionic 1/4 adduct (bottom) on the x,z planes of their unit cells; 25% ellipsoids for the non-hydrogen atoms; symmetry code a and b for $-x+1, -y+1, -z+1$ and $-x+0.5, y, z-0.5$, respectively.

$\text{N-H}(\cdots\text{O})_2$ —that is, of yet another type and bifurcated—links the pyridinium cation with two complex anions and vice versa along a one-dimensional array parallel to the crystallographic z direction.

Some parameters of the covalent and hydrogen-bonding geometry, with H atom positions not corrected for the systematic errors of the X-ray method, are given in Tables 1 and 2. The above assignment of molecular or ionic to structures and species is not only based on which of the alternative positions for the proton in a hydrogen bond has been found occupied in each case, but is substantiated by the particular pattern of distances C–O and angles C–N–C.

With angles of 9 to 51° between the (best) planes through the two molecular or ionic species each involved in a

Table 1. Selected bond lengths [Å] and bond angles [°].

$\text{C}_5\text{H}_5\text{N} \cdot \text{HCOOH}$, molecular:					
C2–N–C6	117.7(2)				
C1–O1	1.198(3)	C1–O2	1.315(3)	O2–H	0.98(4)
$\text{C}_5\text{H}_5\text{N} \cdot 4\text{HCOOH}$, ionic:					
C2–N–C6	123.0(2)				
C1–O1	1.239(3)	C1–O2	1.266(3)	N–H	0.88(4)
C11–O11	1.194(3)	C11–O12	1.296(4)	O12–H12	0.84(4)
C21–O21	1.214(3)	C21–O22	1.290(3)	O22–H22	0.96(5)
C31–O31	1.197(5)	C31–O32	1.314(4)	O32–H32	0.84(4)

Table 2. Distances [Å] and angles [°] of hydrogen bonds.

D–H $\cdots$ A	D–A	H–A	D–H–A
1/1 adduct:			
O2–H $\cdots$ N	2.664(3)	1.69(4)	173(4)
C3–H3 $\cdots$ O1 ^a	3.308(3)	2.37(3)	160(2)
1/4 adduct:			
N–H $\cdots$ O11 ^b	3.020(4)	2.38(4)	130(4)
N–H $\cdots$ O21	2.871(3)	2.14(4)	141(4)
O12–H12 $\cdots$ O2	2.557(3)	1.74(4)	167(4)
O22–H22 $\cdots$ O2	2.542(3)	1.59(5)	170(5)
O32–H32 $\cdots$ O1	2.590(3)	1.76(4)	167(4)

hydrogen bond, the heterodimer as well as the one-dimensional array are not as close to planar as their projections in Figure 1 may infer. In the 1/1 compound, very weak further hydrogen bonding of the unconventional type $\text{C–H}\cdots\text{O}^{[2]}$ leads to a heterodimeric cyclic pair around an inversion center of the space group. Additional interactions of this type, not detailed in this account, can be perceived in either structure.

The few other proven cases, known to the authors, of an adduct with a molecular and another one with an ionic crystal structure in one and the same Brønsted acid/base binary system have all originated in this laboratory. One of them is given by pyridine and hydrogen fluoride instead of formic acid. The respective system contains again a molecular 1/1 and, among several others, an ionic 1/4 adduct, the latter with a complex anion similar to that described above.^[3] Formic acid and hydrogen fluoride, on the other hand, combine in a 1/1 and a 1/3 cocrystal, of which the former was found molecular and the latter at least argued to be ionic, with the proton transfer from the inorganic to the organic acid.^[4]

The remaining examples are those of acid hydrates beside (hydrated) oxonium salts. Trifluoroacetic acid forms a molecular mono- and an ionic tetrahydrate.^[5] Furthermore, the deuterated tetrahydrate is isotopic, that is also ionic, only up to 230 K, whereas a here existing high-temperature form, though in some ways structurally related, is again clearly molecular.^[6] For dichlorofluoroacetic acid, there is a hemi- and a hexahydrate, and also in this case the lower is a true hydrate and the higher an oxonium salt.^[7] Finally, in both the nondeuterated^[8] and the deuterated^[9] system water/hydrogen fluoride there are three adducts, pairwise isotopic and all of them ionic, whereas a singular further phase^[9] of composition $2\text{D}_2\text{O} \cdot 3\text{DF}$ was found to be molecular.

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- [1] Crystal growth by miniature zone-melting^[10] with the samples of stoichiometric composition in glass capillaries of 0.3 mm inner diameter. C₅H₅N·HCOOH: monoclinic, space group *P*₂₁/*n*, *a* = 10.954(6), *b* = 3.817(3), *c* = 15.842(7) Å, β = 104.96(5)°, *V* = 639.9(7) Å³, *Z* = 4, ρ_{calcd} = 1.30 g cm⁻³, μ = 0.10 mm⁻¹; 2θ_{max} = 50°, 1120 independent reflections with *F*_o² > -3σ_{*F*²}, 986 of them with |*F*_o| > 4σ_{*F*} observed; direct methods, 111 variables refined on *F*², *R*(*F*)(obsd) = 0.047, *wR*(*F*²)(all) = 0.167, residual electron density between -0.22 and +0.16 e Å⁻³. C₅H₅N·4HCOOH: orthorhombic, *P*ca2₁, *a* = 16.35(1), *b* = 3.702(3), *c* = 20.23(1) Å, *V* = 1225(1) Å³, *Z* = 4, ρ_{calcd} = 1.43 g cm⁻³, μ = 0.13 mm⁻¹; 2θ_{max} = 60°, 1835 independent reflections with *F*_o² > -3σ_{*F*²}, 1551 of them with |*F*_o| > 4σ_{*F*} observed; direct methods, 216 variables refined on *F*², *R*(*F*)(obsd) = 0.041, *wR*(*F*²)(all) = 0.114, residual electron density between -0.24 and +0.22 e Å⁻³. Siemens Stoe AED 2 diffractometer adapted for low-temperature work, graphite-monochromated MoKα radiation (λ = 0.71073 Å); computer programs SHELXS-86, SHELXL-93 and SHELXTL PLUS.^[11] 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-112269 and CCDC-112270. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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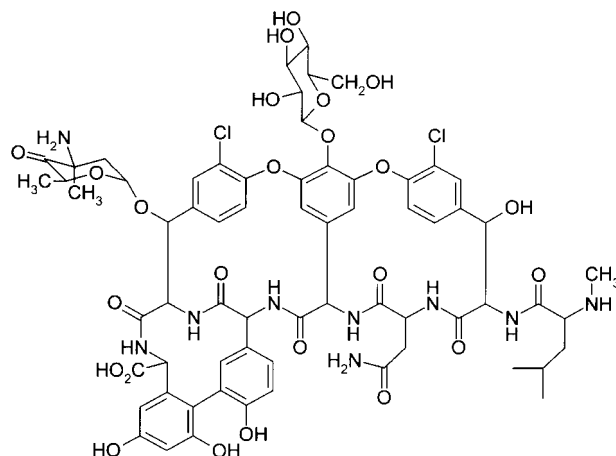
New Advances in the Biosynthesis of Glycopeptide Antibiotics of the Vancomycin Type from *Amycolatopsis mediterranei***

Roderich D. Süssmuth, Stefan Pelzer, Graeme Nicholson, Tilmann Walk, Wolfgang Wohlleben, and Günther Jung*

The glycopeptide antibiotic vancomycin was isolated in the mid 1950s,^[1] and its structure was conclusively determined with spectroscopic methods and by means of crystal structure analysis.^[2] As a drug of last resort, vancomycin is the most important agent after penicillin against Gram-positive bacteria, such as methicillin-resistant staphylococci (MRS).^[3] It is employed in enantiomer analytics as a chiral selector.^[4] The total synthesis of vancomycin is especially challenging owing to the synthetically demanding biphenyl ether and biphenyl bridges.^[5] Although vancomycin has been known for more than 40 years, little is known about the biosynthesis and the intermediates of the aglycon.

Recently the DNA sequence of a gene cluster of the chloroeremomycin producer was described^[6] which is assumed to encode enzymes for glycopeptide biosynthesis. Functional proof by means of expression studies or mutant analysis has so far not been reported. However, an understanding of the biosynthesis on a genetic and on a structural level is important for the development of novel glycopeptide analogs by combinatorial biosynthesis.

Amycolatopsis mediterranei, the producer of balhimycin (Scheme 1),^[7] a glycopeptide antibiotic identical with vanco-



Scheme 1. Structure of balhimycin, an antibiotic of the vancomycin type.

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mycin with regard to the aglycon, was used as a model organism. Through use of a reverse genetic approach the balhimycin biosynthesis cluster was isolated. To prove the involvement of the isolated genes in the biosynthesis of balhimycin, gene inactivation experiments were carried out in *Amycolatopsis mediterranei*.^[8] The null-mutant SP1-1 was generated by integration mutagenesis in the region of the oxygenase genes of the balhimycin biosynthesis gene cluster (Figure 1). From the culture filtrate of this null-mutant, two new biosynthetically accumulated compounds (relative mono-

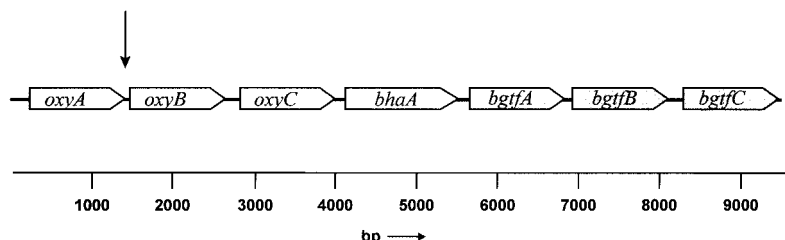


Figure 1. Schematic representation of part of the balhimycin biosynthesis gene cluster from *A. mediterranei* DSM5908. The deduced gene products show significant similarities to the following enzymes: OxyA–C (cytochrome P450-dependent monooxygenases), BhaA (halogenases), BgtfA–C (glycosyl transferases). The arrow symbolizes the targeted integration mutagenesis between the oxygenase genes *oxyA* and *oxyB*.

isotopic masses 969 and 1134) were detected by means of HPLC-ES-MS coupling by comparison with the wild type, whereas the parent ion of balhimycin was no longer detectable.

In addition to an exact mass determination, ES-FT-ICR measurements yielded characteristic chlorine isotope distribution patterns for both compounds (Figure 2). As balhimycin is doubly chlorinated, these compounds had to be

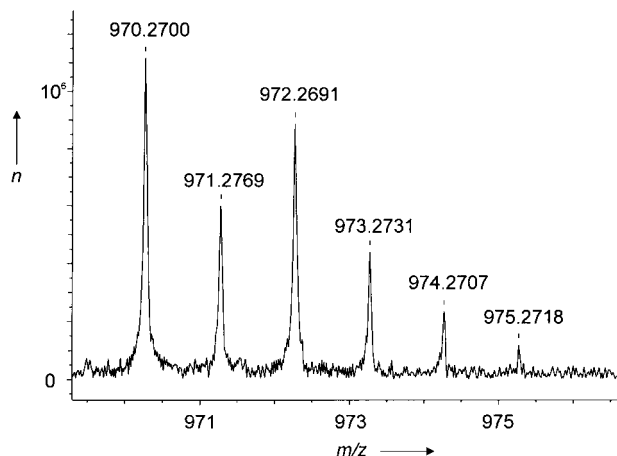


Figure 2. High-resolution ES-FT-ICR mass spectrum of the metabolite SP-969. The isotope distribution is characteristic for compounds of the vancomycin type with two chlorine atoms.

biosynthetic intermediates. The ES-MS daughter ion spectra for the mass signals m/z 970 and 1135 showed a multitude of fragment ion peaks (Figure 3), which made the presence of linear biosynthetic intermediates feasible.

The compounds SP-969 (5.0 mg) and SP-1134 (3.5 mg), named after their molecular masses, were isolated from the culture filtrate with a high degree of purity by means of preparative HPLC. The amino acid analysis with capillary GC-MS on Chirasil-Val showed that L-aspartic acid, D-leucine, and D-4-hydroxyphenylglycine (Hpg) are present in both compounds. In addition, L-3,5-dihydroxyphenylglycine (Dpg) was identified in the hydrolysate of SP-1134; the expected chlorine-containing amino acids could not be detected. The established configurations of the amino acids agreed with those of the vancomycin aglycon. From the daughter ion spectra together with the results of amino acid analytics and knowing the biosynthetic target, the partial sequences Leu-Xxx-Asn-Hpg-Hpg-Yyy (SP-969) and Leu-Xxx-Asn-Hpg-Hpg-Yyy-Dpg (SP-1134) could be deduced (Scheme 2).

The noncyclized structures of the peptides SP-969 and SP-1134 were furthermore established with the Edman degradation procedure. To be able to identify the expected unusual phenylthiohydantoin (PTH) amino acid derivatives, the gas-phase sequencer was directly connected to the ES mass spectrometer.^[9] The number of degradation

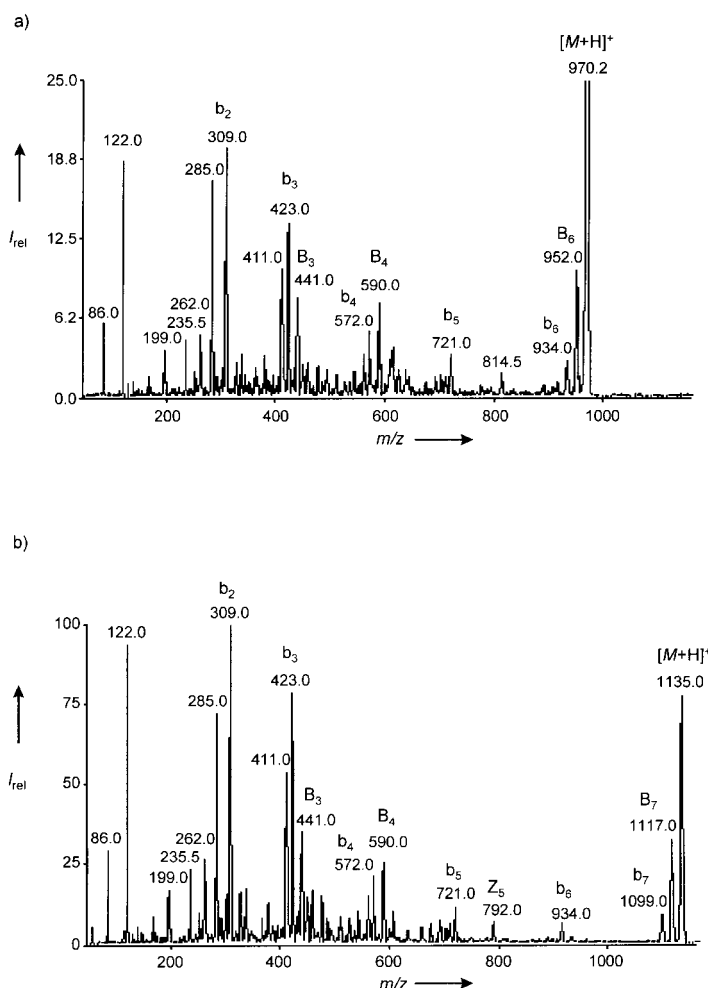
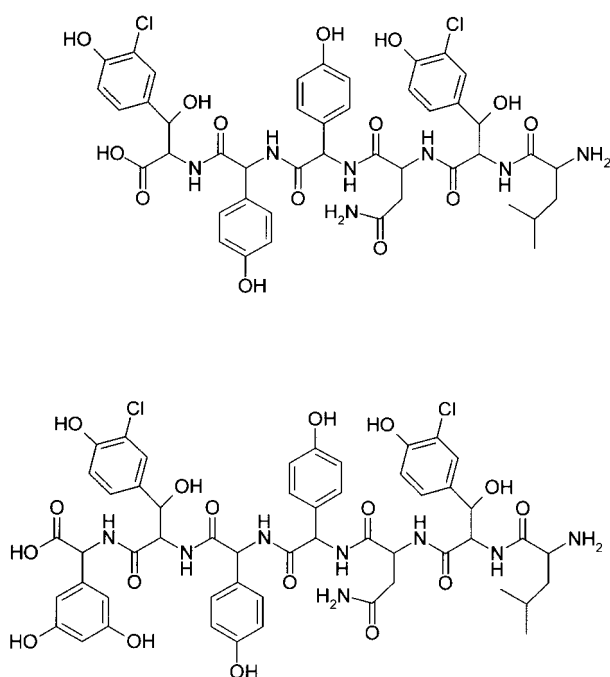


Figure 3. Daughter ion spectra of SP-969 (a) and SP-1134 (b), which show mainly fragments of the B series. The b series marks B fragment ions that emerge after loss of water.



Scheme 2. Structures of SP-969 (above) and SP-1134 (below).

cycles of SP-969 corresponded to six, that of SP-1134 to seven amino acids. For the degradation cycles 1 and 3 of compounds SP-969 and SP-1134, Leu and Asn were detected. The PTH derivatives of the degradation cycles 2 and 6 both agree with the amino acid 3-chloro- β -hydroxytyrosine (Cht) of the aglycon structure with respect to their molecular masses. The occurrence of the PTH derivatives of Gly and 3-chloro-dehydrotyrosine, which were also detected, indicates a retroaldol reaction of the β -hydroxyamino acid to glycine and the

corresponding aldehyde and an elimination of water to furnish the 2,3-didehydroamino acid, respectively. The degradation cycles 4 and 5 correspond to Hpg, which was confirmed by an Hpg standard. For SP-1134, Dpg was additionally identified in degradation cycle 7.

The definite structures of the peptides SP-969 and SP-1134 were confirmed by NMR spectroscopy^[10] (Tables 1 and 2). In the TOCSY spectra (TOCSY = total correlation spectroscopy), five and six spin systems, respectively, were identified in the range of the amide protons. The assignment of aromatic protons in the TOCSY experiment was carried out with two AB spin systems from Hpg (SP-969), three AB spin systems from Hpg and Dpg (SP-1134), and two ABC spin systems each from Cht. In the TOCSY spectrum of the aliphatic region, Leu and Asn were detected. The presence of Asn was verified by a spin system in the TOCSY spectrum for which no ^{13}C contacts could be established in the HSQC experiment (HSQC = heteronuclear single quantum coherence), but for which ROE contacts (ROESY = rotating frame Overhauser effect spectroscopy) to the Asn spin system were proved. In accordance with the signals in the TOCSY spectrum, all further ^1H – ^{13}C contacts of the HSQC spectrum were assigned. The connections of the aromatic rings to the peptide backbone were elucidated with the HMBC spectrum (HMBC = heteronuclear multiple bond correlation). The complete amino acid sequences for SP-969 and SP-1134 could be determined with the ROESY spectra consulted for sequence information.

Peptides SP-969 and SP-1134 are the first known, linear biosynthetic intermediates of antibiotics of the vancomycin type to be isolated. Accordingly, the halogenation of ^2Cht and ^6Cht already takes place before the oxidative ring closures to the biaryl ethers (positions 2, 4, and 6) and the biphenyl

Table 1. ^1H and ^{13}C NMR shifts of the peptide SP-969 ($[\text{D}_6]\text{DMSO}$, $c_{\text{SP-969}} = 8.3 \text{ mg mL}^{-1}$, 305 K).

	NH	C_α	H_α	C_β	H_β	$\delta(^{13}\text{C})_n/\delta(^1\text{H})_n$
^1Leu	–	50.8	3.63	39.7	1.43	23.2/1.45, 21.0/0.80, 21.0/0.80
^2Cht	8.51	58.3	4.58	71.7	4.64	5.64 (β -OH), 133.6 (a), 127.9/7.34 (b), 118.8 (c), 152.0 (d), 115.3/6.86 (e), 126.4/7.10 (f), 10.09 (OH)
^3Asn	8.39	49.0	4.66	37.0	2.38/2.48	6.98 (NH), 7.40 (NH)
^4Hpg	8.00	54.7	5.54	–	–	128.9, 127.5/7.17, 114.7/6.68, 156.3, 9.35 (OH)
^5Hpg	8.77	54.7	5.54	–	–	128.0, 127.7/6.99, 114.4/6.62, 156.4, 9.33 (OH)
^6Cht	8.09	57.7	4.32	70.8	4.91	– (β -OH), 133.7 (a), 127.2/7.21 (b), 118.8 (c), 151.6 (d), 114.6/6.70 (e), 125.3/6.78 (f), 9.98 (OH)

Table 2. ^1H and ^{13}C NMR shifts of the peptide SP-1134 ($[\text{D}_6]\text{DMSO}$, $c_{\text{SP-1134}} = 5.8 \text{ mg mL}^{-1}$, 305 K).

	NH	C_α	H_α	C_β	H_β	$\delta(^{13}\text{C})_n/\delta(^1\text{H})_n$
^1Leu	–	50.6	3.64	39.6	1.43	23.2/1.49, 21.9/0.80, 21.9/0.80
^2Cht	8.56	58.2	4.52	71.6	4.64	5.64 (β -OH), 133.4 (a), 127.9/7.34 (b), 118.5 (c), 151.7 (d), 115.4/6.84 (e), 126.5/7.08 (f), 10.08 (OH)
^3Asn	8.34	49.0	4.62	37.1	2.41/2.41	6.96 (NH), 7.37 (NH)
^4Hpg	8.01	54.8	5.49	–	–	(–), 127.4/7.17, 114.5/6.65, 156.3, 9.36 (OH)
^5Hpg	8.73	55.1	5.46	–	–	(–), 128.1/6.98, 114.5/6.65, 156.4, 9.40 (OH)
^6Cht	7.82	57.7	4.56	71.1	4.84	5.61 (β -OH), – (a), 127.4/7.16 (b), 118.5 (c), 151.1 (d), 114.5/6.65 (e), 125.2/6.70 (f), 9.93 (OH)
^7Dpg	8.57	56.0	5.10	–	–	138.3, 105.3/6.27, 158.0, 101.5/6.16, 9.36 (OH)

(positions 5 and 7). The N-methylation of Leu and the O-glycosylations presumably are later biosynthetic steps. With knowledge of the linear precursor peptides, the synthesis of varied linear peptides which could possibly serve as substrates for the cyclization is now being pursued in a combinatorial approach. Thus the way towards new, semi-synthetic glycopeptide antibiotics would be opened.

Experimental Section

The SP1-1-oxygenase mutant from *Amycolatopsis mediterranei* DSM5908 was fermented in a liquid culture^[11] with 50 µg mL⁻¹ of erythromycin (output: 3.1 mg (SP-969) and 2.2 mg (SP-1134) per L of culture medium). After separation on XAD-16 adsorber resin, the peptides were purified by means of preparative HPLC (Nucleosil C18, 5 µm, 250 × 20 mm, Grom, Herrenberg, Germany), eluent: water (0.1% trifluoroacetic acid) and acetonitrile (0.1% trifluoroacetic acid).

The ES-MS and ES-MS/MS experiments were carried out with a triple quadrupole mass spectrometer (Perkin-Elmer Sciex, Thornhill, Canada) using a pneumatically supported electrospray source and argon collision gas.

The high-resolution mass spectra were recorded with the APEX-II-FT-ICR mass spectrometer 4.7 T (Bruker-Franzen, Bremen, Germany) with electrospray ionization in the positive-ion mode. The calibration took place externally with PPG-1020. The resolution achieved amounts to 17 000 (SP-969) and 30 000 (SP-1134), respectively, with accuracies of mass of 9 and 16 ppm, respectively. The NMR experiments were carried out on a WM-400 spectrometer console (Bruker, Karlsruhe, Germany).

For the GC-MS investigation (Carlo Erba 2900/Varian MAT112S; MAT, Bremen), the peptides were hydrolyzed under a vacuum (6 N HCl, 110 °C, 24 h) and derivatized with methanol/HCl (110 °C, 15 min) and trifluoroacetic anhydride (110 °C, 10 min), respectively. Fused silica capillaries (25 m × 0.25 mm covered with L-Chirasil-Val and Lipodex E (30 %), respectively, in PS255, film thickness 0.13 µm) were used as columns.

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A Highly Luminescent Tetranuclear Silver(I) Cluster and Its Ligation-Induced Core Rearrangement**

Vincent J. Catalano,* Heidi M. Kar, and Joanna Garnas

Gold compounds, in particular gold–phosphane complexes, form inter- and intramolecular aggregates with Au–Au interactions shorter than twice the van der Waals radius.^[1] Such aggregation produces numerous structural motifs that range from extended, linear chains and arrays to macromolecular clusters.^[2] These assemblies often exhibit intense emissions, and the excited states are directly related to the Au···Au interaction. Recently, it was demonstrated that perturbation of this interaction forms the basis of a luminescent sensor by altering the intermolecular Au···Au separations and hence the emission properties.^[3]

While the aurophilicity principle^[4] has been used extensively to describe the strong, closed-shell metal–metal interactions of gold clusters, surprisingly few examples of argentophilic behavior of closely related silver analogues have been reported. In contrast, for silver compounds aggregation is not the rule, and high-nuclearity silver phosphane clusters are rarely formed.^[5] The sparsity of homonuclear silver clusters may be attributed to the weaker metallophilic nature of silver compared to gold. For example, the association energy of Au···Au interactions is comparable to that of typical hydrogen bonds (ca. 5–10 kcal mol⁻¹). This energy is large enough to dictate the structure in solution and the solid state, but the weaker Ag···Ag interactions preclude such directed aggregation.

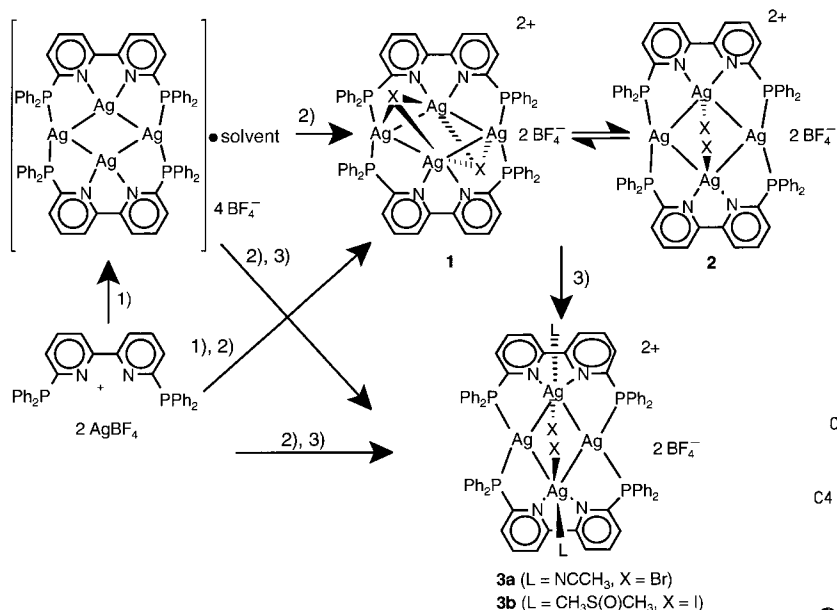
Here we report the synthesis and dynamic behavior of a tetranuclear silver cluster and its unusual core rearrangement upon further ligation. With their strong luminescence and high sensitivity to ligation, these compounds have the ability to act as solution-state sensors.

As shown in Scheme 1, reaction of two equivalents of AgBF₄ with 6,6-bis(diphenylphosphanyl)-2,2'-bipyridyl (P₂-bpy)^[6] in trichloromethane produces a species tentatively identified as [Ag₄(P₂-bpy)₂](BF₄)₄ on the basis of ³¹P{¹H} NMR spectroscopy. At room temperature in CDCl₃, a

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Scheme 1. Reaction of P₂-bpy with AgBF₄. 1) Solvent, 2) 2 LiX, 3) CH₃CN or DMSO.

multiplet at $\delta = 24.7$ ($^1J(^{107}\text{Ag}, \text{P}) \approx 620$, $^1J(^{109}\text{Ag}, \text{P}) \approx 710$ Hz for the [AM]₂[X]₄ second-order spin system)^[7] is observed. However, in CD₃CN a broad resonance without resolvable Ag coupling is observed at $\delta = 11.7$ and is indicative of a dynamic solvent–complex interaction. Addition of Br[−] or I[−] leads to capping of the Ag₄ faces and formation of the pale yellow species **1** and **2**, whereas addition of halide in the presence of a coordinating solvent produces colorless **3a** or **3b** (see below). The order of addition is inconsequential, for dissolution of an intimate mixture of solid LiBr, AgBF₄, and P₂-bpy produces the same species without loss of Ag⁺ as AgBr. At room temperature **1** and **2** appear to interconvert on the NMR time scale.^[8] In view of the solid-state structures, this dynamic behavior may be attributed to the movement of the halide ligand about the Ag₄ face with concurrent rearrangement of the Ag₄ core. Complexes **1** and **2** can be treated with a donor solvent to produce **3a** or **3b**.

Remarkably, **1** and **2** (X = Br) cocrystallize with both species present in the unit cell (Figure 1).^[9] Each molecule is centrosymmetric and contains a crystallographically imposed planar Ag₄ core. Although the two molecules have the same composition, they exhibit significant structural differences. The main structural element of **1** is a diamondlike Ag₄ core, the upper and lower faces of which are capped by triply bridging bromide ions, whereas in **2** the silver ions form a nearly square core with two terminal bromide ligands. These differences are reflected in the interatomic separations, with Ag1–Ag2 and Ag1a–Ag2 distances in **1** of 3.165(2) and 2.965(2) Å, respectively, while the corresponding distances in **2** are slightly shorter (Ag3–Ag4 2.937(2), Ag3a–Ag4 2.978(2)). Although formally nonbonding, these Ag–Ag separations of about 3.0 Å may be considered as strongly interacting.^[5] The shift from the diamond core in **1** to the square core in **2** is reflected in the internal angles at the silver ions. In **1** the Ag1–Ag2–Ag1a and Ag2–Ag1–Ag2a angles are 107.13(5) and 72.87(5)°, respectively. The corresponding angles in **2** (Ag3–Ag4–Ag3a 93.51(5), Ag4–Ag3–Ag4a

86.49(5)°) more closely approach the 90° of a perfectly square core. The bromide ion triply caps the Ag₄ core of **1** with three short Ag–Br distances (Ag1–Br1 2.768(2), Ag2–Br1 2.780(2), Ag2a–Br1 2.658(2) Å), while in **2** the bromide ions are bound to the bipyridine-coordinated Ag ions in a terminal fashion with longer distances to the neighboring Ag atoms (Ag4–Br2 2.614(2), Ag3–Br2 2.978(2), Ag3a–Br1 2.946(2) Å). A further reflection of

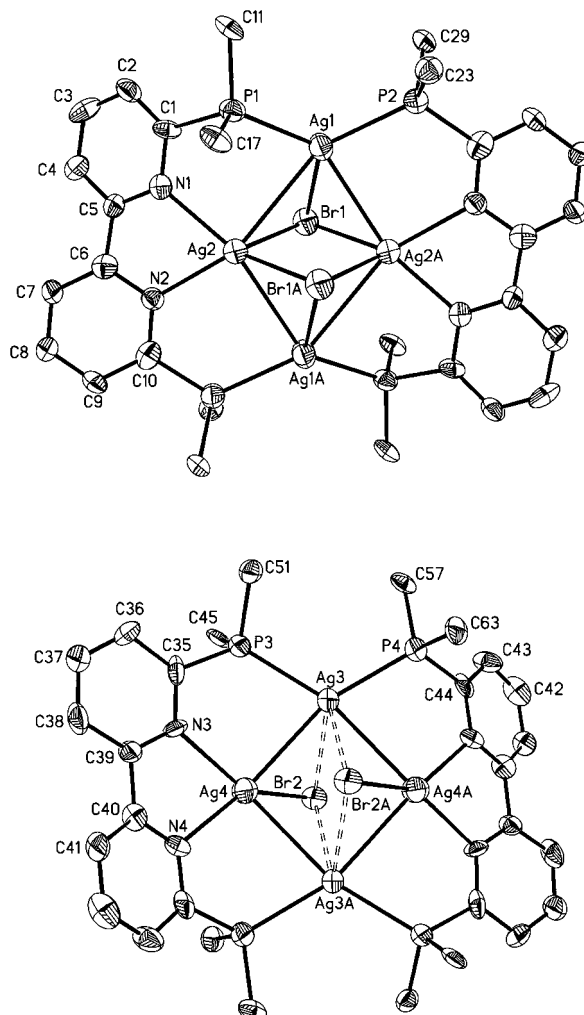


Figure 1. Thermal ellipsoid plot (50% probability) of the cores of **1** (top) and **2** (bottom) with phenyl rings and hydrogen atoms omitted for clarity. Selected bond lengths [Å] and angles [°] for **1**: Ag1–Ag2 3.165(2), Ag1–Ag2a 2.965(2), Ag1...Ag1a 4.933, Ag2...Ag2a 3.644, Ag1–Br1 2.768(2), Ag2–Br1 2.780(2), Ag2a–Br1 2.658(2), Ag1...Br1a 3.558(2), Ag1–P1 2.468(4), Ag1–P2 2.457(4), Ag2–N1 2.301(11), Ag2–N2 2.368(11); P1–Ag1–P2 122.07(14), P2–Ag1–Ag2a 83.46(10), Ag2a–Ag1–Br1 55.11(5), Br1–Ag1–Ag2 55.39(5), Ag2–Ag1–P1 73.44(9), N1–Ag2–Ag1 88.4(3), Ag1–Ag2–Br1 55.04(5), Br1–Ag2–Br1a 95.87(6), Br1a–Ag2–Ag1a 58.68(5), Ag1a–Ag2–N2 91.4(3), N2–Ag2–N1 72.3(4), Ag1–Br1–Ag2a 66.22(5), Ag2a–Br1–Ag2a 84.13(6), Ag2–Br1–Ag1 69.57(5). Selected bond lengths [Å] and angles [°] for **2**: Ag3–Ag4 2.937(2), Ag3–Ag4a 2.978(2), Ag3...Ag3a 4.309, Ag4...Ag4a 4.052, Ag4–Br2 2.614(2), Ag3–Br2 2.978(2), Ag3–Br2a 2.946(2), Ag4...Br2a 3.106(2), Ag3–P3 2.459(4), Ag3–P4 2.458(4), Ag4–N3 2.321(10), Ag4–N4 2.300(11); P3–Ag3–P4 118.76(13), P4–Ag3–Ag4a 76.26(10), Ag4a–Ag3–Br2a 52.36(4), Br2a–Ag3–Ag4 63.73(5), Ag4–Ag3–P3 78.57(9), N3–Ag4–Ag3 91.5(3), Ag3–Ag4–Br2 64.60(5), Br2–Ag4–Ag3a 63.20(5), Ag3a–Ag4–N4 91.7(3), N4–Ag4–N3 71.8(4), Ag4–Br2–Ag3 62.96(5), Ag3–Br2–Ag3a 93.31(5), Ag3a–Br2–Ag4 64.44(5).

these core distortions is the twisting of the bipyridine portions of the P_2 -bpy ligands. In **1** the bipyridine ligand is significantly twisted with a N1–C5–C6–N2 torsion angle of 30.1° , whereas the two pyridine rings of **2** are less distorted (N3–C39–C40–N4 14.3°). All other distances are within expected ranges for similar silver phosphane^[10] and bipyridine complexes.^[11]

Coordination of DMSO to a mixture of **1** and **2** ($X=I$) quantitatively produces the centrosymmetric complex **3b** (Figure 2), which contains a rigid, diamondlike Ag_4 core with a terminal halide ligand.^[12] As in **1** and **2** the crystallographic symmetry dictates a planar Ag_4 core in **3b**. The coordinated

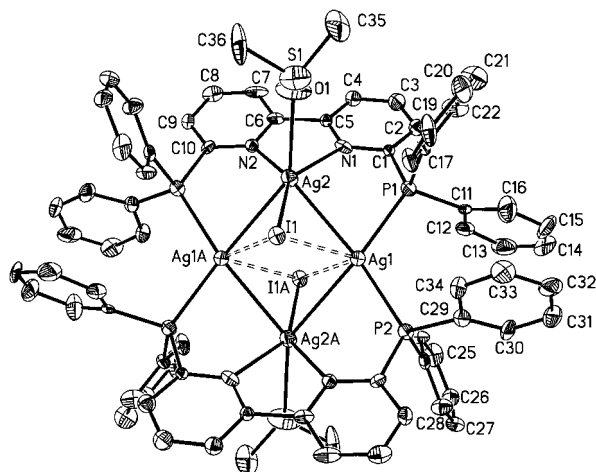


Figure 2. Thermal ellipsoid plot (50% probability) of **3b** with hydrogen atoms omitted for clarity. Selected bond lengths [Å] and angles [$^\circ$]: Ag1–Ag2 2.999(2), Ag1–Ag2a 3.024(2), Ag1...Ag1a 3.953, Ag2...Ag2a 4.545, Ag1–I1 2.9388(14), Ag1–I1a 2.9918(15), Ag2–I1 2.7351(15), Ag2...I1a 3.553(2), Ag1–P1 2.492(4), Ag1–P2 2.487(4), Ag2–N1 2.386(10), Ag2–N2 2.355(9), Ag2–O1 2.532(10); P1–Ag1–Ag2 76.23(9), Ag2–Ag1–I1 54.84(3), I1–Ag1–I1a 96.41(4), I1a–Ag1–Ag2a 54.08(3), Ag2a–Ag1–P2 75.97(9), P2–Ag1–P1 111.54(12), N2–Ag2–Ag1a 87.0(2), Ag1a–Ag2–I1 62.36(4), I1–Ag2–Ag1 61.46(4), Ag1–Ag2–N1 87.9(3), N1–Ag2–O1 78.6(3), O1–Ag2–N2 83.2(3), Ag2–I1–Ag1a 63.56(3), Ag1a–I1–Ag1 83.59(4), Ag1–I1–Ag2 63.70(4).

DMSO molecule binds *cis* to the halide on the bipyridine-coordinated Ag ion and thus alters the coordination geometry towards octahedral. The bipyridine ligands are bent away to accommodate the DMSO molecule, and the Ag_4 core is elongated to 4.545(2) Å along the Ag2...Ag2a vector and shortened to 3.953(2) Å along the Ag1...Ag1a vector. This can be compared to the diamondlike core of **1**, in which the long Ag...Ag axis (4.933(2) Å) lies between the phosphane-coordinated Ag ions. In spite of this marked change, the shorter Ag–Ag distances in **3b** (Ag1–Ag2 2.999(2), Ag1a–Ag2 3.024(2) Å) are quite similar to those of **1**. However, the analogous angles are different: 82.03(4) and 97.97(4) $^\circ$ for Ag1–Ag2–Ag1a and Ag2–Ag1–Ag2a, respectively. A similar arrangement is observed for **3a** ($X=Br$, $L=NCCH_3$); however, severe disorder of the counterion prevented satisfactory refinement, and its structure is not reported here.

The Ag_4 clusters are luminescent in solution and in the solid state. Figure 3 shows the room-temperature emission of the halide-free precursor $[Ag_4(P_2-bpy)_2](BF_4)_4$ in the absence of oxygen. This intense emission is sensitive to both oxygen and coordinating solvents. Exposure to air decreases the emission intensity by a factor of two, while addition of a drop of DMSO

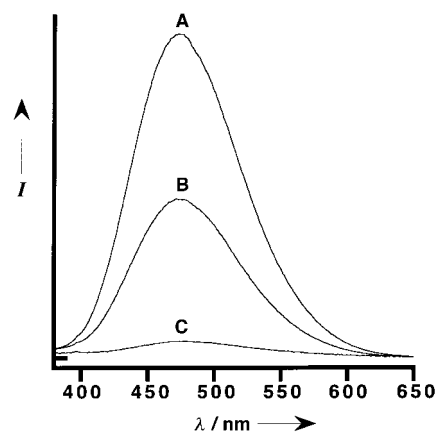


Figure 3. Emission spectra ($\lambda_{ex}=355$ nm, 4×10^{-5} M, CH_2Cl_2) of $[Ag_4(P_2-bpy)_2]^{4+}$ under N_2 (A), saturated with air (B), and with the addition of one drop of DMSO (C). I = intensity.

quenches the intensity by a further factor of ten. Similarly, a solution of **1** and **2** in dichloromethane shows a broad emission at 499 nm with a small shoulder at 410 nm when excited at 355 nm. Addition of acetonitrile quenches the lower energy emission but less effectively than DMSO.

The photoluminescent properties of **1–3** can be attributed to structural rearrangements associated with ligand coordination, as are evident in the their solid-state structures. Emission between d^{10} metal centers is well known^[13] and has been assigned to $ds^* - p\sigma$ transitions for discrete d^{10} dimers,^[14] to $4d - 5s$ transitions for tetranuclear, cubane-like Ag_4 clusters,^[15] to a metal-centered $5d - 6s$ transition for the structurally similar square-planar $[Au(dta)_4]$ (*dta* = dithioacetate),^[16] and to a mixed-metal-centered, ligand-to-metal charge transfer (LMCT) for the closely related $[Ag_4(\mu-dppm)_4(\mu_4-Te)]^{2+}$ species (*dppm* = bis(diphenylphosphanyl)methane).^[17] A MLCT transition can be ruled out because of the large Stokes shift, the lack of an accessible Ag^{II}/Ag^I redox couple, and the fact that the corresponding $[(AuCl)_2(P_2-bpy)]^{18}$ complex is nonemissive. Since the halide-free material is highly luminescent, a LMCT process is unlikely. Therefore, the observed emission can be assigned to a metal-centered state analogous to that of $[Au(dta)_4]$ or of $[Ag_4(\mu-dppm)_4(\mu_4-Te)]^{2+}$ without the LMCT ($Te \rightarrow Ag_4$) contribution.

The ability of $[Ag_4(P_2-bpy)_2]^{4+}$ to act as a sensor is demonstrated in Figure 4. The emission is significantly reduced by the presence of CO and returns to full intensity after purging with N_2 . The IR spectrum of the CO adduct shows only terminal CO ligands (1993 and 2021 cm^{-1}). Interestingly, the same emission is completely quenched by addition of NO but does not return to its initial state after N_2 purging. However, evaporation of this solution in *vacuo* with gentle heating produces the NO-free material. A broad band at 1844 cm^{-1} (CH_2Cl_2) indicates linear NO coordination, but interference between bands at 1550 and 1650 cm^{-1} may mask stretching bands of the bent coordination mode.

As is typical of Ag^I phosphane complexes, none of the compounds reported here exhibited reversible redox behavior in cyclic voltammetry experiments. Instead, only decomposition was observed.

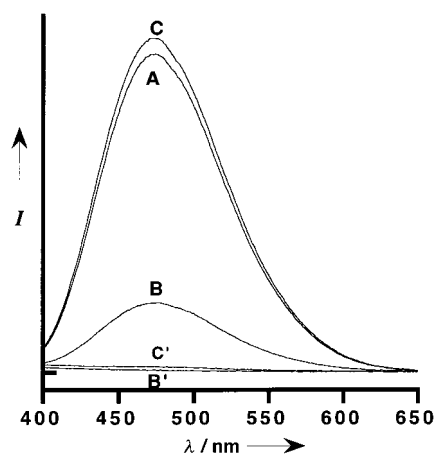


Figure 4. Emission spectra ($\lambda_{\text{ex}}=355$ nm, CH_2Cl_2 , 10^{-5} M) of $[\text{Ag}_4(\text{P}_2\text{-bpy})_2](\text{BF}_4)_4$ under N_2 (A), saturated with CO (B), after purging with N_2 (C), saturated with NO (B'), and after purging with N_2 (C'). I = intensity.

The novelty of the Ag_4 complexes reported here resides in their unusual ligation-induced core rearrangements and the resulting photophysical properties. Unlike the numerous Au–Au compounds, the photophysical responses originate without the destruction of the metal–metal interactions. Rather, they reflect a significant rearrangement of the tetrametallic core with changing ligation. The ability to act as a solution-state sensor is demonstrated by the significant spectroscopic changes. The $[\text{Ag}_4(\text{P}_2\text{-bpy})_2]^{4+}$ species provides a useful template for exploring the metal–metal rearrangements as a function of added capping group and donor ligand. We are currently exploring these reactions and the chemical and physical properties of their products.

Experimental Section

1 and 2: A solution of AgBF_4 (127.7 mg, 0.656 mmol) in methanol (20 mL) was slowly added dropwise to a solution of $\text{P}_2\text{-bpy}$ (172.0 mg, 0.328 mmol) in dichloromethane (25 mL), and the mixture was stirred for 5 min at room temperature. A solution of LiBr (42.7 mg, 0.49 mmol) in methanol (20 mL) was then added very slowly with vigorous stirring, and the solution turned cloudy. After stirring for 10 min, the mixture was evaporated to dryness, the residue dissolved in dichloromethane, and the solution filtered through celite. Light yellow crystals of **1** and **2** were precipitated by addition of hexanes and collected by filtration (187.4 mg, 0.103 mmol, 63 %).

3: A solution of AgBF_4 (75.1 mg, 0.386 mmol) in methanol (20 mL) was slowly added dropwise to a solution of $\text{P}_2\text{-bpy}$ (101.2 mg, 0.193 mmol) in dichloromethane (25 mL), and the mixture was stirred for 5 min at room temperature. A solution of NaI (43.4 mg, 0.290 mmol) in methanol (20 mL) was then added very slowly with vigorous stirring, and the solution eventually turned cloudy and pale yellow. After stirring for 10 min, the mixture was evaporated to dryness, the residue dissolved in dichloromethane, and the solution filtered through celite. After precipitation by addition of diethyl ether, the solid was recrystallized from DMSO/diethyl ether to afford colorless crystals of **3** (127.2 mg, 0.067 mmol, 69 %).

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A Polymer Guest Transforms Clathrate Cages into Channels: The Single-Crystal X-Ray Structure of Tetra-*n*-butylammonium Polyacrylate Hydrate, $n\text{Bu}_4\text{NPA} \cdot 40\text{H}_2\text{O}^{**}$

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Furthering the understanding of macromolecular hydration continues to generate a great deal of experimental as well as theoretical interest from the point of view of the organization of water in close contact with biopolymers as well as with synthetic organic polymers.^[1–3] Although the structural chemistry of biopolymer hydration is well developed,^[4] mainly because of advances in protein crystallography and computer modeling,^[5] it has been difficult to find hydrated polymers with sufficient long-range order to give diffraction structures with resolution characteristic of small-molecule structural determinations. Here we report a first structural study on the incorporation of a polymer, in this instance polyacrylic acid (PA), into a crystalline clathrate with tetra-*n*-butylammonium hydroxide as co-guest.

Following the extensive structural work of Jeffrey and co-workers^[6] on alkylammonium salt hydrates with various small anionic counterions, Nakayama^[7] reported the first hydrates with polymeric anions. He determined the phase diagrams of $n\text{Bu}_4\text{NPA} \cdot \text{H}_2\text{O}$ ^[8] and $i\text{Am}_4\text{NPA} \cdot \text{H}_2\text{O}$, and gave the approximate composition of the hydrates as $n\text{Bu}_4\text{NPA} \cdot 30\text{H}_2\text{O}$ and $i\text{Am}_4\text{NPA} \cdot 42\text{H}_2\text{O}$. Remarkably, Bogatyrov et al.^[9] found that tetra-*n*-alkylammonium polyhydrates also could be formed inside the grains of cross-linked polyacrylic acid resins. So far, structural information on the solid hydrates of simple organic polymers is not available. A model has been proposed^[5, 6] in which the polyacrylate chains are arranged in dodecahedral (D-5¹²)^[10] cavities. However, this model must be incorrect as the proposed structure is a tetragonal form of structure I hydrate, and it is impossible to find a chain of connected D cavities as required by the model. Besides, the presence of a polymer in the small cavities should stabilize the polymer hydrate with respect to the Bu_4NOH hydrate structure where these cavities are known to be empty,^[5] but instead the phase diagram of $n\text{Bu}_4\text{NPA}$ hydrate shows that it is less stable.

We have investigated the structure of $n\text{Bu}_4\text{NPA}$ hydrate with single-crystal X-ray crystallographic methods. The $n\text{Bu}_4\text{N}$ ion in $n\text{Bu}_4\text{NPA} \cdot 40\text{H}_2\text{O}$ (**1**) is located in a four-sectioned T_3P (for explanation of notation see ref. [10]) fused cavity^[9] (Figure 1), and the polymer molecule is in the channel made up from modified T cavities.^[9] Two T cavities sharing a horizontal face form the basic segment of the channel. A vertical stack is formed by stacking the basic segments so that

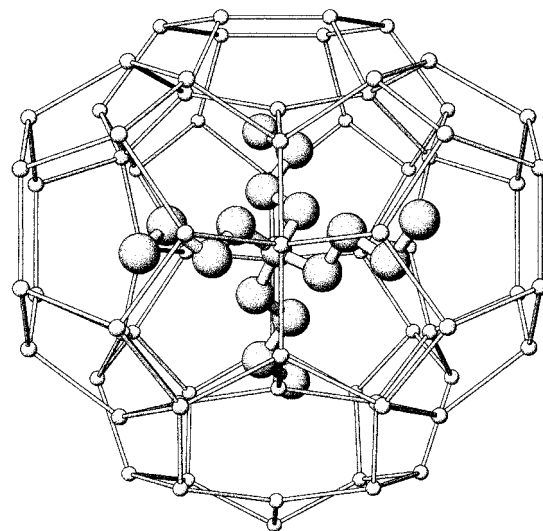


Figure 1. The tetra-*n*-butylammonium cation in the four-sectioned fused T_3P supercage.

alternate segments are at right angles to each other. The actual channel forms upon removal of two water molecules that are shared by every four T cavities, so that we may designate the channel as a $(\text{T}_4)_n$ arrangement. The hydrocarbon chain of the polymeric molecule is disordered over eight possible positions in the channel, and the disordering of the carboxyl groups is yet more complicated. The carbon atom of the carboxyl group is disordered over four possible positions on each hydrocarbon chain and the oxygen atoms also are able to occupy one of two sites. The carbon atoms in the hydrocarbon chain were found on the difference map in the structure refinement. The carbon and oxygen atoms of the carboxyl groups were put in calculated positions and refined isotropically.

The polymer and the hydrate lattices would normally not be expected to be structurally compatible in the sense that they have quite different periodicities. However, the polymer adapts to the hydrate lattice remarkably well by folding into a complex spiral with the appropriate periodicity. This is accomplished through the presence of two conformations of the carbon skeleton: the first is all *anti*, the second is *gauche-anti-anti-anti*. The polymer chain is located in the channel (Figure 2) in such a way that some of the carboxyl groups displace the water molecules in the clathrate framework. On the other hand, some of the carboxyl groups are included in the cavities in a hydrophobic way, namely, lacking hydrogen bonds with water molecules. According to this model some of the large T cavities forming the channel are filled with only carboxyl groups that interact hydrophobically with the cavity. We note that such bimodal forms of hydration have been observed in the recently reported structure of a complex clathrate hydrate incorporating choline hydroxide.^[11]

Approximately 10% of the T cavities are vacant, as are all of the small D cavities. This partial filling of the large cavities by the polymer chain can account for the decrease in the hydrate melting point from 19°C, which is the incongruent melting point of the $n\text{Bu}_4\text{NOH} \cdot 32\text{H}_2\text{O}$, to 14.3°C. The phenomenon of partial filling of the large T cavities is not

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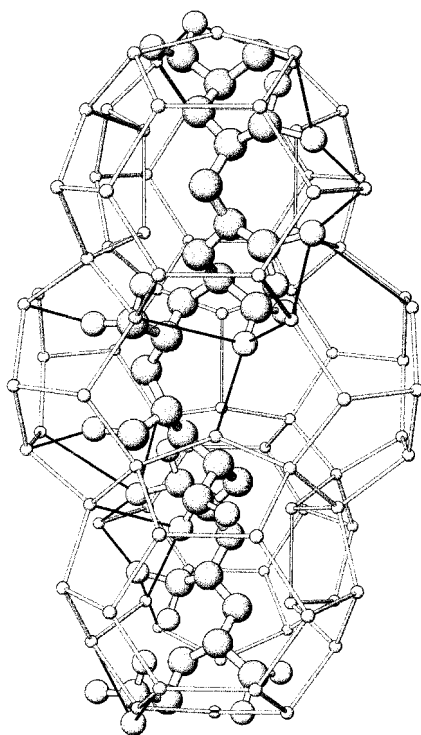


Figure 2. The polyacrylate polymer chain in the channel formed by stacks of T cages. Only one of eight possible disordered chain conformations is shown. Hydrogen-bonded interactions between the "hydrophilic" carboxylate groups and the channel are denoted as thick lines. The remaining carboxylate groups are present as hydrophobic moieties without strong directional interactions with the channel water molecules.

surprising in light of the recent work on a similar tetragonal structure^[12] of bromine hydrate with partially filled cavities.

Since on average each carboxyl group displaces one water molecule in the framework (cases where two water molecules are displaced by one carboxylic group are also possible) the calculated composition of the hydrate will be $\text{Bu}_4\text{NPA} \cdot 40\text{H}_2\text{O}$, which is quite near to Nakayama's results.^[7] Since both $n\text{Bu}_4\text{NOH} \cdot 32\text{H}_2\text{O}$ (this compound is isostructural with $n\text{Bu}_4\text{NF} \cdot 32\text{H}_2\text{O}$ ^[13]) and $n\text{Bu}_4\text{NPA} \cdot 40\text{H}_2\text{O}$ hydrates have the same structure the channel can be filled by combinations of $n\text{Bu}_4\text{N}$ cations and polyacrylic acid molecules. The composition of the hydrate, depending on the experimental conditions, can be described by the formula $n\text{Bu}_4\text{NOH} \cdot 32\text{H}_2\text{O}$ if the channel is completely filled with $n\text{Bu}_4\text{N}$ and $n\text{Bu}_4\text{N}(\text{PA})_{1.2} \cdot 41.6\text{H}_2\text{O}$ if the channel is completely filled with polymer molecules.

A feature of note in this structure is the flexible way that the cages aligned along the *c* axis convert into a channel by the omission of two water molecules for every four adjoining T cavities. In addition, the structure displays a remarkable flexibility in that the carboxylate groups are incorporated either hydrophobically or by replacing one or more water molecules in the cage structure. Whether the polymer contributes significantly to the lattice stability is a moot point. It seems more likely that the presence of the polymer is tolerated at the cost of a small reduction in stability of the lattice. The channel also offers a model for the hydration of the hydrophobic portions of the polymer chain, although it is likely that in solution all carboxylate groups interact with

water molecules by hydrogen bonding. The results presented also show that there should be no particular problems forming a hydrate in the cross-linked polymer^[9] with incorporation of the anionic polymer chain in the hydrate lattice between the cross-links as long as the polymer resin is sufficiently swollen.

Experimental Section

$n\text{Bu}_4\text{NPA} \cdot 40\text{H}_2\text{O}$ (**1**) was crystallized from an aqueous solution of $n\text{Bu}_4\text{NOH}$ and polyacrylic acid ($M_w \approx 1000$) at $+10^\circ\text{C}$. The crystals were colorless with distinct faces and a melting point of $+14.3^\circ\text{C}$. A crystal with the morphology of a tetragonal prism was chosen for the diffraction experiment.^[15]

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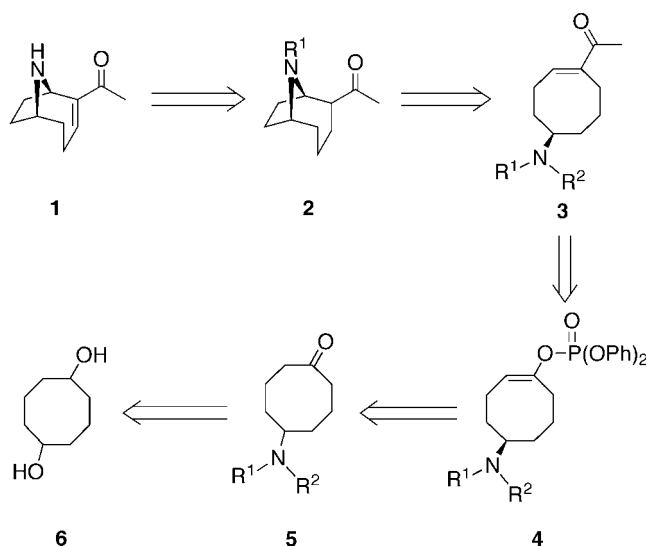
Keywords: clathrates • host–guest chemistry • hydrates • polymers • supramolecular chemistry

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A Formal Asymmetric Synthesis of (+)-Anatoxin-a Using an Enantioselective Deprotonation Strategy on an Eight-Membered Ring**

Varinder K. Aggarwal,* Paul S. Humphries, and Ashley Fenwick

Anatoxin-a (**1**) is a low molecular weight potent neurotoxin present in toxic blooms of the filamentous freshwater blue green algae *Anabaena flos-aquae*, and has been responsible for the fatal poisoning of wildlife in North America and Europe.^[1] Anatoxin-a has attracted much synthetic interest,^[2] primarily because of its potent agonist activity at acetylcholine receptors,^[3] but also because it contains an unusual 9-azabicyclo[4.2.1]nonane skeleton. Herein, we describe a short formal asymmetric synthesis of this natural product, which relies on an enantioselective deprotonation of a cyclooctanone by a chiral lithium amide base (Scheme 1).



Scheme 1. Retrosynthetic analysis of (+)-anatoxin-a (**1**).

The retrosynthetic analysis of (+)-anatoxin-a (**1**) is shown in Scheme 1. The azabicyclic ketone **2** has previously been transformed into **1** by Rapoport et al.,^[4] and could potentially be obtained from **3** by intramolecular conjugate addition of the amine moiety. Enone **3** could be accessed through a Stille reaction from the vinyl phosphate **4**, which itself could be

obtained from ketone **5**. One of the key steps in the synthesis is the enantioselective deprotonation of cyclooctanone **5**.

Desymmetrization of conformationally locked six-membered rings and bicyclic systems have been well explored over the last decade, and high levels of asymmetric induction have been achieved.^[5] However, prior to the start of this work desymmetrization of medium and large rings had not been reported.^[6] This is perhaps because of the commonly held belief that the greater conformational flexibility of these ring systems would result in several low-energy conformations that would lead to low enantioselectivity. However, we were encouraged by the work of Still and Allinger who showed that cyclooctanones exist in conformations having as few trans-annular nonbonded repulsions and high-energy torsional arrangements as possible (namely chair–boat).^[7] Cyclooctanone **5** should therefore adopt conformation **A** in which the carbonyl moiety resides at the position shown (Figure 1) and the amino group occupies an equatorial position. Several

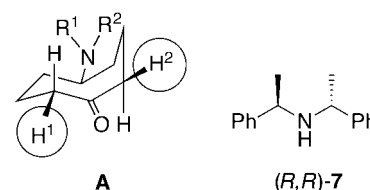


Figure 1. Conformational analysis of prochiral ketone **5** and Simpkins base **7**.

other conformations are populated at room temperature as a result of relatively small barriers to pseudorotation and ring inversion. However, at low temperature one conformation should be largely favored, which would lead to the possibility of high asymmetric induction. The enolizable protons of the low-energy conformation of cyclooctanone **5** are circled in Figure 1. By correlating the sense of asymmetric induction in the desymmetrization of 4-*tert*-butylcyclohexanone with the Simpkins base **7**^[8] we predicted that Li-(*R,R*)-**7** would preferentially abstract H² and provide the enolate required for the synthesis of (+)-anatoxin-a.^[9]

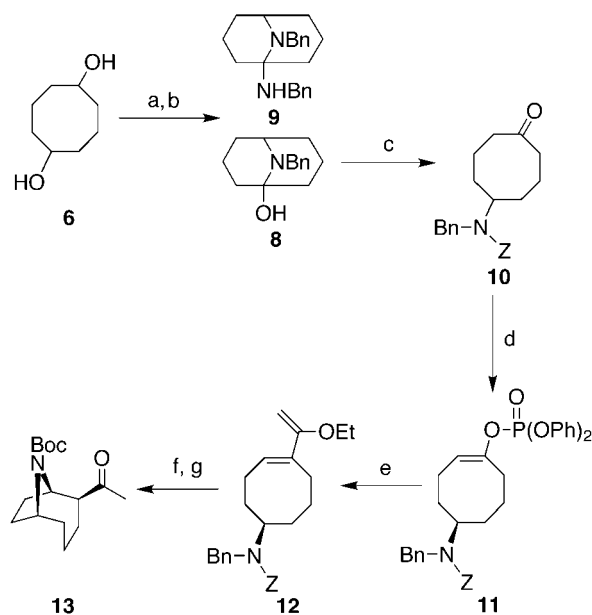
Our route to key intermediate **10** began with *cis*-1,5-cyclooctanediol (**6**), which was oxidized (PDC, CH₂Cl₂) to the intermediate hemiacetal (Scheme 2). Treatment of this hemiacetal with hot aqueous benzylamine gave a mixture of the hemiaminal **8** and amina **9**. Subsequent addition of sulfuric acid converted this mixture into the required hemiaminal in high overall yield.^[11] Attempted ring opening of hemiaminal **8** and protection of the amine moiety with the benzyl carbamate group using standard conditions (PhCH₂OCOC₂H₅, Et₃N, CH₂Cl₂) was ineffective, returning only starting material. However, the addition of catalytic quantities of Sc(OTf)₃ resulted in rapid protection.

The key desymmetrization step required formation of enantiomerically enriched enol phosphate **11**. It had previously been shown by Simpkins and Majewski that it was possible to achieve enantioselective deprotonations utilizing lithium chloride as an external additive to enhance the enantioselectivity.^[12] This was achieved by treatment of prochiral ketone **10** with the base-derived (*R,R*)-**7**·HCl and two equivalents of butyllithium at –100 °C.^[12e] Subsequent

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Scheme 2. Formal total synthesis of (+)-anatoxin-a (**1**). See reference [10] for abbreviations. a) PDC, CH_2Cl_2 , 100%; b) 1. aq PhCH_2NH_2 (40%), *p*-TsOH (30 mol %), Δ ; 2. H_2SO_4 (10%), 81% over two steps; c) PhCH_2OCOC , $\text{Sc}(\text{OTf})_3$ (5 mol %), $i\text{Pr}_2\text{NEt}$, MeCN, 95%; d) (*R,R*)-**7**·HCl, *n*BuLi (2 equiv), $(\text{PhO})_2\text{POCl}$, THF, -100°C , 89%, 89% *ee*; e) $[\text{Pd}(\text{PPh}_3)_4]$, $\text{CH}_2=\text{CH}(\text{OEt})\text{SnBu}_3$, LiCl, THF, Δ , 84%; f) 45% HBr in AcOH, 95%; g) Pd/C, H_2 , MeOH, $(t\text{BuCO})_2\text{O}$, 89%.

quenching of the reaction mixture with diphenyl chlorophosphate gave the enol phosphate **11** with high enantioselectivity (89% *ee*).^[13]

Completion of the formal synthesis was achieved by a Stille reaction of enol phosphate^[14] **11** with $\text{CH}_2=\text{CH}(\text{OEt})\text{SnBu}_3$ in the presence of $[\text{Pd}(\text{PPh}_3)_4]$ and LiCl in THF, followed by a novel cascade reaction. This sequence entailed unmasking the enone moiety with concomitant nitrogen deprotection and intramolecular conjugate addition to give the required bridged azabicyclo.^[15] Changing the protecting group from benzyl to *tert*-butoxycarbonyl gave ketone **13**, which was identical, by ^1H and ^{13}C NMR, and IR spectroscopy and MS, to that reported by the group of Rapoport.^[4] The $[\alpha]_D^{22}$ value we obtained (+47.2, $c=0.8$ in CH_2Cl_2) is consistent with the production of the natural enantiomer ($[\alpha]_D^{22}=+51.9$, $c=0.795$ in CH_2Cl_2).^[4] Ketone **13** has been converted into (+)-anatoxin-a (**1**) by Rapoport et al. in three steps.

In conclusion, this paper describes one of the most concise and efficient routes (34% overall yield, including the final literature steps) to enantiomerically enriched (+)-anatoxin-a. Key steps in our synthesis include a highly enantioselective desymmetrization of an eight-membered ring ketone, and a novel cascade reaction to set up the 9-azabicyclo[4.2.1]nonane skeleton. Such desymmetrization reactions of medium and perhaps large ring ketones could find wide applications in synthesis.

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2,2'-*commo*-Bis[2-ruthena-*nido*-1-(η^5 -pentamethylcyclopentadienyl)ruthenahexaborane(12)]: An Unusual Ruthenaborane Related to Ruthenocene and Exhibiting a Linear Triruthenium Fragment**

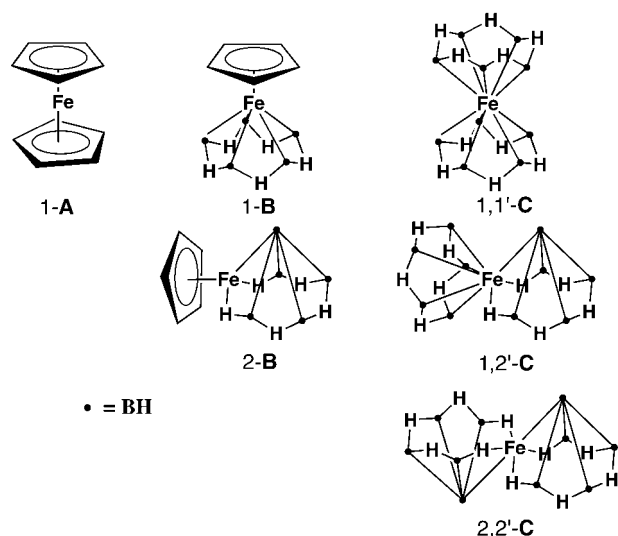
Xinjian Lei, Maoyu Shang, and Thomas P. Fehlner*

The intimate connection between metallaborane chemistry and organometallic chemistry is expressed in the existence of isoelectronic pairs of compounds, for example, $[(\text{CO})_4\text{FeB}_2\text{H}_5]^-$ ^[1,2] versus $[(\text{CO})_4\text{Fe}(\eta^2\text{-C}_2\text{H}_4)]$ and $[(\eta^5\text{-C}_5\text{H}_5)\text{CoB}_4\text{H}_8]$ ^[3] versus $[(\eta^5\text{-C}_5\text{H}_5)\text{Co}(\eta^4\text{-C}_4\text{H}_4)]$.^[4–8] One of the fascinating aspects of these inorganic analogues of organo-

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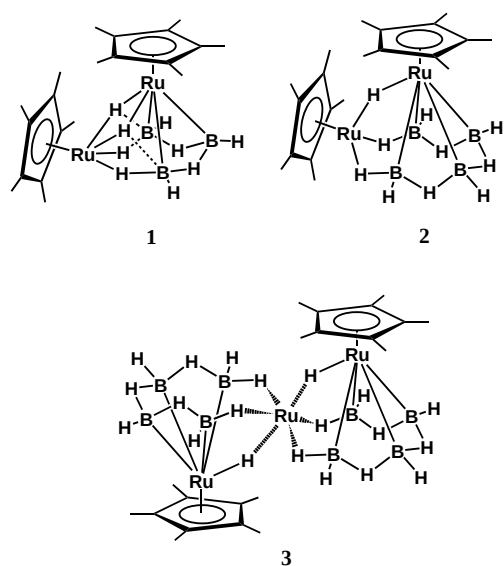
metallic compounds is the greater structural diversity displayed; for example, both $[1-(\eta^5\text{-C}_5\text{H}_5)\text{CoB}_4\text{H}_8]$ and $[2-(\eta^5\text{-C}_5\text{H}_5)\text{CoB}_4\text{H}_8]$ are known.^[3,9] Realizing these connections, one can begin with a known metallocene, such as ferrocene, and generate an array of ferraborane analogues as shown in Scheme 1. Indeed, this is not a purely theoretical exercise as



Scheme 1. Connection between ferrocene and ferraborane analogues.

chemical, spectroscopic, and calculational studies have appeared describing **1-B**, **2-B**, and **2,2'-C**.^[10–13] The last compound is formally similar to $\text{B}_5\text{H}_{10}\text{BeB}_5\text{H}_{10}$, which is the only metal derivative of this type to have been characterized by a solid-state structure.^[14]

Although metallaboranes often have been found as trace products in complex reactions, for example $[1,2\text{-}\{\text{Fe}(\text{CO})_3\}_2\text{B}_3\text{H}_7]$,^[15] their existence created synthetic goals. Thus, a method for the production of dimetallapentaboranes in excellent yields was subsequently developed.^[16] We^[17] and others^[18] have recently described the synthesis of *nido*-[1,2- $\{\text{Cp}^*\text{Ru}\}_2\text{B}_3\text{H}_9]$ (**1**, $\text{Cp}^* = \eta^5\text{-C}_5\text{Me}_5$) from the reaction of monoboranes with $[\{\text{Cp}^*\text{RuCl}_2\}_2]$. Further, **1** cleanly adds a



monoborane fragment to produce *nido*-[1,2- $(\text{Cp}^*\text{Ru})_2(\mu\text{-H})\text{B}_4\text{H}_9]$ (**2**), which can be viewed as a metal analogue of either **1-B** or **2-B** (Scheme 1) depending on which Cp^*RuH fragment is transformed into the isolobal BH fragment.^[17] At the same time, the continuing characterization of unexpected coproducts creates the next generation of synthetic goals. In this vein, we now report *commo*-[1- $(\text{Cp}^*\text{Ru})(\mu\text{-H})\text{B}_4\text{H}_9$]₂Ru (**3**), which is an unusual ruthenaborane analogue of both $\text{B}_5\text{H}_{10}\text{FeB}_5\text{H}_{10}$ ^[12] and $\text{B}_5\text{H}_{10}\text{BeB}_5\text{H}_{10}$.^[14]

A solid-state structure determination of **3** (Figure 1) shows two dimetallahexaborane cages fused in a transoid fashion with one basal ruthenium atom common to both core skeletons.^[19] A consequence of the intercage connection is

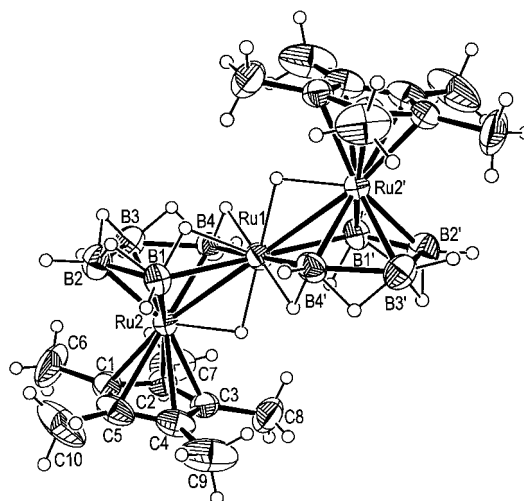


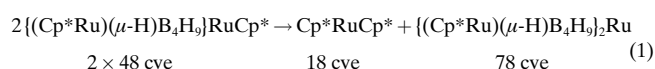
Figure 1. Molecular structure of **3**. Selected bond lengths [Å] and angles [°]: Ru1–B4 2.320(17), Ru1–B1 2.325(16), Ru1–Ru2 2.7930(10), Ru2–B2 2.121(18), Ru2–B3 2.132(19), Ru2–B1 2.143(18), Ru2–B4 2.150(15); Ru2–Ru1–Ru2' 180.00(4).

that the three metal atoms form a linear array. The outer Ru atoms, which occupy the 1-positions of the individual pentagonal pyramids, are η^5 -coordinated to C_5Me_5 ligands. The inner Ru atom, which simultaneously occupies the 2-positions of both pentagonal pyramids, has six nearest neighbor hydrogen atoms in a distorted octahedral geometry. In total, there are six skeletal hydrogen atoms per pentagonal pyramid, five of which occupy the open pentagonal face with the sixth bridging a Ru–Ru edge. Consequently, each of the fused clusters resembles the structure of **2**, and there is a close correspondence between most of the structural parameters of **3** and those of **2**. However, the hydrogen-bridged Ru–Ru distance in **3** is significantly shorter than that in **2** (2.7930(10) vs. 2.8527(4) Å), reflecting the significantly different environment of the unique Ru atom.

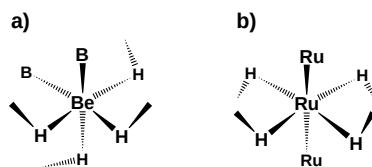
The spectroscopic data of **3** are fully in accord with the solid-state structure, and no evidence of fluxional behavior is observed. The ^{11}B NMR chemical shifts as well as the ^1H shifts (assigned by $^1\text{H}\{^{11}\text{B}\}$ selective decoupling) of the terminal and B–H–B bridging hydrogen atoms correspond closely to those of the comparable atoms of **2**. However, the ^1H resonance of the hydrogen atoms that bridge B1 or B4 and the central ruthenium atom is shifted by 3.3 ppm to higher field, whereas

that of the Ru-H-Ru bridging hydrogen atoms is shifted by 2.9 ppm to lower field, thereby inverting their relative positions in the spectrum compared to **2**. These differences also reflect the distinctly different electronic environment of the unique metal center of **3** relative to **2**.

Counting electrons in **3** becomes transparent once one realizes that the basal Ru atom in **2** receives five electrons from its Cp* ligand as well as five from the {(Cp*Ru)(μ-H)B₄H₉} fragment. Thus, replacement of the basal Cp* ligand of **2** with a {(Cp*Ru)(μ-H)B₄H₉} fragment retains the 18-electron count on the central Ru atom of **3**. Alternatively, **3** is analogous to B₅H₁₀BeB₅H₁₀ in that the two-electron Be atom is mimicked by the unique Ru atom of **3**, which can be thought to possess two valence electrons and six nonbonding electrons (the t_{2g} set). Finally, in terms of counting cluster valence electrons (cve),^[20] the formal disproportionation in Equation (1) based on the cage condensation principle is self-evident.



Although analogous to B₅H₁₀BeB₅H₁₀, the structure of **3** differs as illustrated in Scheme 2. The dihedral angle between the two basal planes of the two boron cages of the



Scheme 2. Comparison of the nearest neighbor environments of a) B₅H₁₀BeB₅H₁₀^[14] and b) **3**.

beryllaborane is 66°, placing the apical boron fragments in a *gauche* orientation (a) in terms of the six-coordinate Be center. However, in **3** the basal planes are coplanar, leading to a transoid arrangement of the fused pentagonal pyramids (b) and a linear array of metal atoms. Hence, compound **3** offers an alternative strategy for the formation of linear multinuclear metal complexes. Indeed **3** (*d*(Ru–Ru) = 2.7930(10) Å) can be compared with [Ru₃(dpa)₄Cl₂] (dpa = bis(2-pyridyl)amido anion), containing a linear multiply bonded Ru₃ moiety (*d*(Ru–Ru) = 2.2537(5) Å).^[21] However, the Ru–Ru–Ru interaction in **3** is qualitatively different in that it is best described as a Ru^{II} ion coordinated to two ruthenaborane ligands as indicated above. On the other hand, as **3** constitutes an isomeric form of a sandwich complex, it suggests the accessibility of variants of multidecker sandwich compounds^[22–29] with linear chains of metal atoms.

The pathway for the formation of **3** is unknown; however, note that **1** contains a [Cp*Ru(μ-H)₄] fragment with a Ru center nearly the same as those in [(Cp*Ru(μ-H)₂)₂].^[30] In the presence of chlorinated solvents, the latter generates coordinatively unsaturated species, ultimately leading to [Cp*Ru(arene)]⁺ complexes. Thus, it is possible that **1** is similarly activated by loss of a one-electron Cp*Ru metal fragment in the presence of the radical generator [Co₂(CO)₈]. Research to establish the origin of **3** continues.

Experimental Section

3: All the preparative work was carried out under an Ar atmosphere. [Co₂(CO)₈] (0.19 g, 0.57 mmol) in hexane (20 mL) was added dropwise to a solution of **1** (0.29 g, 0.57 mmol) in hexane (20 mL). The reaction mixture was stirred for 15 h at room temperature. The products are [1-(Cp*Ru)-2-{Cp*Ru(CO)}-3-{Co(CO)₂}(μ₂-CO)B₅H₉], [1-{Cp*RuH(CO)}B₅H₉], [Co₄(CO)₁₂], [(Cp*Ru(CO))₂], and small amounts of **3**.^[17] Column chromatography (room temperature, hexane) gave a dark brown band which contained [1-{Cp*RuH(CO)}B₅H₉], [Co₄(CO)₁₂], and **3**, with the other products eluting in subsequent fractions.^[17] Repeated fractional crystallization at –40°C removed nearly all of the [Co₄(CO)₁₂] (according to IR spectroscopy). The supernatant was then concentrated and kept at –40°C for several days, after which needlelike crystals of **3** mixed with some black crystals of [Co₄(CO)₁₂] appeared on the bottom of the flask. The black crystals were removed by washing with hexane, and 12 mg of orange needles of **3** remained (≈8% yield based on available boron in **1**). MS (EI): *m/z*: 684 (isotopic pattern for 3 Ru and 8 B atoms); calcd for a weighted average of isotopomers lying within the instrument resolution: 684.1787, found: 684.1751; ¹¹B NMR (hexane, 22 °C): δ = 4.7 (d, ¹J(B,H) = 130 Hz, 4B), 19.3 (d, ¹J(B,H) = 120 Hz, 4B); in the [¹H]¹¹B NMR spectrum these two signals appear as singlets; ¹H NMR: ([D₆]benzene, 22 °C): δ = 3.72 (partially collapsed quartet (pcq), 4H, BH_i), 2.83 (overlapping pcq, 4H, BH_i), 1.72 (s, 30H, C₅(CH₃)₅), –2.88 (br, 6H, B–H–B), –10.64 (s, 2H, Ru–H–Ru), –16.66 (br, 4H, B–H–Ru).

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have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-112935. 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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Liquid Crystals Based on Hypervalent Sulfur Fluorides: Pentafluorosulfuranyl as Polar Terminal Group**

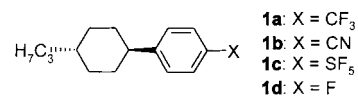
Peer Kirsch,* Matthias Bremer, Michael Heckmeier, and Kazuaki Tarumi

Recently, active matrix liquid crystal displays (AM-LCD or thin film transistor LCD, TFT-LCD) have been evolving rapidly towards becoming the major technology for flat panel displays.^[1] To save costs in the mass production of display panels, there is a strong tendency to reduce the driving voltage of active matrix LCD. On the materials side, the consequence of this trend is the demand for liquid crystals with a higher dielectric anisotropy ($\Delta\epsilon$) in order to obtain an electrooptical response.^[2, 3]

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In designing liquid crystals the most efficient way to increase $\Delta\epsilon$ is by using a polar terminal group with the maximum possible dipole moment. The current limit for AMD-compatible materials is achieved with the trifluoromethyl group (e.g. **1a**: $\Delta\epsilon = 8.6$). Unfortunately, the much



more polar and nematogenic materials based on a terminal cyano group (e.g. **1b**, PCH-3: $\Delta\epsilon = 21.1$) cannot be used for active matrix displays. The cyano group tends to solvate ubiquitous ionic impurities^[4] resulting in a low voltage holding ratio^[5] and observable flicker and contrast loss in the display panel.

To further increase the dielectric anisotropy ($\Delta\epsilon$) of the materials, a polar ring such as 1,3-dioxane or 1,3-dithiane can be introduced. Fluorine substituents in *ortho*-position to the terminal group also result in an increase of $\Delta\epsilon$, but at the cost of a drop of the clearing points by 30–40 K per lateral fluorine atom.^[6, 7] For polar two-ring materials in particular this latter option leads to unacceptably low clearing points.

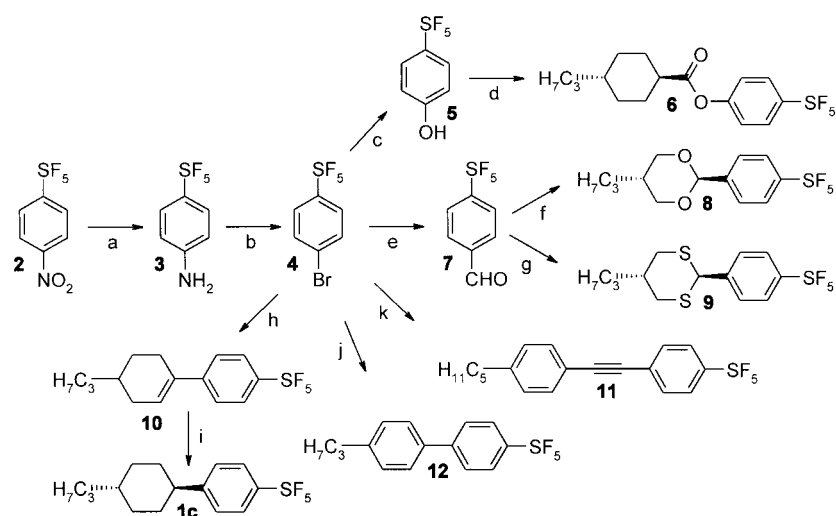
Following and anticipating the technological trend to lower driving voltage, we concentrated on the identification and evaluation of a new, more polar terminal group that is compatible with active matrix technology and does not require lateral fluorination to obtain an acceptably high dielectric anisotropy ($\Delta\epsilon$). Our interest was especially focused on two-ring materials with low rotational viscosity (γ_1).^[3] Other prerequisites for practical display applications are high chemical and photochemical stability of the liquid crystalline materials.

A most interesting candidate as a highly polar head group for liquid crystals is the pentafluorosulfuranyl group.^[8] This functional group is known to be even more stable than trifluoromethyl against hydrolytic agents, also at elevated temperatures. In addition, the dipole moment μ of 3.44 D of pentafluorosulfuranylbenzene^[9] (compared to 2.60 D for benzotrifluoride) indicates that liquid crystals with very high dielectric anisotropy can be obtained. A semiempirical calculation^[10] (PM3) on 4-(propylcyclohexyl)pentafluorosulfuranylbenzene (**1c**) gave a $\Delta\epsilon_{PM3}$ value of 22.2, exceeding even that of the cyano-based based PCH-3.

The chemistry of pentafluorosulfuranylbenzene derivatives was first studied thoroughly at the beginning of the 1960s by W. A. Sheppard,^[9] and its use for liquid crystals was the subject of a preliminary investigation in our group at the end of the 1980s.^[11] These studies were impeded by the inconvenient synthesis of pentafluorosulfuranylbenzene derivatives, which was based on a two-step reaction of aromatic disulfides with silver difluoride in the severely ozone-depleting solvent 1,1,2-trichloro-1,2,2-trifluoroethane ("Freon 113"). The recent introduction of the direct fluorination of deactivated aromatic disulfides made the precursor **2** commercially available in kilogram quantities.^[12] This finally enabled us to develop a suitable synthesis, systematically screen pentafluoro-sulfuranyl-derived liquid crystals, and compare them with conventional cyano- and fluoro-based materials.

The synthesis of all the pentafluorosulfuranyl-substituted liquid crystals started from 4-nitropentafluorosulfuranylbenzene (**2**), which was hydrogenated to the aniline **3** and subsequently converted into the bromide **4** under Sandmeyer conditions (Scheme 1).^[9b] Compound **4** is a versatile synthetic

typical liquid crystal core structures, we synthesized also the three-ring materials **15–17**.^[13] Due to their higher clearing

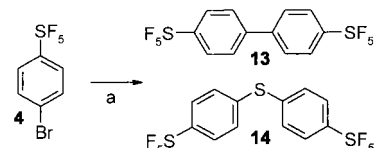


Scheme 1. Synthesis of pentafluorosulfuranyl-based two-ring liquid crystals: a) H_2 , 5% Pd/C, THF; b) 1. HBr, NaNO₂; -5°C ; 2. CuBr; room temperature $\rightarrow 80^\circ\text{C}$ (46%); c) 1. *t*BuLi, Et₂O; -70°C ; 2. B(OMe)₃; $-70 \rightarrow -20^\circ\text{C}$; 3. HOAc, H₂SO₄, H₂O₂ (30%); $-20 \rightarrow -35^\circ\text{C}$, 1 h (58%); d) 4-propylcyclohexylcarbonyl chloride, pyridine, 0.1 equiv dimethylaminopyridine, CH₂Cl₂ (24%); e) 1. *t*BuLi, Et₂O; -78°C ; 2. *N*-formylpiperidine; $-40^\circ\text{C} \rightarrow$ room temperature (76%); f) 2-(trimethylsilyloxymethyl)-1-trimethylsilyloxypentane, cat. Me₃SiOTf, CH₂Cl₂; -78°C , 30 min (67%); g) 2-thiomethylpentane-1-thiol, BF₃·Et₂O; room temperature, 1 h (28%); h) 1. *t*BuLi, Et₂O; -78°C ; 2. 4-propylcyclohexanone; $-78^\circ\text{C} \rightarrow$ room temperature; 3. cat. TsOH, toluene; azeotropic removal of water (55%); i) H_2 , 5% Pd/C, THF (25% pure *trans* isomer); j) 4-propylphenylboronic acid, cat. [Pd(PPh₃)₄], toluene, 2N NaOH; room temperature, 2 d (23%); k) 1-ethynyl-4-propylbenzene, cat. [Pd(PPh₃)₄], pyrrolidine; room temperature, 18 h (33%).

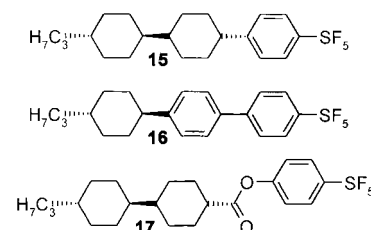
building block which can be easily converted into a variety of liquid crystals^[13] either directly or via the aldehyde **7** or the phenol **5**. The purity of the newly synthesized compounds was verified to be at least 99.5% by GC or HPLC.

The only unexpected “complication” during the syntheses was the sensitivity of **4** towards *n*-butyllithium. In Sheppard’s original paper^[9b] no significant side reactions under Grignard conditions were reported. Nevertheless, our attempts to metalate **4** with *n*-butyllithium resulted in an immediate dark coloration of the solution, even at -78°C . After workup only the products **13** and **14** were isolated (Scheme 2). Apparently, the pentafluorosulfuranyl group with sulfur in its highest oxidation state was undergoing a redox reaction. Fortunately, the initial problem could be solved by using *tert*-butyllithium, which produced no unwanted side reactions.

To evaluate the influence of the pentafluorosulfuranyl group on the actual, not extrapolated, mesophase behavior of



Scheme 2. Products isolated from the reaction of **4** with *n*BuLi: a) *n*BuLi, THF; -78°C (**13**: 6%, **14**: 4%).



points, fluorinated three-ring liquid crystals have a much stronger tendency to display mesophases than the two-ring materials.^[14] To our surprise, the experimentally determined dielectric anisotropies of the newly synthesized pentafluorosulfuranyl derivatives (**1c**: $\Delta\epsilon_{\text{exp}} = 12.0$) were far lower than predicted by our otherwise very reliable method^[10] (**1c**: $\Delta\epsilon_{\text{PM3}} = 22.2$). Even if our quite ambitious initial target was not met, the physical evaluation (Table 1) shows that pentafluorosulfuranyl-based liquid crystals are still the most polar class of liquid crystals that is compatible with active matrix display technology. The dielectric anisotropy can even be enhanced with a concomitant slight increase of the clearing point, if in the phenylcyclohexane derivative **1c** the cyclohexane ring is replaced by a 1,3-dioxane (**8**: $\Delta\epsilon_{\text{exp}} = 20.3$) or a 1,3-dithiane unit (**9**: $\Delta\epsilon_{\text{exp}} = 22.3$). The polarities of **8** and **9** are even in the range of typical cyano-functionalized compounds such as **1b** (PCH-3). The trifluoromethyl-substituted liquid crystals are slightly less polar than the pentafluorosulfuranyl derivatives.

A less favourable property of the pentafluorosulfuranyl-based liquid crystals is their relatively high rotational viscosity

Table 1. Physical properties of the pentafluorosulfuranyl derivatives and some selected conventional fluorinated liquid crystals.

Entry	(Meso)phases	$T_{\text{N,extr}}$ [$^\circ\text{C}$]	$\Delta\epsilon$	Δn	γ_1 [mPas]
1a	C 22 I	-90.0	8.6 ^[b]	0.036 ^[b]	–
1b	C 45 N 46.1 I	10.1	21.1	0.136	116
1c	C 11 I	-96.8	12.0	0.087	133
1d	C 31 I	-54.0	4.3 ^[b]	0.1 ^[b]	–
6	C 70 I	-31.6	12.4	0.091	–
8	C 69 I	-79.9	20.3	0.091	145
9	C 93 I	-66.4	22.3	0.096	–
10	C 29 I	-84.9	14.2	0.124	–
11	C 68 I	-16.8	16.4	0.224	–
12	C 68 I	-84.5	15.8	0.161	–
15	C 121 I	97.8	11.6	0.094	612
16	C 109 N (87.8) I	94.8	14.3	0.154	442
17	C 90 N 152.8 I	132.9	12.3	0.087	–

[a] Clearing points ($T_{\text{N,extr}}$), dielectric anisotropies ($\Delta\epsilon$), birefringences (Δn), and rotational viscosities (γ_1) are extrapolated from the Merck liquid crystal mixture ZLI-4792.^[14] C = crystalline, N = nematic, I = isotropic. Numbers in parentheses denote monotropic phase transitions. [b] Values were extrapolated from ZLI-1132.

(γ_1), which is in the range of that of the cyano materials (**1c** vs **1b**). Another disadvantage with regard to technical application is the relatively high melting point of most of the newly synthesized materials.

Why are the predicted dielectric anisotropies so much higher than the experimental data? An X-ray crystal structure of **8**^[15] was supposed to help us identify the possible reasons and develop a more reliable prediction method. The X-ray structure (Figure 1) shows that the equatorial fluorine atoms

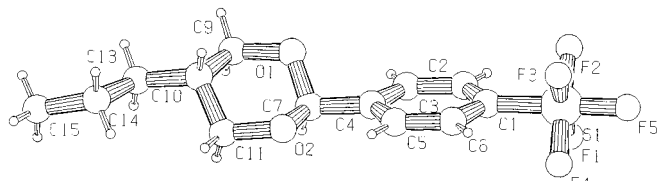


Figure 1. X-ray crystal structure of **8**.^[15] For selected bond lengths and angles see Table 2.

of the pentafluorosulfuranyl group are staggered relative to the *ortho* hydrogen atoms of the benzene moiety, resulting in local C_{2v} symmetry. The only significant difference with regard to the molecular dipole moment between the PM3-optimized structure and the X-ray structure is the C_{ar} -S- F_{eq} angle: $\alpha_{PM3} = 95.6^\circ$ vs $\alpha_{X-ray} = 92.3^\circ$. At first glance we thought that this difference was too small to account for the deviation of ten units from our predicted $\Delta\epsilon$.

Intuitively, one expects that materials such as **1c** and **8** derive their high dielectric anisotropy mostly from the one sulfur–fluorine bond directed along the long molecular axis of the liquid crystal.^[2] The other four S- F_{eq} dipoles are directed like a ring symmetrically in nearly perpendicular directions, due to the octahedral geometry at the hypervalent sulfur atom. Therefore, they should not contribute very much to the molecular dipole moment. The electronegativities (EN)^[16] are 4.1 for fluorine, 2.44 for sulfur, and 2.5 for carbon. Accordingly, under the assumption of similar polarizability of the aromatic part of the molecule, the dipole moments of **1d** (C-F dipole; $\Delta EN = 1.6$; bond length ~ 136 pm) and **1c** (S- F_{ax} dipole; $\Delta EN = 1.66$; bond length ~ 158 pm) should differ by no more than $\sim 20\%$. Contrary to the expectations based on this rather crude model, the measured $\Delta\epsilon$ values are 4.3 for **1d** and 12.0 for **1c**. This big difference in polarity is too large to be explained only by the stronger-than-usual polarization due to the hypervalent character of only one axial S- F_{ax} bond.

So what is the reason for the strong dipole moment and the high dielectric anisotropy of aromatic pentafluorosulfuranyl derivatives? We approached the problem from two sides: our first goal was a better structure prediction, especially for the presumably sensitive C_{ar} -S- F_{eq} angle α . The second goal was to find out more about the dependence of the molecular dipole moment on α .

The best fit between X-ray data and a calculated structure was obtained when the structure was optimized on the HF/6-31G* level.^[17a] While the C_{ar} -S- F_{eq} angle α was very close to the experimental value, the dipole moments and the polarizabilities based on ab initio calculations tended to be far too

large ($\mu_{ai} = 6.75$ D, $\Delta\epsilon_{ai} = 33.3$, $\Delta n_{ai} = 0.025$ for **8**). Therefore, on the supposedly realistic HF/6-31G*-optimized structure of **8** we calculated a PM3 single point in order to obtain the dipole moment and the polarizabilities required for the calculation of $\Delta\epsilon$ and Δn .^[2, 10] This method finally led to a good correlation of experimental with calculated electro-optical parameters, and a reliable computational model for hypervalent sulfur fluorides became available (Table 2). A calculation by the same method predicts for **1c** a $\Delta\epsilon_{calc}$ value of 13.0 and a Δn_{calc} of 0.083, also in excellent agreement with the experimentally determined data ($\Delta\epsilon_{exp} = 12.0$, $\Delta n_{exp} = 0.087$, see also Table 1.)

Table 2. Comparison of calculated and experimentally determined parameters for **8**.^[a]

Parameter	Experimental	PM3//PM3	6-31G*//6-31G*	PM3//6-31G*
C_{ar} -S [Å]	1.8066(18)	1.833	1.799	–
S- F_{eq} [Å] ^[b]	1.5827(13)	1.590	1.583	–
S- F_{ax} [Å]	1.5791(12)	1.609	1.577	–
C_{ar} -S- F_{eq} [°] ^[b]	92.31(7)	95.6	92.57	–
μ [D]	–	7.07	6.75	5.58
$\Delta\epsilon$	20.3	35.9	33.3	21.3
Δn	0.091	0.091	0.025	0.088

[a] The experimental bond lengths and angles are based on the X-ray structure; the dielectric anisotropy ($\Delta\epsilon$) and birefringence (Δn) were extrapolated from ZLI-4792.^[14] The calculated data were obtained by three different methods: PM3//PM3: PM3 structure optimization, polarizabilities, and dipole moment.^[10] 6-31G*//6-31G*: Ab initio (HF/6-31G*) structure optimization, polarizabilities, and dipole moment.^[17b] PM3//6-31G*: HF/6-31G* structure optimization, subsequent PM3 single point for polarizabilities and dipole moment as required for the electrooptical data. [b] Average values for all four F_{eq} atoms.

An ab initio model study on pentafluorosulfuranylbenzene indicates the reason for the grossly overestimated dipole moments μ and $\Delta\epsilon$ values as predicted from PM3-optimized structures: The dipole moment depends strongly on the C_{ar} -S- F_{eq} angle α , which yields readily to steric pressure (Figure 2). A deformation from its minimum ($\approx 92.5^\circ$) by 1° leads to an additional dipole moment of roughly 0.3 D and requires less

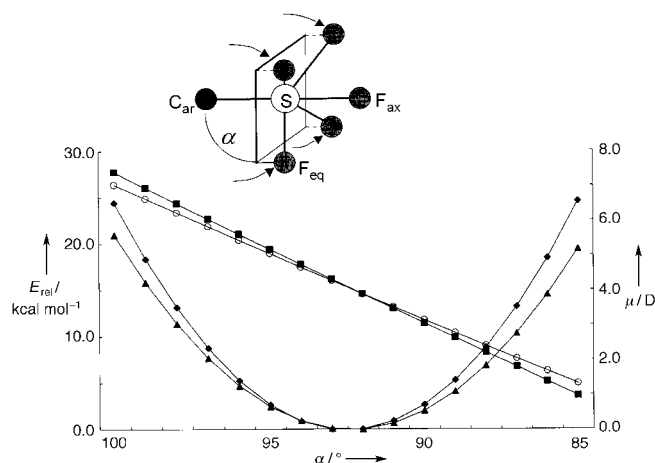


Figure 2. Correlation between dipole moment μ , the deformation energy, and the twist angle α for pentafluorosulfuranylbenzene, calculated by an ab initio (HF/6-31G*) and a DFT method (B3LYP/6-31G*).^[17b] ♦ = HF/6-31G*, ▲ = HF/B3LYP/6-31G*, ■ = dipole HF/6-31G*, ○ = dipole B3LYP/6-31G*.

than 1 kcal mol⁻¹ for moderate deformations. A density functional theory calculation (DFT; B3LYP/6-31G*) predicts slightly lower strain energies also for larger deformations.

The calculations in combination with the X-ray structure data indicate that the relatively high dipole moment μ of the aromatic pentafluorosulfuranyl group is linked to two features. First, a twist of the equatorial fluorine atoms by roughly 2.5° out of the equatorial plane results in an additional component to the overall dipole moment in the direction of the long molecular axis. Second, the S–F bonds are highly polarized due to the hypervalence of the sulfur atom.

An interesting point with regard to materials design is the theoretical possibility to “tune” the dipole moment of the pentafluorosulfuranyl group by pushing the “soft” equatorial fluorine atoms into the desired direction with specific steric pressure of neighboring groups.

The pentafluorosulfuranyl group as a polar terminal group for liquid crystals leads to a new class of liquid crystals with the highly attractive combination of very strong dielectric anisotropy ($\Delta\epsilon$) and reasonably high extrapolated clearing points. A computational model was developed with the aim to understand and predict the electrooptic properties of liquid crystals based on hypervalent sulfur fluorides. Due to its chemical robustness and its polar but lipophilic character, the pentafluorosulfuranyl group surely also has some unexplored potential as a structural component not only liquid crystals but also in polymers and pharmaceuticals.

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Identification of Sequence-Selective Receptors for Peptides with a Carboxylic Acid Terminus**

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The ability to prepare synthetic receptors for specific ligands is a highly desirable goal. In particular selective receptors for specific peptide sequences would have potential applications for separation of peptide mixtures, biosensors, and new therapeutics, as well as providing model systems for biological protein–peptide complexes. Much recent work in the area of peptide receptors has focused on “tweezer” receptors,^[1,2] which, despite their inherent flexibility, have proved to be highly selective for certain peptide sequences in both nonpolar^[3] and aqueous solvent systems.^[4] Many of these tweezer receptors have been screened against combinatorial libraries of resin-bound peptides and have allowed a rapid evaluation of the binding properties of the receptor.^[3,4a] The reverse process, that is, screening of a library of receptors with a chosen peptide substrate, has been less well developed, apart from the pioneering work of Still et al. They have screened libraries of receptors with a steroid core for selective peptide recognition,^[5] and more recently have screened libraries of receptors for the kinetic resolution of cyclic amino acid derivatives.^[6]

The basic design of tweezer receptors incorporates a “head group” or “hinge” bearing two side arms that incorporate appropriate functionality for binding with the backbone of suitable substrates (Figure 1). In contrast to many of the

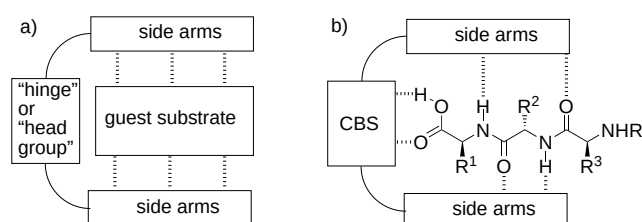
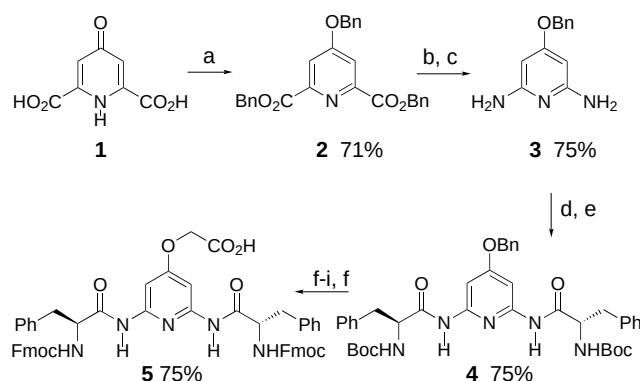


Figure 1. a) Schematic representation of a tweezer receptor with a hinge or head group and side arms that provide binding interactions with a suitable guest substrate. b) Schematic representation of a tweezer receptor with a carboxylic acid binding site (CBS) as the head group.

tweezer receptors reported to date, we are interested in incorporating a head group with a specific recognition site for the terminal functional group of the peptide guests.^[3a] Incorporation of such a binding site or “anchor point”, in addition to binding interactions from the tweezer side arms, should greatly increase the binding affinity of such tweezer receptors with suitable substrates, and should ultimately lead to receptors for the C-terminal sequence of larger peptides.

Here we describe a novel tweezer structure that incorporates a diamidopyridine unit^[7] as the head group to provide a binding site for the carboxylic acid terminus of peptide guests. The arms of the tweezer are themselves simple peptides which can potentially provide selective interactions with the backbone of the peptide guests. A small (2197 membered) resin-bound library of such tweezer receptors has been prepared using the “split and mix” strategy,^[8] and has been used to demonstrate the potential of such libraries to identify selective receptors for selected tripeptide guests with a carboxylic acid terminus.

In order to prepare libraries of tweezer structures we synthesised a diamidopyridine derivative suitably functionalized with a carboxylic acid moiety to allow attachment to resin beads. Such a diamidopyridine derivative **5** was prepared by first converting chelidamic acid **1** into the tribenzyl derivative **2**, followed by aminolysis of the resulting benzyl esters and a Hofmann degradation to give diaminopyridine **3** in 53% overall yield (Scheme 1).^[9] Treatment of **3** with *N,O*-bis(trimethylsilyl)acetamide,^[10] followed by Boc-protected phenylalanine acid fluoride^[11] gave **4** in 75% yield. Hydrogenolysis of the benzyl ether, alkylation with benzyl bromoacetate, exchange of the Boc protecting group for an Fmoc group, and finally further hydrogenolysis of the benzyl ester gave **5** in 75% overall yield from **4**.



Scheme 1. a) K₂CO₃, BnBr, acetone, reflux; b) NH₃, MeOH; c) KOH, Br₂, 90 °C; d) *N,O*-bis(trimethylsilyl)acetamide; e) BocPheF; f) 10% Pd/C, NH₄CO₂H, MeOH; g) BrCH₂CO₂CH₂Ph, K₂CO₃, DMF; h) 20% CF₃CO₂H, CH₂Cl₂; i) FmocONSu, Na₂CO₃, H₂O, dioxan. Boc = *tert*-butoxycarbonyl; Fmoc = (9*H*-fluoren-9-ylmethoxy)carbonyl.

In initial studies the Boc-protected diamidopyridine derivative analogous to **5** was successfully coupled directly to TentaGel resin (without a linker). After removal of the Boc protecting groups a standard solid-phase split and mix synthesis with Fmoc-protected amino acids gave a library of tweezer receptors with tetrapeptide side arms. Attempted sequencing of individual beads by Edman degradation was, however, only partially successful (typically only the first two amino acids could be unambiguously identified) and we concluded that under the conditions of the Edman degradation the amide bonds to the diamidopyridine were being cleaved, leading to the loss of the peptide side arm.^[12] Thus we adopted a strategy using a peptide-coding strand and with the tweezer attached through the Rink-amide linker,^[13] which

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would be cleaved and washed away under the conditions for the Edman sequencing of the coding strand. The peptide coding strand was conveniently incorporated by first coupling Aloc-protected phenylalanine (Aloc = allyloxycarbonyl) onto 10 % of the amine sites on TentaGel-NH₂ resin beads (130 μ m beads, 0.29 mmol g⁻¹) followed by coupling the Fmoc-Rink amide linker^[13] to the remaining 90 % of amine sites (Scheme 2). Removal of the Fmoc group from the Rink linker was followed by the coupling of diamidopyridine **5** and further deprotection of both the Fmoc and Aloc protecting groups to yield resin **6** ready for library generation. A 2197 member library of Fmoc-protected tweezers **7** was prepared by a threefold coupling of thirteen Fmoc-protected amino acids (Gly, L-Ala, L-Val, L-Phe, L-Leu, L-Lys(Boc), L-Pro, L-Glu(OtBu), L-Ser(OtBu), L-Met, L-Trp, L-Asn, L-Gln) to the free amine groups by using the split and mix strategy. A second library of tweezers **8**, with free terminal amino groups,

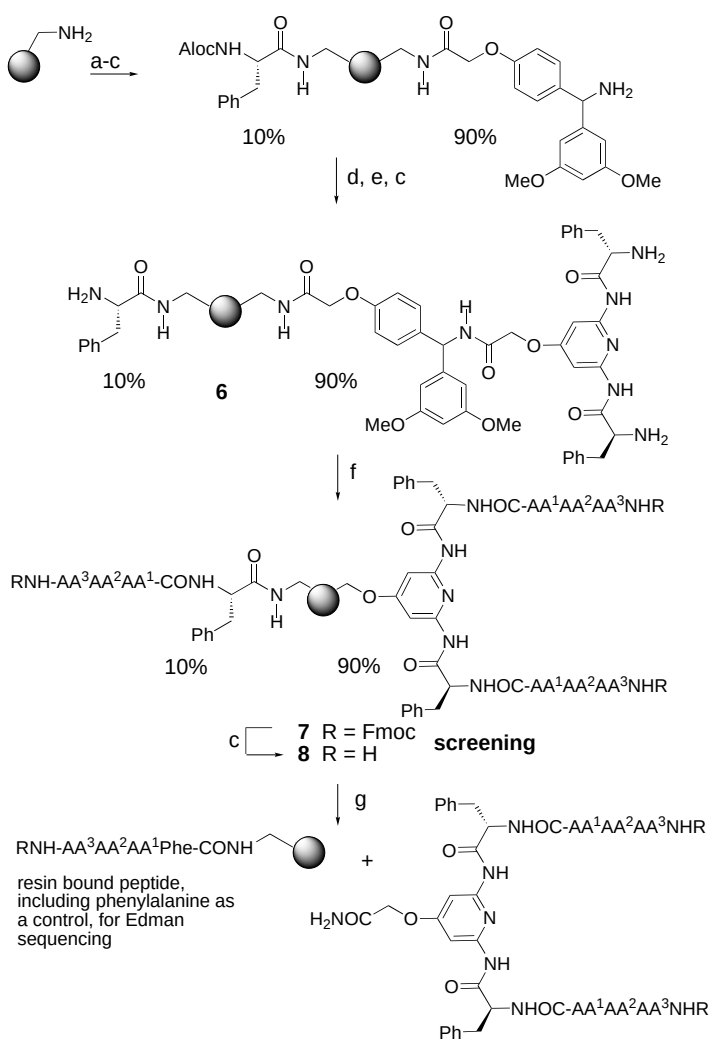
was prepared by treatment of a portion of library **7** with piperidine.

The dansyl-labeled tripeptide with a free carboxylic acid terminus, DNS-L-Glu(OtBu)-L-Ser(OtBu)-L-Val-OH (DNS = dansyl = 5-(dimethylamino)-1-naphthalenesulfonyl), was used as the guest in screening experiments.^[14] A sample of typically 4–5 mg (4000–5000 beads) of the library was equilibrated in a chosen solvent system (160 μ L) for 24 h, followed by the addition of the dansylated tripeptide as a solution in the same solvent system (160 μ L), and incubation for a further 24 h. The beads were analyzed in flat-bottomed glass pots under a Leica inverted DML microscope (magnification $\times$ 40) with a filter cube that contained a suppression filter at 425 nm and an excitation filter at 340–380 nm.

Control tests were carried out for each screening experiment. Thus the dansylated tripeptide guest was incubated with TentaGel resin alone, and samples of the tweezer libraries **7** and **8** were equilibrated in the solvent system in the absence of guest. In these experiments no selective fluorescence was observed, which confirms that all observed fluorescence in the actual screening experiments was derived from the interaction of the guest with selected library members. In addition, in order to show that the observed selectivity was not a consequence of an interaction of the tripeptide guest simply with the peptide side arm of the tweezer receptor, or with the coding strand on the library beads, a simple 2197 membered peptide library directly attached to TentaGel resin (analogous to the coding strand) was prepared. Incubation of this library with the dansylated peptide guests again showed no selective fluorescence.

The screening experiments are simple and allow the rapid evaluation of the binding potential and selectivity of the libraries with the guest in a range of solvent systems. In practice we observed no selective binding with any of the guest-library combinations in a range of buffered aqueous solvents. With chloroform as the solvent, however, high selectivity was observed for DNS-L-Glu(OtBu)-L-Ser(OtBu)-L-Val-OH with both the protected and deprotected libraries (approximately 1 % of the beads were highly fluorescent in both cases against a background of low fluorescence beads).

Highly fluorescent beads were selected from each of the successful screening experiments and sequenced by Edman degradation.^[15] (Edman sequencing was carried out in all cases for a fourth cycle and always showed the presence of phenylalanine for all beads, which was expected as it was introduced by the synthesis, and provided a useful check that the coding strand was operating correctly). The results of the sequencing experiments had the highest level of consensus for screening of DNS-L-Glu(OtBu)-L-Ser(OtBu)-L-Val-OH against the protected library **7** (Table 1) with the sequence Val-xxx-Trp found for six of the beads analyzed, and more specifically Val-Leu-Trp was found for three of the beads. A strong consensus for valine or leucine at the second and third positions was also found with the deprotected library **8** (Table 2), but the terminal amino acid residue was less well defined, with phenylalanine detected for four of the beads. Significantly tryptophan was not detected at the terminal position for any of the beads from the deprotected library **8**.



Scheme 2. a) AlocPhe, HOBT, DIC, DMAP, CH₂Cl₂ (10 % of amine sites); b) Fmoc-Rink amide linker, HOBT, DIC, DMAP, CH₂Cl₂; c) 20 % piperidine, DMF; d) **5**, HOBT, DIC, DMAP, CH₂Cl₂; e) [Pd(PPh₃)₄], dimedone, CH₂Cl₂, THF; f) 3-fold split and mix Fmoc-peptide synthesis using Gly, L-Ala, L-Val, L-Phe, L-Leu, L-Lys(Boc), L-Pro, L-Glu(OtBu), L-Ser(OtBu), L-Met, L-Trp, L-Asn, L-Gln; g) Edman sequencing. HOBT = 1-hydroxy-1*H*-benzotriazole; DIC = 1,3-diisopropylcarbodiimide; DMAP = 4-dimethylaminopyridine; DMF = *N,N*-dimethylformamide.

Table 1. Sequencing data for ten fluorescent beads selected from the screening experiment of DNS-L-Glu(OrBu)-L-Ser(OrBu)-L-Val-OH with the protected library **7** in CHCl₃.

Bead	AA ¹	AA ²	AA ³
1	Val	Leu	Trp
2	Val	Leu	Trp
3	Val	Leu	Trp
4	Val	Ala	Trp
5	Val	Val	Trp
6	Val	Met	Trp
7	Val	Gly	Phe
8	Val	Ala	Val
9	Val	Gly	Val
10	Gln	Val	Gln

Table 2. Sequencing data for ten fluorescent beads selected from the screening experiment of DNS-L-Glu(OrBu)-L-Ser(OrBu)-L-Val-OH with the deprotected library **8** in CHCl₃.

Bead	AA ¹	AA ²	AA ³
1	Val	Leu	Met
2	Val	Leu	Met
3	Val	Leu	Leu
4	Val	Leu	Leu
5	Val	Gly	Ala
6	Val	Met	Met
7	Leu	Val	Phe
8	Leu	Val	Phe
9	Phe	Val	Phe
10	Ala	Ala	Phe

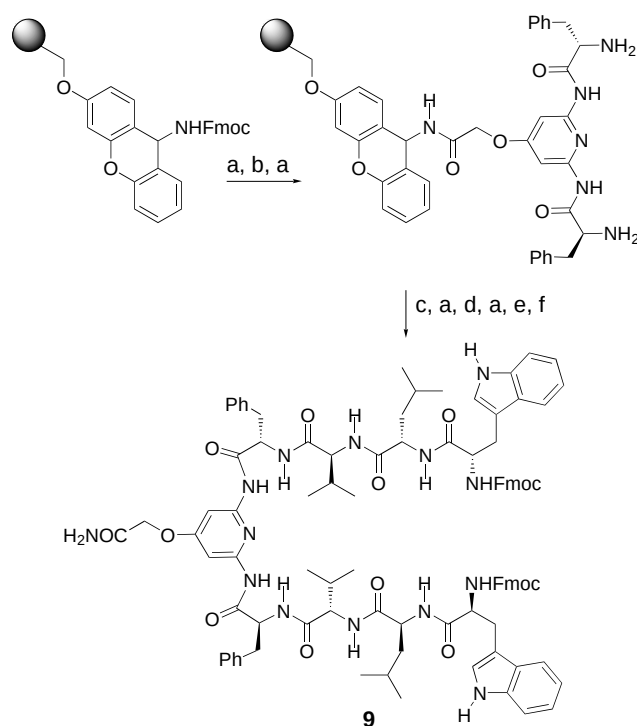
The effect of solvent polarity on the binding selectivity of these libraries could be readily established by the simple addition of more-polar solvents to the screening experiments set up in CHCl₃ solution. Thus addition of DMSO led to a rapid loss of selective fluorescence, with both protected and deprotected libraries **7** and **8**, when the solvent composition reached 10:90 DMSO:CHCl₃. Likewise, addition of 10% MeOH effectively destroyed the selectivity. Addition of CH₃CN, however, did not have any noticeable effect on the selectivity observed for the screening of DNS-L-Glu(OrBu)-L-Ser(OrBu)-L-Val-OH with the Fmoc-protected library **7**, although addition of 50% CH₃CN did destroy the selectivity for the screening with the deprotected library **8**. Indeed, screening DNS-L-Glu(OrBu)-L-Ser(OrBu)-L-Val-OH against the Fmoc-protected library **7** in neat CH₃CN showed high selectivity (again, approximately 1% of beads were highly fluorescent). The sequencing of five highly fluorescent beads selected from this screening experiment by Edman degradation gave the identical consensus sequence Val-Leu-Trp for two of the five beads, as found for the same screen in CHCl₃, and Val-Met-Trp for a third bead (Table 3). The strong

Table 3. Sequencing data for five fluorescent beads selected from the screening experiment of DNS-L-Glu(OrBu)-L-Ser(OrBu)-L-Val-OH with the protected library **7** in CH₃CN.

Bead	AA ¹	AA ²	AA ³
1	Val	Leu	Trp
2	Val	Leu	Trp
3	Val	Met	Trp
4	Phe	Val	Ala
5	Phe	Met/Val	Met/Trp

consensus for tryptophan at the terminal position when screening with the Fmoc protected library **7** in both CHCl₃ and CH₃CN, the absence of tryptophan at the terminal position when screening with the deprotected library **8** in CHCl₃, and absence of any selectivity for the latter screen in CH₃CN suggests that the combination of the Fmoc protecting group and the tryptophan residue play an important role in the binding of the peptide guest.

To establish that the observed binding with resin-bound tweezers was also operating in free solution, tweezer **9** was prepared by solid-phase synthesis as a single compound with tetrapeptide side arms Phe-Val-Leu-Trp, which corresponds to the most commonly found sequence in the screening experiments. The synthesis of **9** was most easily achieved with the Sieber amide resin,^[16] which ultimately allowed cleavage of the tweezer from the resin with 1% CF₃CO₂H in CH₂Cl₂ (Scheme 3). Unfortunately tweezer **9** was essentially insoluble



Scheme 3. a) 20% piperidine, DMF; b) **5**, HOBT, DIC, DMAP, CH₂Cl₂; c) Fmoc-L-Val-OH, HOBT, DIC, DMAP, CH₂Cl₂; d) Fmoc-L-Leu-OH, HOBT, DIC, DMAP, CH₂Cl₂; e) Fmoc-L-Trp-OH, HOBT, DIC, DMAP, CH₂Cl₂; f) 1% CF₃CO₂H, CH₂Cl₂.

in neat CDCl₃ or neat CD₃CN and therefore did not allow NMR studies on the formation of a complex with DNS-L-Glu(OrBu)-L-Ser(OrBu)-L-Val-OH. A 500 μ M solution of **9** in DMSO:CHCl₃ (2:98) could, however, be prepared and allowed us to study the complexation of **9** with DNS-L-Glu(OrBu)-L-Ser(OrBu)-L-Val-OH by fluorescence spectroscopy. The intensity of the fluorescence emission maximum (at 494 nm) for the dansyl group of the peptide guest decreased as aliquots of the tweezer receptor **9** were added, with the drop in intensity exhibiting typical saturation. The data from this experiment showed a good fit for the presumed 1:1 binding and allowed an estimation of the binding constant

as $2.6 \times 10^5 \text{ M}^{-1}$ ($-\Delta G_a = 30.4 \text{ kJ mol}^{-1}$).^[17] Thus, as anticipated the incorporation of a specific binding site for the carboxylic acid terminus of peptide guests into a tweezer structure provides binders with considerably higher affinity than tweezers with a nonspecific head group.

The selectivity of the library was further probed by carrying out identical screening experiments to those described above, but with Ac-L-Lys(DNS)-D-Ala-D-Ala-OH as the guest tripeptide.^[18] No selectivity was observed for this guest when it was screened with the Fmoc-protected library **7**, but high selectivity was again observed when screened against the deprotected library **8** (approximately 1–2% of beads were highly fluorescent, although there were also a number of beads with intermediate levels of fluorescence, which suggested that the library was less selective for this peptide guest than for DNS-L-Glu(OrBu)-L-Ser(OrBu)-L-Val-OH). Indeed Edman sequencing of nine selected beads from this latter screen showed a lower overall consensus compared to screening with DNS-L-Glu(OrBu)-L-Ser(OrBu)-L-Val-OH, but there was considerable consensus for the second position, with seven of the nine sequences having Asn or Gln (Table 4). Significantly, the observed sequences were essentially com-

Table 4. Sequencing data for nine fluorescent beads selected from the screening experiment of Ac-L-Lys(DNS)-D-Ala-D-Ala-OH with the deprotected library **8** in CHCl_3 .

Bead	AA ¹	AA ²	AA ³
1	Trp	Asn	Gly
2	Trp	Asn	Gly
3	Trp	Val	Phe
4	Leu	Val	Phe
5	Leu	Gln	Leu
6	Val	Gln	Leu
7	Phe	Gln	Val
8	Gln	Gln	Gln
9	Met	Gln	Lys

pletely different to those found for the screening with DNS-L-Glu(OrBu)-L-Ser(OrBu)-L-Val-OH, which confirms that the tweezer receptors are selective for different peptide substrates.

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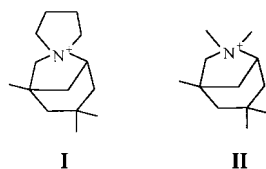
ITQ-7: A New Pure Silica Polymorph with a Three-Dimensional System of Large Pore Channels**

Luis A. Villaescusa, Philip A. Barrett, and Miguel A. Camblor*

Zeolites find widespread uses in a number of technologically important areas, including adsorption, separation, and catalysis.^[1] For some of these applications it is desirable that the zeolite possesses a three-dimensional system of large pores, since this greatly facilitates the diffusion of relatively large molecules into and out of the zeolite void space and in turn minimizes pore blockage. Additionally, it may also be important that the zeolite can be prepared with high Si/Al ratios, since this improves the chemical, thermal, and hydrothermal stability as well as the hydrophobicity, while at the same time modifying the reactivity and distribution of the active sites. However, despite considerable synthetic efforts throughout the last three decades, zeolite Beta is the only known zeolite to possess a three-dimensional system of large pores that can be directly synthesized with Si/Al ratios higher than around 5.^[2] Zeolite Beta is a complex intergrown structure of at least two polymorphs, with all of them possessing large pores defined by windows that contain 12 $\text{TO}_{4/2}$ tetrahedra (12MR windows, where T = Si or Al).^[3]

Our ongoing project on the synthesis of pure and high-silica zeolites in a fluoride-containing medium has produced a number of new pure silica materials during the last three years, including structures with a remarkably low framework density and a high void space.^[4] We report here the synthesis and structure of ITQ-7 (Instituto de Tecnología Química number 7), a new high-silica zeolite with a three-dimensional system of large pore 12MR channels. This material has a very low framework density ($FD = 15.37$, $FD = \text{number of Si per } 1000 \text{ \AA}^3$) which ranks it as the least dense phase ever reported amongst pure silica polymorphs along side chabazite ($FD = 15.40$). ITQ-7 is structurally related to zeolite Beta ($FD = 15.6$)^[5] and its successful synthesis has a certain degree of a priori design.

The synthesis was performed with a new rigid and well-defined organic structure directing agent (SDA) in the presence of fluoride ions. The SDA spyro cation **I** (1,3,3-trimethyl-6-azoniumtricyclo[3.2.1]dodecane) is a modification of the SDA used to produce ITQ-3, ITQ-9, SSZ-31, and some materials that may be intergrowths of zeolite Beta and SSZ-31 (1,3,3,6,6-pentamethyl-6-azoniumbicyclo[3.2.1]octane, SDA



II).^[6] The modification consists of the quaternization of the nitrogen atom of the parent amine with $\text{Br}(\text{CH}_2)_4\text{Br}$ rather than CH_3I in order to form a new ring that gives the whole SDA a larger volume while maintaining a rather rigid conformation. This change was aimed at altering the structure-directing effect to produce a zeolite with an enhanced void space or pore dimensionality as well as to make it more specific, since rigidity and volume are considered amongst the most important features that endow a particular SDA with structure-direction specificity.^[7]

The strategy of using this large, rigid cation in a hydrothermal synthesis in an aqueous fluoride-containing medium afforded the pure silica ITQ-7. A new three-dimensional 4-connected framework of SiO_4 tetrahedra was produced after calcination. ^{29}Si MAS NMR spectroscopic analysis (Figure 1)^[8] shows calcined ITQ-7 contains essentially no Si-O-Si connectivity defects, since it is hard to detect any

resonance around $\delta = -102$ in the spectrum that can be assigned to Si-OH species. Furthermore, the spectrum shows some resolution of $\text{Si}(\text{OSi})_4$ sites, which are assigned to Si species in crystallographically distinct environments in the structure. Calcined ITQ-7 has a large micropore volume (around $0.22 \text{ cm}^3 \text{ g}^{-1}$, determined by N_2 adsorption with the t-plot method) and large pores ($6.8\text{--}6.9 \text{ \AA}$, determined by Ar adsorption with the Horvath-Kawazoe method).

The XRD pattern of calcined ITQ-7 can be indexed as tetragonal ($a = 12.88 \text{ \AA}$, $c = 25.27 \text{ \AA}$) and shows systematic absences consistent with space group $P4_2/mmc$ (and other subgroups). The pattern itself resembles that of the structure C described by Newsam et al. as a hypothetical framework related to polymorphs A and B of zeolite Beta.^[3] Furthermore, the cell dimensions and symmetry of structure C (tetragonal, $a = 12.81$, $c = 13.00 \text{ \AA}$, space group $P4_2/mmc$) suggests a close structural relationship between both topologies. Structure C has a three-dimensional system of large 12MR channels that intersect perpendicularly. In contrast to polymorphs A and B, all the channels in structure C are linear.^[3] We thus adopted a model building approach starting from structure C and looking for a transformation that could account for the doubling of the c edge in ITQ-7. It became clear that structure C can be easily constructed from the

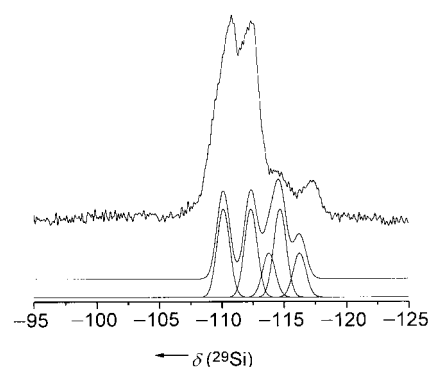


Figure 1. ^{29}Si MAS NMR spectra of calcined pure silica ITQ-7: experimental spectrum (top) and simulations using the equation of Thomas et al.^[8] and the average Si-O-Si angles for each of the crystallographic sites found in this work (bottom).

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simple stacking of a given sheet normal to the *a* or *b* direction, without any translation or rotation. Since the connection between each layer is accomplished through double four member ring units (D4MR), when consecutive sheets are rotated 90°, full connectivity between the layers can still be accomplished and a new structure different from structure C (and the related polymorphs of the zeolite Beta) can be formed. The maximum topological symmetry of this new topology is maintained at $P4_2/mmc$ and a doubling of the *c* edge of the cell is required to describe the structure, which suggests that it could be ITQ-7.^[9] The relationship between the topologies of ITQ-7 and structure C is illustrated in Figure 2. The model obtained for ITQ-7 was then optimized by a distance least squares

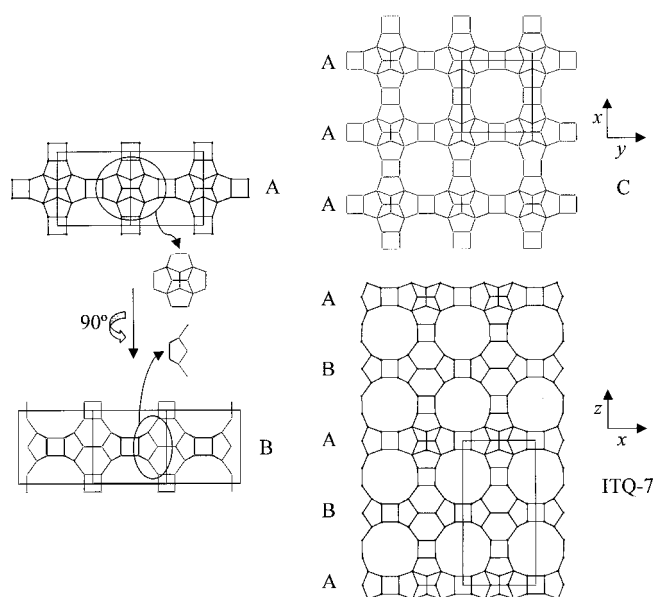


Figure 2. The relationship between the structures of ITQ-7 and the hypothetical structure C proposed by Newsam et al. for zeolite Beta.^[3] A tertiary building unit common to ITQ-7, and the polymorphs A, B, and structure C proposed by Newsam et al.^[3] (center of the left hand side) may be used to build a layer that is common to ITQ-7 and structure C. Connection of these layers without rotation through double four member rings yields structure C. The ITQ-7 topology is obtained when successive layers are rotated by 90° and connected in the same way.

procedure (program DLS76),^[10] and the refined coordinates produced were used to simulate the powder XRD pattern, which was immediately recognized as that of ITQ-7. Finally, Rietveld refinement of this model in the topological symmetry ($P4_2/mmc$) against the XRD data of calcined ITQ-7 obtained with conventional $\text{Cu}_{K\alpha}$ radiation was undertaken.

ITQ-7 has a new zeolite topology (Figure 3) which is closely related to those of polymorphs A, B, and the hypothetical structure C proposed by Newsam et al. for zeolite Beta.^[3] All these topologies may be built from the tertiary building unit shown in Figure 2. Different arrangements of this tertiary building block give rise to three-dimensional 4-connected nets containing a three-dimensional system of 12MR pores. All

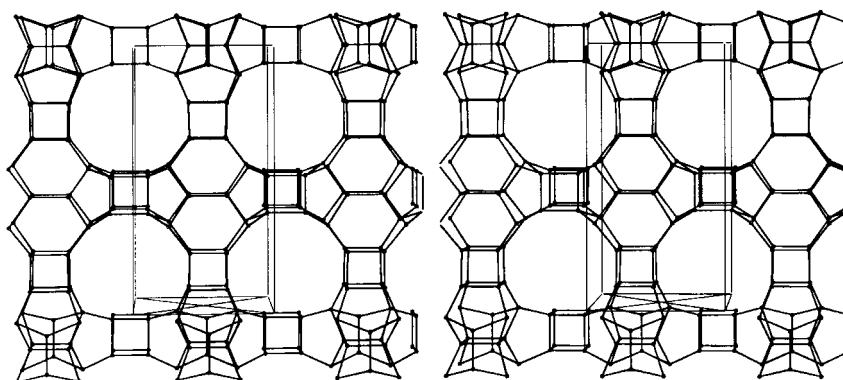


Figure 3. Stereo representation of the structure of ITQ-7 along [100]. Only connectivity between tetrahedral atoms is depicted.

four topologies have linear pores along two directions, while the third pore is sinusoidal in polymorphs A and B but linear in structure C. The channel running along the *c* direction in ITQ-7 is sinusoidal but has large linear sections of around 21 Å. This may give rise to different catalytic behavior of the corresponding aluminosilicates, as the linear sections in the *c* direction will put different steric restrictions on transition states in ITQ-7 relative to zeolite Beta.

An interesting feature in ITQ-7 is the presence of double four member ring cages, a structural unit that was usually considered to be very unfavorable for high-silica zeolites. However, pure silica octadecasil synthesized in the presence of fluoride ions presents a D4MR with the fluoride ion located inside this cage. This material gives a characteristic ^{19}F MAS NMR resonance at $\delta = -38.2$ or -38.5 , depending on the nature of the counteranion.^[11] The ^{19}F MAS NMR spectrum of noncalcined ITQ-7 suggests the F^- ions are also located inside the D4MR, as evident from its chemical shift (Figure 4). Actually, there are two symmetrically different types

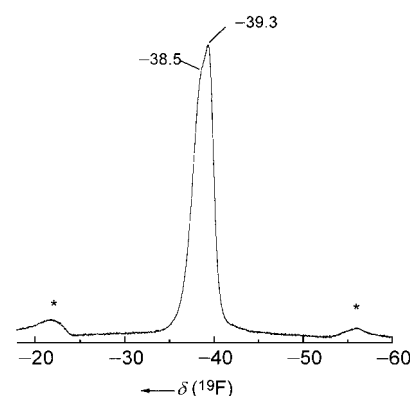


Figure 4. ^{19}F MAS NMR spectrum of noncalcined pure silica ITQ-7. Spinning side bands are marked with an asterisk.

of D4MR cages in ITQ-7 and the ^{19}F MAS NMR clearly consists of two overlapping resonances centered around $\delta = -38.5$ and -39.3 , which strongly suggests that F^- ions are located in both types of cages. Chemical analysis (F content: 1.69%) points to the full occupancy of the cages by F^- ions.

However, the ^{29}Si MAS NMR spectrum shown in Figure 1 does not completely agree with that expected (five signals for

Si(OSi)₄ sites with relative intensities 2:2:2:1:1), which, in our view, may be a strong suggestion that the actual symmetry of ITQ-7 is lower than *P4₂/mmc* (possibly *P4₂22* or *P4₂*). However, we don't find any evidence for this symmetry lowering in the low resolution XRD data, and attempts to refine the model in a lower symmetry were less satisfactory. High resolution XRD data with synchrotron radiation will be necessary to clarify this point.

Finally, preliminary energy minimization calculations of the SiO₂ framework using the GULP code^[12] and the potential model of Sanders et al.^[13] gave no evidence for a lower symmetry of ITQ-7, with the energy and cell dimensions being essentially equivalent in *P4₂/mmc*, *P4₂22*, and *P1* space groups. On the other hand, the calculations gave the following order of stability for SiO₂ frameworks: polymorph A > structure C > ITQ-7. We believe the successful synthesis of ITQ-7 is the result of combining two main structure-direction effects that allow us to overcome the energy penalty for this topology. The fluoride ion may show a preference towards structures containing very small cavities such as the D4MR,^[6] and thus it may exert some structure-direction effect in favor of ITQ-7 rather than zeolite Beta.^[14] On the other hand the shape, size, and rigidity of the SDA apparently favors ITQ-7 rather than structure C (which also contains D4MR units), possibly as a consequence of the bent shape of the cation and the different tortuosity of the channels in both topologies. We are currently investigating the structure-direction issues in the synthesis of ITQ-7, as crystallizations in different conditions may change the phase selectivity of the crystallization from ITQ-7 to zeolite Beta. Additionally, we have found evidence that, depending on the precise synthetic conditions, ITQ-7 samples may present intergrowths, the nature of which are currently under investigation.^[18]

Experimental Section

Synthesis: Pure silica ITQ-7 was synthesized by using the hydroxide form of **I** as a structure directing agent. This was prepared by quaternization of the parent compound 1,3,3-trimethyl-6-azabicyclo[3.2.1]octane (Aldrich) with 1,4-dibromobutane (99%, Aldrich) in a NaOH solution under reflux. The organic product was extracted with CH₂Cl₂. Evaporation of this solvent gave a solid product (93% yield), which was washed with diethyl ether and recrystallized from a mixture of methanol and ethyl acetate. The bromide salt was finally exchanged to the hydroxide form on an anion exchange resin. The zeolite was crystallized under hydrothermal conditions (PTFE-lined stainless-steel autoclaves, 150 °C, rotation (60 rpm), 15 days, pH 6.9, composition: SiO₂:C₁₄H₂₆NOH:HF:H₂O = 1:3:3:5.4) and contained the organic cation intact, as revealed by ¹³C MAS NMR and chemical analysis. The analysis also shows the charge is neutralized by occluded F⁻ ions (elemental analysis: found: C 14.84, N 1.23, H 2.40, F 1.69; calculated for the ideal unit cell of composition [C₁₄H₂₆NF]₄[SiO₂]₆₄: C 14.14, N 1.18, H 2.20, F 1.60). After calcination of the sample at 580 °C both the SDA and the fluoride ions are removed from the material.

Structure analysis: An X-ray powder diffraction pattern of calcined pure silica ITQ-7 was collected at room temperature with a step size of 0.01° for scan times of 11 s and 44 s per step over the ranges 2° ≤ 2θ ≤ 40° and 40° ≤ 2θ ≤ 85°, respectively, using conventional CuK_α radiation (Philips X'Pert diffractometer in flat plate mode). Profile refinement of the structural model derived from model building (see above) was performed by the Rietveld method^[15] with the GSAS program^[16] without the use of geometrical constraints. The profile was matched in the range 12° ≤ 2θ ≤ 75° because of the large asymmetry of the peaks at low 2θ angles and the very poor signal to noise ratio of the data at very high angle. A pseudo Voigt function, together with a manually interpolated background, was used to

describe the peak shape.^[17] A preferred orientation correction along the [001] direction and a peak broadening correction were applied. The final refinement used 62 variables and gave *R_{wp}* = 10.76, *R_p* = 8.27, *R_b* = 6.26, and χ² = 2.897. The average Si–O bond length and Si–O–Si and O–Si–O angles are 1.59 Å, 109.3°, and 151.9°, respectively. The final atomic coordinates are given in Table 1 and Figure 5 shows the Rietveld plot. Further details on the

Table 1. Atomic coordinates of ITQ-7 ([SiO₂]₆₄, tetragonal, *P4₂/mmc*, *a* = 12.8528(4), *c* = 25.2136(8) Å).

Atom	<i>x</i>	<i>y</i>	<i>z</i>
Si(1)	0.1192(5)	0.1146(5)	0.06251(17)
Si(2)	0.3062(5)	0.2511(5)	0.90459(22)
Si(3)	0.5	0.1167(6)	0.06146(26)
Si(4)	0.3822(5)	0.3849(5)	0.18813(18)
Si(5)	0.3793(7)	0.3880(6)	0
O(6)	0.1426(12)	0.1313(12)	0
O(7)	0.1512(11)	0	0.0788(6)
O(8)	0	0.1379(10)	0.0742(6)
O(9)	0.2002(9)	0.1888(9)	0.0870(3)
O(10)	0.3996(6)	0.1685(9)	0.9116(3)
O(11)	0.3197(8)	0.3334(9)	0.9486(3)
O(12)	0.3205(8)	0.3027(9)	0.8453(4)
O(13)	0.5	0	0.0759(6)
O(14)	0.5	0.1373(15)	0
O(15)	0.5	0.3733(14)	0
O(16)	0.3327(18)	0.5	0
O(17)	0.3584(7)	0.3584(7)	0.25
O(18)	0.5	0.3626(12)	0.1782(6)
O(19)	0.3552(12)	0.5	0.1725(6)

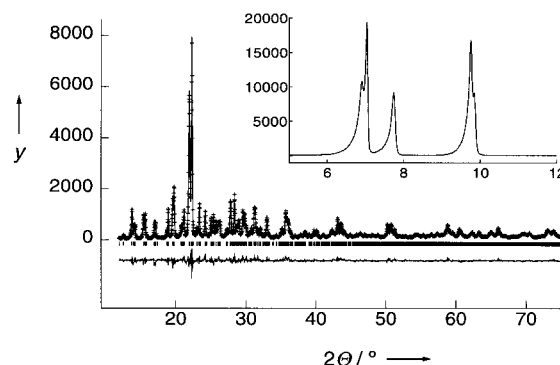


Figure 5. Observed (+) and calculated (solid line) X-ray diffraction patterns for calcined pure silica ITQ-7 (CuK_α radiation) following Rietveld refinement. Vertical ticks indicate the positions of allowed reflections in the *P4₂/mmc* space group. The lower trace is the difference plot, *y* = intensity (counts). The insert shows the low 2θ angle region, which was excluded from the refinement because of the extreme asymmetry of the peaks caused by axial divergence of the X-ray beam.

crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666, e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository number CSD-410723.

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Interaction of a PDZ Protein Domain with a Synthetic Library of All Human Protein C Termini**

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Through increasingly efficient sequencing of genes and whole genomes, the amino acid sequences of unknown proteins are being deduced at an accelerating rate. This means that questions about the function of these identified proteins in the corresponding organisms are becoming increasingly important. Alongside the “classical” approaches to analyzing the roles of these proteins by molecular biological/biochemical methods, through the use of bioinformatics it is possible to gain information about the new protein by sequence comparisons with proteins of known function. At the same time it has been demonstrated that large globular proteins are usually built up of individual domains that show independent folding and functions. Many of these domains are protein modules which occur in a number of, often unrelated, proteins.^[1] Protein sequence comparison not only reveals known protein domains, but also may identify novel, previously undetected stretches of homologous sequence in the new proteins.^[2] While known domains potentially point to the role of a protein, the function of new domains must be derived experimentally. Generally, protein domains can be produced by recombinant technology and folding in vitro. Many of them play a role in signal transduction and regulation processes where the identification of binding partners is a basic step towards discovering their function. To accomplish this, usually biologically generated protein libraries are enlisted to screen for the potential binding partner of the newly discovered domain.^[3]

We report here the chemical synthesis of a new kind of library, designed to discover binding partners for a PDZ

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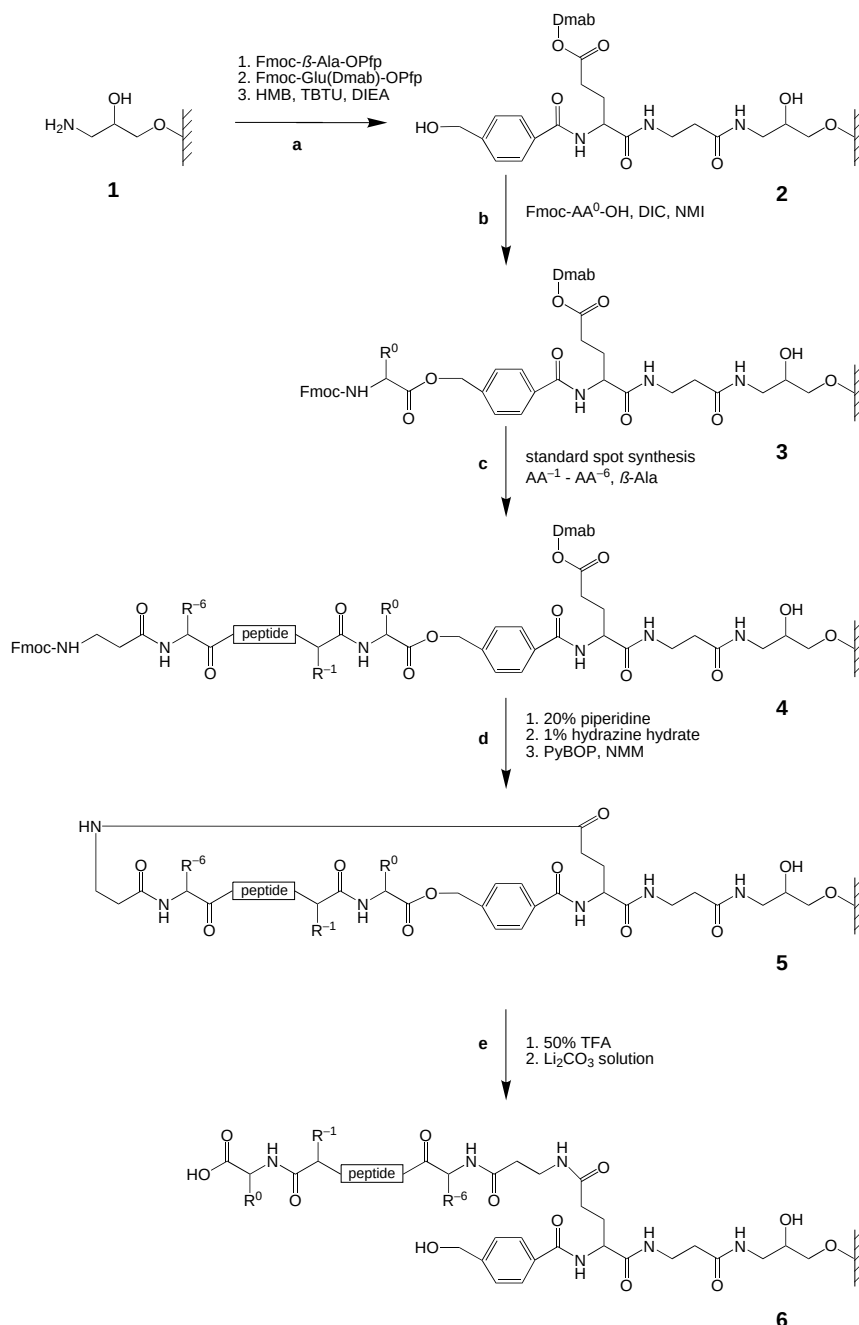
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domain comprising cellulose membrane-bound peptides with free carboxy termini (C termini). PDZ domains, the name being derived from the first letter of the proteins in which they were first discovered (*post synaptic density*, *disc large*, *zonula occludens*), are protein modules about 100 amino acids long which mainly occur in proteins of the cytoskeleton and play a role in signal transduction.^[4] Besides the observed heterodimerization^[5] PDZ domains interact predominantly with the C termini of their binding partners, the terminal carboxyl group being essential for this binding.^[6]

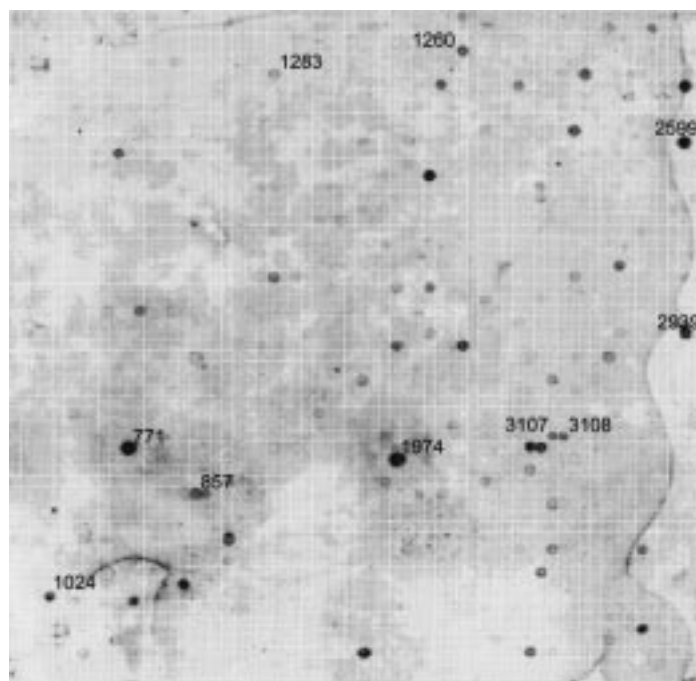
To be able to investigate these interactions using cellulose-bound peptide libraries, a method was developed for positionally directed generation of membrane-bound, inverted peptides. The peptides were synthesized by spot synthesis,^[7] cyclized, and finally linearized again to generate a free C terminus^[8] (Scheme 1). In this way the C termini of all known human proteins (SWISSPROT databank, release 34, 3514 sequences) were synthesized and tested for binding to a syntrophin–PDZ domain^[9] (Figure 1). A total of 38 clearly binding C termini were identified, and from chosen peptides the dissociation constants of the peptide–PDZ complexes were determined (Table 1).

In addition to a voltage-gated sodium channel already identified as a PDZ binding partner by two different approaches,^[10, 11] the strongest binding C termini belong to the α_2 subunit of soluble guanylate cyclase and the α -1A adrenergic receptor. An interaction with the α -1A adrenergic receptor is quite plausible since this membrane protein, like syntrophin, exists in heart muscle.^[12] The potential interaction of soluble guanylate cyclase^[13] is particularly interesting since as yet no binding partner for this enzyme is known; its gene was identified by cDNA sequencing. The binding of the native cyclase with the syntrophin–PDZ domain was confirmed using coprecipitation and subsequent Western blots (Figure 2A). The cyclase is activated by nitric oxide (NO) and produces the signal molecule cyclic guanosine monophosphate (cGMP). Therefore, functionally this interaction is plausible and also very interesting (Figure 2C) since NO synthase also interacts with syntrophin^[5] and, moreover, the cGMP-dependent protein kinase has been identified on the cytoplasmic side of the cell membrane.^[14]



Scheme 1. Synthesis of inverted peptides on cellulose membranes. Aminopropyl ether cellulose^[17] (**1**) was used as the matrix. a) β -Alanine serves both as a spacer and to define the synthesis position.^[7] Dmab-glutamic acid is coupled as a bivalent linker followed by hydroxymethylbenzoic acid (HMB) as a base-labile cleavage site ($\rightarrow$ **2**). b) The intended C-terminal amino acid is coupled through an ester bond ($\rightarrow$ **3**). c) Remaining amino acids and β -alanine as spacer are coupled ($\rightarrow$ **4**). d) The Fmoc and Dmab protecting groups on the N terminus and the side chains of the glutamic acids are cleaved off and the construct cyclized ($\rightarrow$ **5**). e) After removal of the side chain protecting groups, hydrolysis of the ester bond linearizes the construct and generates a free C terminus ($\rightarrow$ **6**). Dmab = α -4-[N-{1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl}amino]benzyl, Fmoc = 9-fluorenylmethoxycarbonyl, HMB = 4-hydroxymethylbenzoic acid, TBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, DIEA = *N,N*-diisopropylethylamine, DIC = diisopropylcarbodiimide, NMI = *N*-methylimidazole, PyBOP = benzotriazole-1-yloxytripyrrolidinophosphonium hexafluorophosphate, NMM = *N*-methylmorpholine, TFA = trifluoroacetic acid.

This spatial proximity of the three components of the NO/cGMP-mediated signal transduction cascade could facilitate higher local concentrations of the enzyme activators NO and cGMP and therefore efficient transfer of the signal.



Investigations into the binding specificity of this syntrophin–PDZ domain was made possible by the novel method of synthesizing membrane-bound peptide libraries with free C termini.^[8] A selection of binding peptides in noninverted form on cellulose showed no, or severely reduced, binding (not shown). For a more universal application of this method to identify linear binding sites which do not lie on the N or C terminus, the synthesis and testing of considerably greater numbers of peptides would be necessary. However, through selection of sequences which, for example, are found on the surface of known protein structures, the number of peptides can be reduced to a range that in the near future will be synthetically feasible.

Figure 1. Library of carboxy-terminal peptides from all known human proteins. The cellulose-bound library contains the C termini (heptamers) of all human proteins from the SWISSPROT databank (release 34). The figure shows the chemiluminescence intensity measured following incubation with horseradish peroxidase labeled syntrophin–PDZ domain. The numbers correspond to the peptides for which the dissociation constant was measured. The 38 strongest binders are listed in Table 1.

Table 1. Syntrophin–PDZ-binding peptides from human C termini.^[a]

Spot	Sequence	K_D	Acc. no.	Protein description
95	AKRCGCI		P39905	glial cell line-derived neurotrophic factor precursor
250	CHSETVV		P01135	transforming growth factor alpha precursor
771	FLRETSI	6 μ M	P33402	guanylate cyclase soluble, alpha-2 chain (EC 4.6.1.2)
857	GDRFFTI	1.3 mM	P18440	arylamine N-acetyltransferase, monomeric (EC 2.3.1.5)
940	GLNQRR		P07766	T-cell surface glycoprotein CD3 epsilon chain precursor
1016	GTRLTVV		P01733	T-cell receptor beta chain precursor V region (YT35)
1024	GVKESLV	76 μ M	P35499	sodium channel protein, skeletal muscle alpha-subunit
1260	IRTVVKI	804 μ M	P11766	alcohol dehydrogenase class III chi chain (EC 1.1.1.1)
1283	ITTKKYI	500 μ M	P51587	breast cancer type 2 susceptibility protein
1318	KAVETDV		P22459	potassium channel protein KV1.4
1477	KRISKRI		P07148	fatty acid-binding protein, liver (L-FABP)
1643	LKFRTEI		P49888	estrogen sulfotransferase (EC 2.8.2.4)
1677	LLRHERI		P16415	zinc finger protein ZFP-36 (fragment)
1774	LRHWLKV		Q01959	sodium-dependent dopamine transporter
1780	LRRESEI		P48049	inward rectifier potassium channel 2
1974	NLRETDI	3 μ M	P25100	alpha-1A adrenergic receptor
2331	PSQGHQP		P28329	choline O-acetyltransferase (EC 2.3.1.6)
2396	QSLETSV		P48050	calcium-transporting ATPase plasma membrane, isoform 4
2470	RGMRGKI		P15248	interleukin-9 precursor (IL-9)
2484	RIRHFKV		P02765	alpha-2-HS-glycoprotein precursor (FETUIN)
2499	RKSRRTI	–	P01589	interleukin-2 receptor alpha chain precursor
2569	RRSAGFI		P48645	neuromedin U-25 precursor (contains: NMU-25)
2599	RTRITFV	45 μ M	P33993	DNA replication licensing factor CDC47 homolog
2666	SERISSV		P28335	5-hydroxytryptamine 2C receptor
2813	SQKETS		P53618	coatamer beta subunit (beta coat protein) (beta-COP) (fragment)
2939	TCRTSII		P47872	secretin receptor precursor(SCR-R)
2972	THFLPRI		P10767	fibroblast growth factor-6 precursor (FGF-6) (HBGF-6)
3007	TKNRFVV		P06730	eukar. initiation factor 4 E (EIF-4E)
3107	TRVTVLG	246 μ M	P01702	Ig lambda chain V-I region (NIG-64)
3108	TRVTVLG	354 μ M	P01705	Ig lambda chain V-II region (NEI)
3125	TSRETDL		P22460	potassium channel protein KV1.5 (HK2) (HPCN1)
3126	TSRITTL		P30872	somatostatin receptor type 1 (SS1R) (SRIF-2)
3165	VAAFNKI		P24534	elongation factor 1-beta (EF-1-BETA)
3181	VCYOCKI		P26371	keratin, ultra high-sulfur matrix protein (UHS keratin)
3307	VQDKRFI		Q07001	acetylcholine receptor protein, delta chain precursor
3346	VTVETVV		P04201	MAS proto-oncogene
3455	YLRRSDV		P35080	profilin II
3485	YRRESAI		P48050	inward rectifier potassium channel 4

[a] The table shows the spot number corresponding to the position in the library (Figure 1), the amino acid sequence, the databank number (Acc. no.) of the appropriate SWISSPROT entry, and the protein description of the 38 identified C termini. For chosen binders the dissociation constant (K_D) was determined.

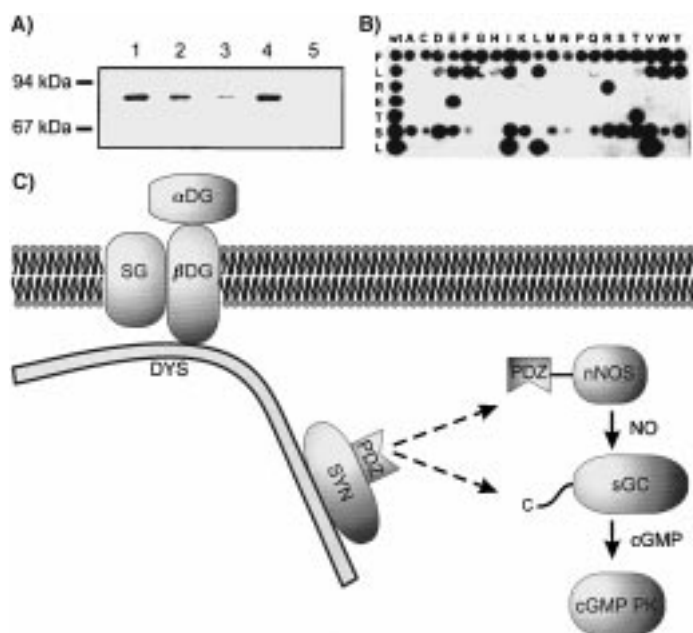


Figure 2. A) Interaction between soluble guanylate cyclase and syntrophin. Cell lysate containing guanylate cyclase^[20] was incubated with sepharose-bound syntrophin–PDZ domain.^[10] The PDZ domain was precipitated by sedimentation, and the whereabouts of cyclase analyzed by Western blotting.^[22] In a control experiment the C-terminal peptide FLRETSL–COOH was added to the cyclase. Lane 1: cell lysate, lane 2: supernatant, lane 3: pellet, lane 4: supernatant of the control experiment, lane 5: pellet of the control experiment. B) Substitutional analysis of the C terminus of cyclase. The peptide FLRETSL and its single-exchange analogues were synthesized on the cellulose membrane and tested for binding to the syntrophin–PDZ domain. The first column (wt) contains identical peptides with the sequence FLRETSL as controls. At every position the corresponding amino acid (rows) was substituted by all 20 L-amino acids (columns). This led to the identification of amino acids arginine (R), glutamic acid (G), and threonine (T) as key residues which cannot be exchanged without loss of binding activity. At the C-terminal position, in addition to leucine (L), isoleucine (I) or valine (V) was also tolerated. C) Syntrophin in the cell (model). Dystrophin (DYS) is anchored to the sarcolemma at the neuromuscular junction through α- and β-dystroglycan (αDG, βDG) and sarcoglycan (SG).^[23] Syntrophin (SYN) attaches to dystrophin^[24] and brings the neuronal NO synthase (nNOS) and the soluble guanylate cyclase (sGC) within proximity of the dystrophin–glycoprotein complex. nNOS, sGC, and the cGMP-dependent protein kinase (cGMP PK), which is also associated with the sarcolemma, build a signal transduction cascade with the help of the mediators NO and cGMP.

With the newly developed method, the door is opened to the future identification of binding partners for other PDZ domains in addition to derivation of a detailed description of their binding specificity by substitutional analyses (Figure 2B). Further protein–peptide interactions where terminal carboxyl groups play a role—for example, as described for some monoclonal antibodies^[15]—will similarly be open to investigation. Moreover, variations of this method will allow the synthesis of peptides with modified C termini,^[16] facilitating ways of studying the biological function of C-terminal modifications using peptide libraries.

Experimental Section

Library synthesis (Scheme 1): Library peptides with a chain length of seven were chosen since in the PDZ–peptide complexes identified to date the

last seven C-terminal amino acids are sufficient for the interaction. Whatman 50 filter paper (20 × 30 cm) which was amino functionalized through the anchor molecule 3-amino-2-hydroxy-propyl ether was used as the matrix.^[17] The peptide combinations were synthesized by the spot technique.^[7] a) 0.3 M Fmoc-β-alanine-OPfp in DMSO, double coupling, 2 × 15 min; Fmoc cleavage, 20% piperidine in DMF, 2 × 5 min; 0.3 M Dmab-glutamic acid-OH, 0.6 M DIEA, 0.3 M DIC, 0.3 M Pfp in *N*-methylpyrrolidine (NMP), double coupling, 2 × 15 min; Fmoc cleavage, 20% piperidine in DMF, 2 × 5 min; 0.3 M HMB, 0.6 M DIEA, 0.3 M TBTU, fourfold coupling, 4 × 15 min. b) 0.3 M Fmoc-AS-OH, 0.3 M DIC, 0.6 M NMI, fourfold coupling, 4 × 15 min. c) 0.3 M Fmoc-AS-OPfp, standard spot synthesis.^[7] d) Fmoc cleavage, 20% piperidine in DMF, 2 × 5 min; Dmab cleavage, 1% hydrazine hydrate in DMF, max. 2 × 3 min (to prevent ester hydrazinolysis); cyclization with 300 mg of PyBOP and 100 mL of NMM in 40 mL of DMF, 1 × 4 h, 1 × 16 h. e) Cleavage of the side chains, 50% TFA, 1% phenol, 2% water, 3% triisobutylsilane (TIBS) in CH₂Cl₂, 2.5 h; ester hydrolysis, saturated Li₂CO₃ solution in H₂O, 16 h. Due to the essential stable ether bridges with the cellulose the peptides are not amenable to analysis. Therefore, to confirm the procedure, analogous synthesis steps were carried out on Tentagel S RAM resin (Rapp Polymere, Tübingen, Germany). Usually syntheses on cellulose and on resin results in peptides of comparable purity.^[7,17] Following cleavage from the resin the products were analyzed by RP-HPLC and MALDI-TOF mass spectrometry. Compound 6 with the peptide sequence NYKQTSV was obtained with 87% purity, and the mass spectrum showed the calculated mass of this combination (not shown).

Detection of PDZ domain interactions with the membrane-bound peptide library: The PDZ domain as a glutathione-S-transferase (GST) fusion protein^[10] was attached through the sugar group to horseradish peroxidase.^[18] The library was washed with ethanol, then with TBS (50 mM Tris buffer, 100 mM NaCl, pH 8.0), blocked for 2 h with blocking buffer (CRB, Northwich, UK), and finally incubated with 1 mg mL^{−1} labeled domain in blocking buffer at 4 °C for 16 h. After threefold washing of the library with TBS, the bound domain was detected following addition of the chemiluminescent substrate Lumi-Light Plus Western Blotting substrate in a Lumi-Imager (Boehringer, Mannheim, Germany).

ELISA measurements of the dissociation constants: Microtiter plates were coated with streptavidin (125 ng per well in 0.1 M carbonate buffer, pH 9.6), washed three times with PBS (10 mM phosphate buffer, 150 mM NaCl, pH 7.3)/0.1% Tween 20 (Merck, Darmstadt, Germany), and 50 mL of peptide biotin-βA-βA-FLRETSL-OH (βA: β-alanine) (100 mM) in water were added. After threefold washing with PBS/0.1% Tween 20, horseradish peroxidase-labeled syntrophin–PDZ domain in the presence of various concentrations of the peptides (0.5–250 mM) in PBS/0.1% Tween 20/6% Gelifundol (Biotest, Dreieich, Germany) was added to the wells. Following three washes with PBS/0.1% Tween 20 the enzyme activity was measured using 5.5 mM *o*-phenylenediamine dihydrochloride (Fluka, Buchs, Switzerland)/8.5 mM H₂O₂ in 0.1% citrate buffer (pH 5.0). The reaction was stopped by the addition of 1 M sulphuric acid/0.05 M sodium sulphite. The absorption was measured at 492 nm and 620 nm (reference). The inhibition constants were calculated according to Friguet et al.^[19] and the values obtained by ELISA confirmed by grating coupler measurements (F. Kleinjung, not shown).

Expression of soluble guanylate cyclase and precipitation experiments: The α₂β₁ isoform of soluble guanylate cyclase was expressed in Sf9 insect cells.^[20] The cells were collected by centrifugation at 800 g for 10 min, resuspended in buffer (2 mM dithiothreitol, 2 mM EDTA, 0.2 mM benzamidine, 0.5 mM PMSF, 1 mM pepstatin A, and 50 mM triethanolamine/HCl, pH 7.4) and lysed by sonication; the cytoplasm was extracted by ultracentrifugation (200 000 g for 30 min at 4 °C). The syntrophin–PDZ–GST fusion protein was coupled to glutathione sepharose CL-4B (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. Protein concentrations were determined following the method of Bradford.^[21] Precipitation of the guanylate cyclase was carried out with 2 mg of Sf9 cytoplasmic cell protein and 1 mg of sepharose-bound PDZ domain in a volume of 500 μL (75 mM NaCl, 1 mM EDTA, 2 mM DL-dithiothreitol, 50 mM TEA, pH 7.4) for 10 min at room temperature. Sedimentation of the sepharose was carried out on ice (5 min), and separation of the proteins from the relevant fractions achieved by 7.5% SDS-PAGE. To detect the cyclase an immunoblot was carried out as described by

Harteneck et al.^[22] The antibodies used are directed against the C-terminal peptide of the α_2 subunit (KKVSYNIGTTMFLRETSL).

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N₅⁺: A Novel Homoleptic Polynitrogen Ion as a High Energy Density Material**

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Dedicated to Professor George Olah

Polynitrogen compounds are of significant interest as high energy density materials (HEDM) for propulsion or explosive applications.^[1–3] In spite of numerous theoretical studies predicting that certain all-nitrogen compounds might be stable, only a few unsuccessful experimental studies aimed at their actual synthesis have been undertaken.^[4] Presently, only two homoleptic polynitrogen species are known that can be prepared on a macroscopic scale: dinitrogen, N₂, which was independently isolated in pure form from air in 1772 by Rutherford, Scheele, and Cavendish, and the azide anion, N₃[−], discovered in 1890 by Curtius.^[5] Other species such as N₃[•], N₃⁺, and N₄⁺ have been observed only as free gaseous or matrix-isolated ions or radicals.^[6–8] In view of the extensive theoretical studies indicating that molecules such as N₄, N₈, N(N₃)₂[−], N(N₃)₃, and N(N₃)₄⁺ are vibrationally stable,^[4] the lack of a single successful synthesis of a new species on a macroscopic scale is surprising and may be a testament to the great experimental difficulties resulting from their high endothermicities, which give rise to instability and unpredictable explosiveness.

The high energy content of polynitrogen candidates stems from the N–N single and double bonds they possess. The average bond energies of 160 and 418 kJ mol^{−1}, respectively, are much less than one-third or two-thirds the N₂ triple bond energy of 954 kJ mol^{−1}.^[9] Therefore, any transformation of a polynitrogen compound to N₂ molecules is accompanied by a very large energy release, and any new metastable polynitrogen compound will be isolable and manageable only if it possesses a sufficiently large energy barrier to decomposition.

In view of the dearth of potential synthetic pathways for the construction of homoleptic polynitrogen rings or polycycles, and because many chain- or branch-type polynitrogen compounds are calculated to be lower in energy than their cyclic or polycyclic isomers,^[3] our efforts are focused on the synthesis of catenated polynitrogen species, which may be more readily accessible. The weakest link in a chain always

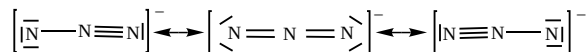
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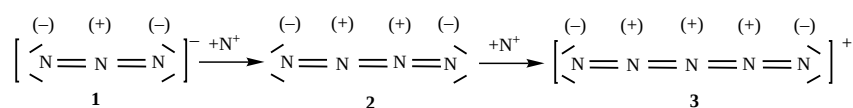
determines the overall strength, so it is imperative to search for target compounds devoid of any isolated N–N single bonds that cannot gain partial multiple bond character through resonance with neighboring bonds.

The building principle and unique resonance stabilization of the well-known and exceptionally stable azide anion (**1**, Scheme 1), in which each N–N bond has full double bond



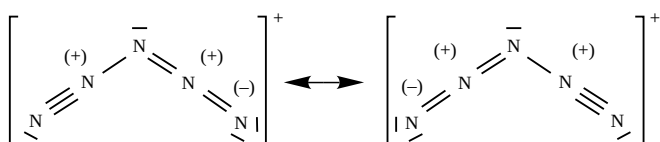
Scheme 1. Resonance structures of N_3^- (**1**).

character, might be further extended by the addition of nitrogen atoms containing only four valence electrons, that is, N^+ ions. This leads first to neutral N_4 (**2**) and then to the N_5^+ cation (**3**, Scheme 2).



Scheme 2. Addition of N^+ to N_3^- (**1**) to form N_4 (**2**) and N_5^+ (**3**) as well as the electronic charge distributions in **1**–**3**.

Although **2** and **3** contain, like azide, only cumulated linear N=N bonds, the electronic charge distributions shown in Scheme 1 are only favorable for N_3^- , whereas the neighboring positive charges render structures **2** and **3** energetically unfavorable. However, the problem of neighboring equal-sign charges can be remedied for N_5^+ with the resonance structures shown in Scheme 3, which result in a bent structure of C_{2v} symmetry with a bond order of 1.5 for the central N–N bonds. For linear N_4 , analogous structures cannot be written, and therefore N_5^+ was chosen as the prime target of our synthesis program.



Scheme 3. Resonance structures of N_5^+ (**3**).

Only one previous report on N_5^+ was found in the literature, a theoretical study of a series of ABCBA-type compounds by Pyykkö and Runeberg.^[10] Based on MP2/6-31G* calculations, they predicted a planar C_{2v} -symmetric structure with a B–C–B angle of 110.7° . The possible synthesis of N_5^+ was considered in 1992 by G. Rasul in his Ph.D. proposal at the University of Southern California, but it was not pursued.^[11] Theoretical calculations were used to predict whether the candidate is vibrationally stable, and IR, Raman, and NMR spectra were calculated to aid in the identification and characterization. For N_5^+ , these calculations predict the stable C_{2v} structure depicted in Figure 1. We report now the synthesis and characterization of $\text{N}_5^+\text{AsF}_6^-$, which constitutes only the third known compound containing a homoleptic polynitrogen moiety that is preparable on a macroscopic scale.

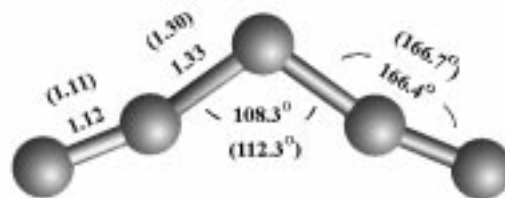
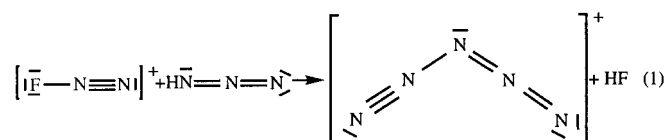


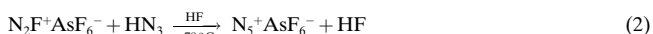
Figure 1. Optimized structures of N_5^+ calculated at the B3LYP (values given in parentheses) and CCSD(T)/6-311 + G(2d) levels of theory.

In designing a synthesis, it is useful to find energetic starting materials that already possess the energy-enhancing weakened bonds, the required formal charges, and suitable ligands that allow for an exothermic and facile coupling reaction. If the target molecule is a cation such as N_5^+ , the presence of a formal positive charge in one of the starting materials is very important in view of the high first ionization potential of N_2 (1503 kJ mol^{-1}). Equation (1) shows that the N_2F^+ cation and HN_3 are ideal starting materials for the synthesis of N_5^+ because they already possess the desired types of bonds, N_2F^+ provides the formal positive charge, and, in view of the weak N–F and strong H–F bond, the HF elimination reaction is expected to be exothermic.



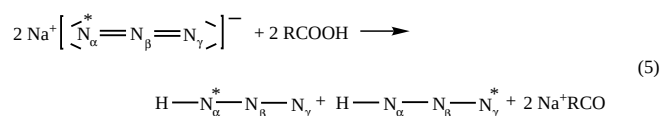
A final important point is the need for a reaction medium that offers good solubility at low temperatures, can act as a heat sink for the exothermic reaction, and can stabilize a product that is potentially very shock sensitive. For N_5^+ , anhydrous HF was chosen because of its high dipole moment, low melting point (-80°C), and high volatility.

Application of these principles led to a surprisingly straightforward synthesis of N_5^+ according to Equation (2). A

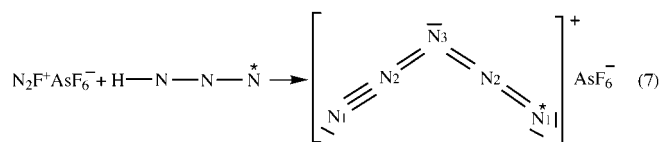
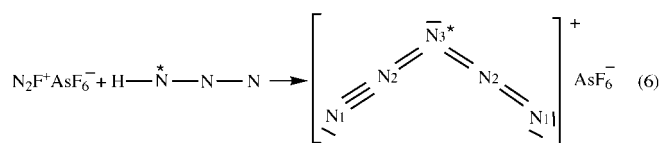


small excess of HN_3 was used to ensure complete conversion of the $\text{N}_2\text{F}^+\text{AsF}_6^-$. The only detectable by-product was less than 20 mol% of protonated HN_3 ,^[12] formed according to Equation (3). The AsF_5 required for the protonation of HN_3 to proceed could have formed by decomposition of some $\text{N}_5^+\text{AsF}_6^-$, or less likely by hydrolysis of $\text{N}_5^+\text{AsF}_6^-$ with traces of water in the system [Eq. (4)].

For the synthesis of ^{15}N -labeled N_5^+ , ^{15}N -labeled HN_3 was prepared from stearic acid and ^{15}N -labeled Na^+N_3^- [Eq. (5)].



The reaction of labeled HN_3 with $\text{N}_2\text{F}^+\text{AsF}_6^-$ produced a roughly equimolar mixture of N_5^+ with ^{15}N in either the 1- or 3-position [Eqs. (6), (7)].



The $\text{N}_5^+\text{AsF}_6^-$ salt is a white solid that is sparingly soluble in anhydrous HF. It is marginally stable at 22°C and can be stored for weeks at -78°C without noticeable decomposition. It can be handled both in HF solution or as a solid and, in our experience, has not exploded during careful normal handling or when squashed with a stainless steel spatula at -196°C . It has survived numerous exposures to a focused 488-nm Ar-ion laser beam (1.5 watt) at -130°C , although a 5-mg sample did explode on one occasion with sufficient force to destroy our low-temperature Raman device. It is a powerful oxidizer, capable of igniting organic substances such as foam rubber even at low temperatures. The reaction of $\text{N}_5^+\text{AsF}_6^-$ with water is violently explosive and should be avoided. This is not surprising in view of the facts that O_2^+ is a powerful oxidizer and the first ionization potential of N_2 (1503 kJ mol^{-1}) is significantly higher than that of O_2 (1206 kJ mol^{-1}); the electron affinity of N_5^+ is the subject of a further computational study. The high energy density of N_5^+ was also confirmed by a calculation using the G2 method^[13] that gave formation enthalpies of $\Delta H_f^0 = 1478$ and $\Delta H_f^{298} = 1469 \text{ kJ mol}^{-1}$ for free gaseous N_5^+ .

Solid ^{15}N -labeled $\text{N}_5^+\text{AsF}_6^-$ in a quartz tube (3 mm outer diameter) was warmed in a stepwise manner from -78 to $+22^\circ\text{C}$ under a vacuum of 10^{-7} Torr while monitoring the volatile products with a mass spectrometer; the principal decomposition product detected was N_2 . After pumping at 22°C for 20 min, however, most of the solid remained and was identified by low-temperature Raman spectroscopy as $\text{N}_5^+\text{AsF}_6^-$, thus demonstrating that the compound has reasonable stability at room temperature. In samples prepared from an excess of HN_3 and containing some $\text{H}_2\text{N}_3^+\text{AsF}_6^-$ ^[12] as a by-product, HN_3 and its fragments, HF, and AsF_5 were also observed in the mass spectra.

The ^{14}N and ^{15}N NMR spectra of N_5^+ labeled in either the 1- or 3-position and the ^{14}N NMR spectrum of unlabeled N_5^+ were recorded at -63°C in anhydrous HF solution that was acidified with about 2 mol % of AsF_5 to slow down a potential exchange between the cation and the solvent.^[14] The spectra of the ^{15}N -labeled mixture are shown in Figure 2, and the observed and calculated chemical shifts are compared in Table 1.

The signals due to N1 and N3 were observable in the ^{15}N spectra at $\delta = -237.3$ and -100.4 , respectively, in excellent

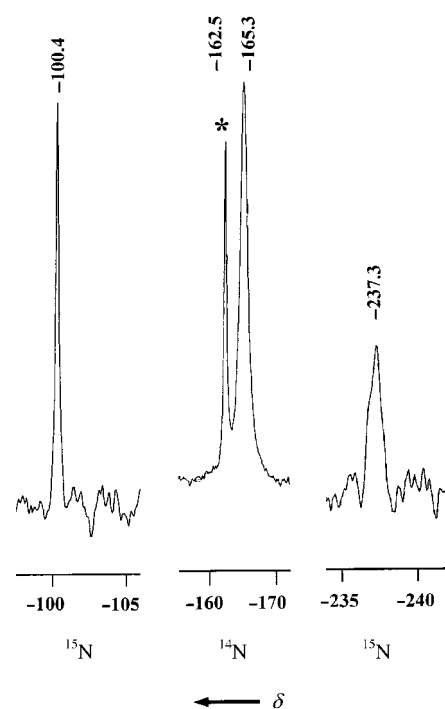


Figure 2. Sections of ^{14}N and ^{15}N NMR spectra for an equimolar mixture of singly ^{15}N -labeled $[\text{N}_5^+]$ and $[\text{N}_5^+]\text{AsF}_6^-$ recorded at -63°C in anhydrous HF solution that was acidified with 2 mol % AsF_5 . The resonance marked by an asterisk is due to H_2N_3^+ (see text for details).

Table 1. Observed and calculated ^{15}N and ^{14}N NMR data for N_5^+ .

	δ ^[a]				
	observed ^[b] ^{15}N	^{14}N	calculated ^[c] N1	N2	N3
$[\text{N}_5^+]$					
$[\text{N}_5^+]$	-237.3				
		-165.3 ^[d]	-235	-166	-95
$[\text{N}_5^+]$					
$[\text{N}_5^+]$	-100.4				

[a] Chemical shifts are given relative to that of neat CH_3NO_2 as external standard. [b] The spectra were recorded ^1H -coupled at -63°C in anhydrous HF solution which was acidified with AsF_5 . [c] Calculated at the CCSD(T)/QZP level of theory. An empirical correction of -20 ppm was applied to all calculated values, based on a comparison between the calculated and observed shifts of a number of closely related molecules and ions. [d] The other two resonances are badly broadened owing to exchange and were not detected in the ^{14}N spectrum.

agreement with the calculated values of $\delta = -235$ and -95 . The signal-to-noise ratio of the ^{15}N spectrum was low due to the poor solubility of $\text{N}_5^+\text{AsF}_6^-$ in HF at -63°C , and a long delay time (60 s) was needed because of the slow relaxation rates. The area ratio of the two signals was about 1:1, indicating that the synthesis of HN_3 from end-labeled N_3^- and stearic acid had resulted in about equimolar quantities of N_α^- and N_γ^- -labeled HN_3 . In addition to the two N_5^+ signals, two weaker signals at $\delta = -312.0$ (t, $^1J(^1\text{H}, ^{15}\text{N}) = 100.7 \text{ Hz}$) and -111.4 (s) were observed in the ^1H -coupled ^{15}N NMR spectrum that are attributable to N_α and N_γ , respectively, of $[\text{H}_2\text{N}_\alpha\text{N}_\beta\text{N}_\gamma]^+$.^[12] This was verified by recording the spectrum of a known sample of $\text{H}_2\text{N}_3^+\text{AsF}_6^-$ in HF solution.

In the ^{14}N spectrum of labeled and unlabeled $\text{N}_5^+\text{AsF}_6^-$ a single resonance at $\delta = -165.3$ was observed and assigned to

N2 of N_5^+ based on the calculated value of $\delta = -166$. The signals due to N1 and N3 could not be observed in the ^{14}N spectra under these conditions due to excessive quadrupole broadening. The N_β signal of $[\text{H}_2\text{N}_\alpha\text{N}_\beta\text{N}_\gamma]^+$ was also observable in the ^{14}N spectra of the labeled and unlabeled cations as a sharp resonance at $\delta = -162.5$, while signals for N_α and N_γ were strongly quadrupole broadened. It is fortunate that N2 of N_5^+ gives rise to a sharp ^{14}N signal and that the single ^{15}N substitution provided us with an equal mixture of ^{15}N labels on N1 and N3, thus allowing the unambiguous observation of all three signals of N_5^+ . Their excellent agreement with the calculated values provides positive proof for the presence of a C_{2v} -symmetric N_5^+ cation.

Additional unambiguous proof for the presence of C_{2v} - N_5^+ was provided by the vibrational spectra of $N_5^+\text{AsF}_6^-$ and the ^{14}N – ^{15}N isotopic shifts observed for the mixture of $^{15}\text{N1}$ - and $^{15}\text{N3}$ -labeled $N_5^+\text{AsF}_6^-$. The low-temperature Raman spectra of unlabeled and a mixture of labeled $N_5^+\text{AsF}_6^-$ are shown in Figures 3 and 4, respectively, and the observed frequencies are summarized in Tables 2 and 3. The vibrational assignments for octahedral AsF_6^- in Table 2 are well established^[15] and do not

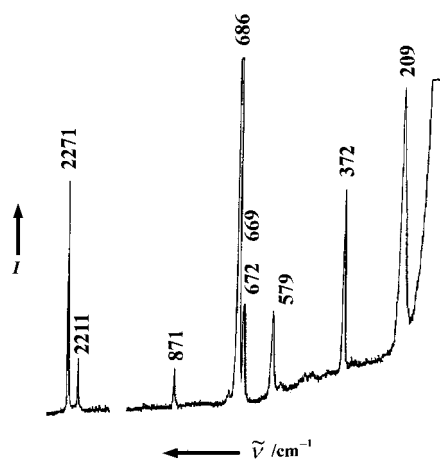


Figure 3. Low-temperature Raman spectrum of unlabeled solid $N_5^+\text{AsF}_6^-$.

require any further discussion; those for N_5^+ are based on our calculations. As can be seen, the four N–N stretching modes were observed with the predicted frequencies and intensities.

The spectra of $^{15}\text{N1}$ - and $^{15}\text{N3}$ -labeled N_5^+ allowed accurate measurements of the isotopic shifts for modes $\nu_2(\text{A}_1)$, $\nu_7(\text{B}_2)$, and $\nu_1(\text{A}_1)$. Again, the agreement between experiment and theory is very good and confirms the validity of the predicted structure given in Figure 1. Since the observed frequencies of N_5^+ are intermediate between those predicted at the CCSD(T) and the B3LYP levels of calculation, the actual geometry of N_5^+ is probably also intermediate between the CCSD(T) and B3LYP values of Figure 1. Therefore, the following geometry is interpolated for $[\text{N1-N2-N3-N2-N1}]^+$: $r(\text{N1-N2}) = 1.11 \text{ \AA}$, $r(\text{N2-N3}) = 1.315 \text{ \AA}$, $\angle(\text{N1-N2-N3}) = 166.6^\circ$, and $\angle(\text{N2-N3-N2}) = 110.3^\circ$.

The results from a normal coordinate analysis of N_5^+ are summarized in Table 4. They show that the A_2 , B_1 , and B_2 vibrations and $\nu_1(\text{A}_1)$ are all highly characteristic, but that $\nu_2(\text{A}_1)$ is a mixture of stretches and bends.

The internal force constants of greatest interest are the stretching force constants f_r and f_R of the terminal and the central N–N bonds, respectively. Interpolation of the data in Table 4 and adjustments for the observed frequencies give values of 20.08 and 6.59 $\text{mdyn \AA}^{-1}$ for the terminal and the central N–N stretching force constants, respectively. The

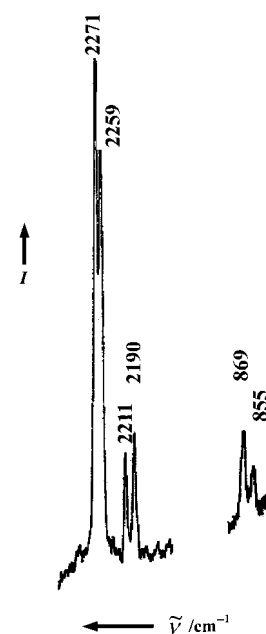


Figure 4. Low-temperature Raman spectrum of an equimolar mixture of solid $[\text{N}_5^+\text{AsF}_6^-]$ and $[\text{N}_5^+\text{AsF}_6^-]$.

Table 2. Low-temperature Raman and IR bands of solid $^{14}\text{N}_5^+\text{AsF}_6^-$ and their assignments based on the calculated harmonic frequencies of free gaseous N_5^+ .

Obsd frequencies [cm^{-1}] ^[a]		Assignment (point group)		Calcd frequencies [cm^{-1}] ^[b]	
Raman ^[c]	IR ^[d]	$^{14}\text{N}_5^+(\text{C}_{2v})$	$\text{AsF}_6^-(\text{O}_h)$	B3LYP	CCSD(T)
2271 [44]	2270 (m)	$\nu_1(\text{A}_1)$		2336 (22)	2229 (13) [215] ^[e]
2211 [8]	2210 (s)	$\nu_7(\text{B}_2)$		2282 (147)	2175 (105) [42]
	1088 (s)	$\nu_8(\text{B}_2)$		1167 (141)	1032 (138) [2]
871 [7]	872 (w)	$\nu_2(\text{A}_1)$		850 (4)	818 (0.5) [5]
	704 (vs)		$\nu_3(\text{F}_{1u})$		
686 [100]	680 (sh)		$\nu_1(\text{A}_{1g})$		
672 [17], 669 [18]		$\nu_3(\text{A}_1)$	or part of $\nu_1(\text{A}_{1g})$ or $\nu_3(\text{F}_{1u})$	678 (1)	644 (2) [1]
579 [16]	575 (w)		$\nu_2(\text{E}_g)$		
	420 (sh)	$\nu_5(\text{A}_2)$		502 (0)	475 (0) [1]
		$\nu_6(\text{B}_1)$		424 (7)	405 (6) [0]
		$\nu_9(\text{B}_2)$		436 (0.6)	399 (1) [0.5]
	394 (vs)		$\nu_4(\text{F}_{1u})$		
372 [32]	360 (sh), 380 (sh)		$\nu_5(\text{F}_{2g})$		
209 [44]		$\nu_4(\text{A}_1)$		193 (0.5)	181 (0.3) [6]
127 [55]		lattice vibration			

[a] Relative IR and Raman intensities given in parentheses and brackets, respectively. [b] Frequencies calculated using a 6–311 + G(2d) basis set. IR intensities given in parentheses [km mol^{-1}], and Raman intensities given in brackets [$\text{\AA}^4 \text{amu}^{-1}$]. [c] $T = -130^\circ\text{C}$. [d] $T = -196^\circ\text{C}$. [e] The Raman intensities were calculated at the RHF level.

Table 3. Comparison of the calculated (B3LYP) and observed ^{15}N isotopic shifts for N_5^+ .

N_5^+ isotopomer	$\nu_4(\text{A}_1)$	$\nu_9(\text{B}_2)$	$\nu_6(\text{B}_1)$	Calcd frequencies (shifts) [cm^{-1}]					$\nu_8(\text{B}_2)$	$\nu_7(\text{B}_2)$	$\nu_1(\text{A}_1)$
				$\nu_5(\text{A}_2)$	$\nu_3(\text{A}_1)$	$\nu_2(\text{A}_1)$					
$^{14}\text{N}_1\text{-}^{14}\text{N}_2\text{-}^{14}\text{N}_3\text{-}^{14}\text{N}_2\text{-}^{14}\text{N}_1$	193.1(0)	424.1(0)	436.3(0)	502.4(0)	678.1(0)	850.0(0)		1116.9(0)	2281.7(0)	2336.3(0)	
$^{14}\text{N}_1\text{-}^{14}\text{N}_2\text{-}^{15}\text{N}_3\text{-}^{14}\text{N}_2\text{-}^{14}\text{N}_1$	191.8(1.3)	422.0(2.1)	436.0(.2)	502.4(0)	677.5(.7)	833.3(16.7)		1138.0(28.9)	2281.2(.5)	2336.3(.1)	
$^{15}\text{N}_1\text{-}^{14}\text{N}_2\text{-}^{14}\text{N}_3\text{-}^{14}\text{N}_2\text{-}^{14}\text{N}_1$	190.8(2.2)	422.3 (1.8)	434.7(1.5)	500.7(1.7)	674.4(3.7)	847.4(2.6)		1163.4(3.5)	2259.8(21.9)	2324.5(11.9)	

N_5^+ isotopomer	Obsd frequencies (shifts) [cm^{-1}] ^[a]					
	$\nu_2(\text{A}_1)$, IR	$\nu_2(\text{A}_1)$, RA	$\nu_7(\text{B}_2)$, IR	$\nu_7(\text{B}_2)$, RA	$\nu_1(\text{A}_1)$, IR	$\nu_1(\text{A}_1)$, RA
$^{14}\text{N}_1\text{-}^{14}\text{N}_2\text{-}^{14}\text{N}_3\text{-}^{14}\text{N}_2\text{-}^{14}\text{N}_1$	872(0)	871(0)	2210(0)	2211(0)	2271(0)	2271(0)
$^{14}\text{N}_1\text{-}^{14}\text{N}_2\text{-}^{15}\text{N}_3\text{-}^{14}\text{N}_2\text{-}^{14}\text{N}_1$	858(14)	855(16)	2209.8	2211(1)	2270.8	2271(0)
$^{15}\text{N}_1\text{-}^{14}\text{N}_2\text{-}^{14}\text{N}_3\text{-}^{14}\text{N}_2\text{-}^{14}\text{N}_1$	870(2)	869(2)	2189.0(21)	2190(21)	2259.0(12)	2259(12)

[a] RA = Raman.

Table 4. Results from the normal coordinate analysis^[a] of N_5^+ .

Approx. mode in point group C_{2v}			Frequency [cm^{-1}]		Symmetry force constants ^[b] CCSD(T) (B3LYP)				Potential energy distribution ^[a] [%]
			obsd	calcd	F_{11}	F_{22}	F_{33}	F_{44}	CCSD(T)
				CCSD(T) (B3LYP)					
A_1	ν_1	in-phase terminal stretches	2270	2229 (2336)	F_{11}	19.573(21.314)			93(1) + 6(2)
	ν_2	sym. central stretch	872	818 (850)	F_{22}	0.702(0.843)	5.546(6.952)		62(2) + 23(3) + 13(4) + 2(1)
	ν_3	central bending		644 (678)	F_{33}	-0.085(-0.137)	1.377(1.535)	1.540(1.427)	39(3) + 33(2) + 23(4) + 5(1)
	ν_4	in-phase terminal bends	209	181 (193)	F_{44}	0.167(0.171)	0.204(0.312)	0.120(0.108)	64(4) + 37(3) - (2)
A_1	ν_5	torsion		475 (502)	F_{55}	0.0266(0.0281)			100(5)
B_1	ν_6	torsion		405 (424)	F_{66}	0.0236(0.0246)			100(6)
					F_{77}		F_{88}	F_{99}	
B_2	ν_7	out-of-phase term stretch	2210	2175 (2282)	F_{77}	19.491(21.272)			96(7) + 4(8)
	ν_8	asym. central stretch	1088	1032 (1167)	F_{88}	1.197(1.359)	4.780(5.927)		95(8) + 4(7)
	ν_9	out-of-phase terminal bends		399 (436)	F_{99}	0.200(0.195)	0.085(0.159)	0.358(0.423)	99(9) + 1(8)

[a] The following symmetry coordinates were used for $[\text{N}_1\text{-N}_2\text{-N}_3\text{-N}_2'\text{-N}_1']^+$: $\text{S}_1 = \nu(1-2) + \nu(1'-2')$; $\text{S}_2 = \nu(2-3) + \nu(2'-3)$; $\text{S}_3 = \delta(2-3-2')$; $\text{S}_4 = \delta(1-2-3) + \delta(1'-2'-3)$; $\text{S}_5 = \tau(1-2-3-2') + \tau(2-3-2'-1')$; $\text{S}_6 = \tau(1-2-3-2') - \tau(2-3-2'-1')$; $\text{S}_7 = \nu(1-2) - \nu(1'-2')$; $\text{S}_8 = \nu(2-3) - \nu(2'-3)$; $\text{S}_9 = \delta(1-2-3) - \delta(1'-2'-3)$. [b] The two most important internal force constants, estimated from the calculated symmetry force constants and the observed frequencies are $f_{(1-2)} = 20.08 \text{ mdyn } \text{\AA}^{-1}$ and $f_{(2-3)} = 6.59 \text{ mdyn } \text{\AA}^{-1}$. Stretching constants in $\text{mdyn } \text{\AA}^{-1}$, deformation constants in $\text{mdyn } \text{\AA}^{-1} \text{ rad}^{-2}$, and stretch-bend interaction constants in mdyn rad^{-1} .

former value is significantly lower than the $22.4 \text{ mdyn } \text{\AA}^{-1}$ found for the $\text{N}\equiv\text{N}$ bond in N_2 ,^[15] whereas the latter value is between those found for typical $\text{N}-\text{N}$ single ($f_{\text{N}-\text{N}} = 3.6 \text{ mdyn } \text{\AA}^{-1}$) and double bonds ($f_{\text{N}=\text{N}} = 10.2 \text{ mdyn } \text{\AA}^{-1}$).^[15] The strengthening of the $\text{N}-\text{N}$ central bonds at the expense of the terminal bonds, as suggested by the resonance structures, explains the relative stability of N_5^+ toward N_2 elimination. Reliable calculations of the energy barrier for N_2 elimination from N_5^+ will be the subject of a separate study.

Experimental Section

Caution! $\text{N}_5^+\text{AsF}_6^-$ is a highly energetic, strongly oxidizing material that can detonate violently. It should be handled only on a very small scale while using appropriate safety precautions (face shields, leather gloves, and protective clothing).

The $\text{N}_2\text{F}^+\text{AsF}_6^-$ was prepared from *cis*- N_2F_2 and AsF_5 as previously described.^[16a-c] The HN_3 was generated by heating NaN_3 with a threefold excess of stearic acid to about 80°C under a dynamic vacuum and collecting the evolved HN_3 in a trap cooled with liquid N_2 . The HN_3 was purified by fractional condensation through a series of traps at -64 , -95 , and -196°C , with the material retained at -95°C being used. Singly ^{15}N -labeled NaN_3 (Cambridge Isotope Laboratories, 99% ^{15}N label) was used for the preparation of a roughly 50:50 mixture of HN_3 that was singly ^{15}N -labeled in either the α or γ position. The HF (Matheson Co.) was dried by storage over BiF_3 .^[17]

The HN_3 was generated and handled on a Pyrex glass vacuum line equipped with grease-free Kontes glass-Teflon valves. The HF was handled on a previously described^[18] stainless steel-Teflon FEP vacuum line. The $\text{N}_5^+\text{AsF}_6^-$ samples were handled at -196°C in the dry N_2 atmosphere of a glove box.

Low-temperature Raman spectra were recorded on a Cary Model 83GT spectrometer using the 488-nm line of an Ar-ion laser for excitation, a previously described cooling device,^[19] and quartz tubes (3 mm outer diameter) as sample containers. For measurements of the $^{14}\text{N}-^{15}\text{N}$ isotopic shifts, the signal was scale expanded on an external strip chart recorder. The low-temperature IR spectra were recorded on a Mattson Galaxy FTIR

spectrometer using a demountable low-temperature cell equipped with CsI windows. The ^{14}N and ^{15}N NMR spectra were recorded at 36.13 and 50.69 MHz, respectively, on a Bruker AMX 500 spectrometer using saturated solutions of $\text{N}_5^+\text{AsF}_6^-$ in HF/AsF_5 at -63°C and heat-sealed 5-mm Teflon-FEP liners (Wilma Glass Co.) as sample containers, delay times of 60–120 s for the recording of the ^{15}N spectra, and neat CH_3NO_2 and aqueous ^{15}N -labeled urea referenced as $\delta = -305.0$ relative to CH_3NO_2 as external standards. The spectra were recorded both ^1H -coupled and ^1H -decoupled to determine if there was a nuclear Overhauser effect.

Preparation of $\text{N}_5^+\text{AsF}_6^-$: In a typical experiment, $\text{N}_2\text{F}^+\text{AsF}_6^-$ (1.97 mmol) was loaded in the drybox into a Teflon-FEP ampule (1.9 cm outer diameter) that was closed by a stainless steel valve. On the metal vacuum line, anhydrous HF (≈ 3 mL) was added at -196°C , and the mixture was warmed to room temperature to dissolve the $\text{N}_2\text{F}^+\text{AsF}_6^-$. The ampule was connected to the glass line, and HN_3 (2.39 mmol) was added at -196°C . The ampule was reconnected to the metal line and allowed to warm to -78°C , where it was kept for three days with occasional gentle agitation. The ampule was then cooled to -196°C to check for the presence of volatile products. Nitrogen (0.76 mmol) was identified by mass spectrometry. All material volatile at -64°C was pumped off for 8 h, leaving behind a white solid residue that was identified by low-temperature vibrational and ^{14}N and ^{15}N NMR spectroscopy as a mixture of $\text{N}_5^+\text{AsF}_6^-$ (≈ 80 mol %) and $\text{H}_2\text{N}_3^+\text{AsF}_6^-$ (≈ 20 mol %).

Preparation of ^{15}N -labeled $\text{N}_5^+\text{AsF}_6^-$: The procedure was identical to that used for the synthesis of unlabeled $\text{N}_5^+\text{AsF}_6^-$, except for the use of a mixture of HN_3 that was ^{15}N -labeled in either the N_α or N_γ position.

Computational Methods. Hartree–Fock (HF), density functional theory (DFT), and single- and double-excitation coupled cluster calculations^[20] that include a noniterative treatment of connected triple excitations (denoted CCSD(T))^[21] were carried out employing several atomic basis sets. The DFT calculations employed the B3LYP functional.^[22] Geometries, IR and Raman spectra, and nuclear magnetic resonance shieldings were calculated for N_5^+ as well as (for calibration purposes) N_2F^+ , H_2N_3^+ , and the standard CH_3NO_2 , the geometries and NMR parameters of which are experimentally known.^[12, 14, 16] The vibrational spectra reported in this work were computed using the 6-311 + G(2d) atomic basis set.^[23] The NMR shieldings were computed at the CCSD(T)/6-311 + G(2d) geometries employing the gauge-including atomic orbital (GIAO) solution to the gauge-invariance problem^[24] and density matrices obtained from second-order many-body perturbation theory [MBPT(2)], CCSD, or CCSD(T) calculations.^[25–27] The results reported in this work used quadruple-zeta plus polarization (QZP) atomic basis sets derived from the QZ sets of Schäfer et al.,^[28] supplemented with a d function with exponent 1.0. As discussed by Gauss and Stanton,^[25–27] the accurate calculation of nitrogen NMR shieldings frequently requires an extensive electron correlation treatment such as that provided by the CCSD(T) method. Hartree–Fock and even MBPT(2) shielding calculations for species such as N_5^+ with many free valence electron pairs yield extremely poor results. The GAMESS,^[29] Gaussian,^[30] and ACES II^[31] program systems were used for these calculations on IBM RS/6000 work stations.

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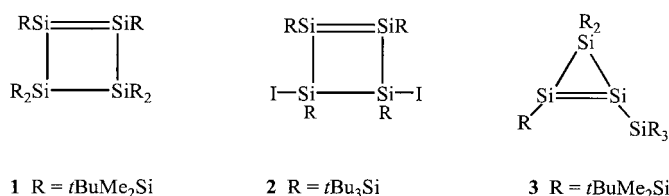
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Chalcogenatetrasilacyclopentenes: Five-Membered Rings with Endocyclic Si–Si Double Bonds**

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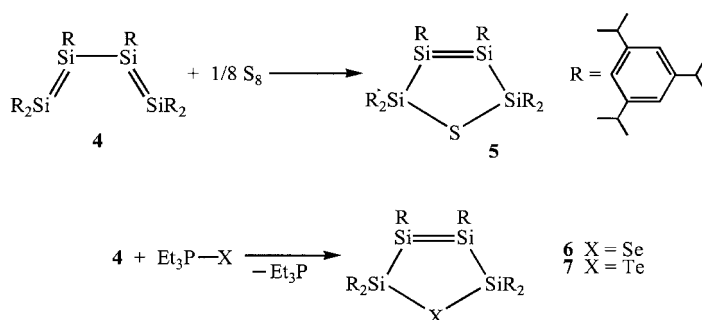
*Dedicated to Professor Gerhard Fritz
on the occasion of his 80th birthday*

Acyclic disilenes are now well-established molecules, and their properties have been summarized in several review articles.^[1] However, molecules containing an endocyclic Si–Si double bond were virtually unknown until recently. The first compound containing this structural element, the cyclotetrasilene **1**, was obtained by Kira et al. by the coupling reaction of two oligosilanes in very low yield (1.5%).^[2] In contrast, Wiberg et al. were able to isolate the cyclotetrasilene **2** in high yield from the reaction of a *tetrahedro*-tetrasilane with iodine.^[3] Very recently the formation of the cyclotrisilene **3** was described, whose constitution was indicated by spectroscopic data.^[4] We now report on the synthesis, properties, and structures of the first five-membered rings containing Si–Si double bonds.



After the successful synthesis of the tetrasilabutadiene **4**^[5] we presumed that this molecule should be ideally suited for [4 + 2] cycloadditions, since it adopts the *s-cis* form in the solid state as well as in solution.^[6] However, all attempted reactions of **4** with either electron-poor and electron-rich olefins or with acetylenes have remained unsuccessful so far. The reason might be the steric overcrowding of **4** as well as the large 1,4-separation of the silicon atoms of 540 pm.

Thus, it was even more surprising that treatment of **4** with sulfur afforded the five-membered ring **5** in a formal [4 + 1] cycloaddition. Under addition of a small amount of triethylphosphane, selenium and tellurium reacted with **4** to give the analogous rings **6** and **7** in high yield. As opposed to the



reactions of disilenes with sulfur,^[7] selenium, and tellurium,^[8] from which the chalcogenadisilacyclopropanes can be obtained at room temperature, the additions to the tetrasilabutadiene **4** demanded heating in toluene. The reaction time increased appreciably from sulfur to tellurium.

First indications on the constitution of **5–7** were provided by the ²⁹Si NMR spectra (Table 1), in which only two singlets were detected. The low-field signals are found in the characteristic region for 1,2-diaryl-1,2-disilyldisilenes,^[1] while the high-field signals are consistent with the decreasing electronegativity from sulfur to tellurium. A similar tendency was observed in three-membered rings containing two silicon atoms and a chalcogen atom.^[7b] The electron spectra of **5–7** show an analogous trend, with a bathochromic shift of the longest wavelength absorption from sulfur to tellurium.

Compounds **5–7** crystallize isotypically, each with two molecules of toluene. Figure 1 depicts the framework of the sulfur compound, and Figure 2 reflects the steric crowding of

Table 1. Selected data of the ring compounds **5–7**.

Compd.	M.p. [°C]	δ(²⁹ Si) ^[a]	λ _{max} [nm] (ε) ^[b]
5	> 300 (decomp)	86.0, –19.3	423 (16 240)
6	279–281	90.3, –27.7	431 (10 140)
7	228–229 (decomp)	97.8, –53.5	459 (11 440)

[a] ²⁹Si NMR in [D₆]benzene. [b] UV/Vis in *n*-hexane.

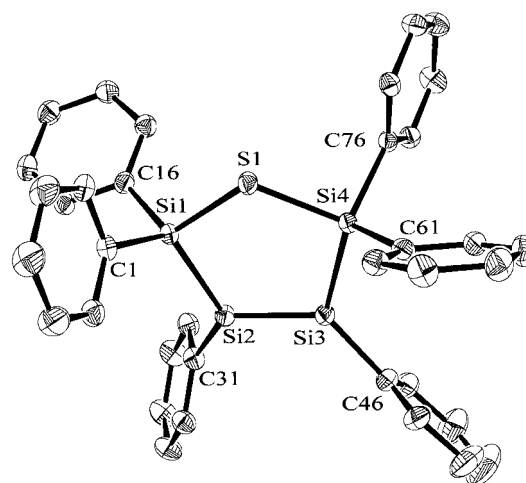


Figure 1. Structure of **5** in the crystal (hydrogen atoms and isopropyl groups omitted, ellipsoids represent the 50% probability level). Selected bond lengths [pm] and angles [°]: Si2–Si3 217.0(1), Si1–Si2 235.5(1), Si3–Si4 235.6(1), Si1–S 217.3(1), Si4–S 217.8(1); Si1–Si2–Si3 108.34(4), Si2–Si3–Si4 109.44(4), Si3–Si4–S 101.96(4), Si4–Si1–S 116.93(4), S–Si1–Si2 102.80(4).

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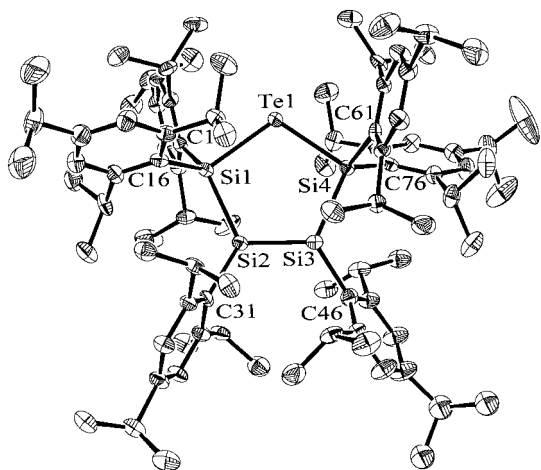


Figure 2. Structure of **7** in the crystal (hydrogen atoms omitted, ellipsoids represent the 50% probability level). Selected bond lengths [pm] and angles [°] for **6** and **7**: Si2–Si3 218.1(1), Si1–Si2 235.5(1), Si3–Si4 235.9(1), Si1–Se 230.77(9), Si4–Se 231.47(8); Si1–Si2–Si3 110.45(4), Si2–Si3–Si4 111.51(4), Si3–Si4–Se 101.37(4), Si4–Se–Si1 113.99(4), Se–Si1–Si2 102.27(4). **7**: Si2–Si3 219.8(2), Si1–Si2 236.1(2), Si3–Si4 236.4(2), Si1–Te 254.2(1), Si4–Te 254.9(1); Si1–Si2–Si3 114.71(7), Si2–Si3–Si4 114.74(7), Si3–Si4–Te 99.73(5), Si4–Te–Si1 110.40(4), Te–Si1–Si2 100.04(5).

these compounds represented by **7**. The five-membered rings are nearly planar (sum of angles 539.5°) with a slight deviation from planarity towards the twist form. A slight increase is observed in the Si–Si double bond lengths from the sulfur compound **5** to the tellurium compound **7**; all data are consistent with acyclic disilenes of a similar substitution pattern.^[1] The highest ring strain should be present in compound **5**, since the large 1,4-separation of the silicon atoms leads to a lengthening of the S–Si bond and a notable widening of the Si–S–Si angle. Because of the larger Si–Se and Si–Te bond lengths the corresponding angles Si–X–Si are significantly smaller and at the same time allow larger Si–Si–Si angles than those observed in **5**.

The new ring compounds are thermally very stable and chemically inert, for example towards air. However, the tellurium compound shows the expected light sensitivity and deposition of tellurium occurs in daylight.

Although the mechanism of formation of **5–7** cannot be proven experimentally, the following proposal seems to be reasonable. In analogy to the reaction of disilenes,^[7] the reaction sequence could be initiated by a [2 + 1] cycloaddition of a chalcogen atom to one of the Si–Si double bonds, followed by rearrangement of these intermediates into the presumably less strained five-membered rings **5–7**. All attempts to isolate **5** or its possible precursors by reaction of **4** with thiiranes remained unsuccessful, since no reaction occurred.

Experimental Section

5: Sulfur (1 g, large excess) was added to a solution of **4** (0.45 g, 0.34 mmol) in toluene (50 mL) and the mixture was heated for 3 h under reflux. Sulfur was removed by cooling to –70 °C and filtration, and the solution was concentrated. Cooling at 4 °C provided yellow needles of **5** (0.40 g, 86% yield). ¹H NMR (Bruker ARX 500, [D₆]benzene, 60 °C): δ = 0.35 (d, 6H, *J* = 6.5 Hz), 0.40 (d, 6H, *J* = 6.2 Hz), 0.49 (d, 6H, *J* = 6.3 Hz), 0.53 (d, 6H, *J* = 6.3 Hz), 0.61 (d, 6H, *J* = 6.6 Hz), 1.08 (d, 6H, *J* = 6.3 Hz), 1.12 (d, 6H,

J = 6.7 Hz), 1.13 (d, 6H, *J* = 6.9 Hz), 1.15 (d, 6H, *J* = 6.0 Hz), 1.16 (d, 6H, *J* = 7 Hz), 1.26 (d, 6H, *J* = 6.7 Hz), 1.28 (d, 6H, *J* = 6.8 Hz), 1.30 (d, 6H, *J* = 7 Hz), 1.36 (d, 6H, *J* = 6.5 Hz), 1.42 (d, 6H, *J* = 6.5 Hz), 1.46 (d, 6H, *J* = 6.5 Hz), 1.51 (d, 6H, *J* = 6.5 Hz), 1.87 (d, 6H, *J* = 6.6 Hz), 2.69 (sept, 4H), 2.83 (sept, 2H), 3.10 (sept, 2H), 3.66 (sept, 4H), 3.73 (sept, 2H), 4.05 (sept, 2H), 4.52 (sept, 2H), 7.08 (m, 12H); ¹³C NMR (125 MHz, [D₆]benzene, 60 °C): δ = 21.29, 21.90, 23.83, 23.92, 24.07, 24.27, 24.50, 24.59, 25.36, 26.27, 26.44, 26.50, 27.34, 28.67, 29.30, 31.72, 34.57, 35.09, 35.43, 37.43, 38.06, 38.29, 122.02, 122.26, 122.90, 122.97, 123.62, 123.96, 128.28, 128.49, 129.27, 129.79, 135.15, 142.05, 149.13, 149.98, 150.52, 151.34, 154.74, 154.87, 155.26, 156.29, 156.89; elemental analysis (%): calcd. for C₉₀H₁₃₈SSi₄: C 79.20, H 10.19, S 2.35; found: C 78.98, H 10.02, S 2.31.

6: To a solution of **4** (0.35 g, 0.26 mmol) in toluene (30 mL), an excess of selenium (1.0 g) and a few drops Et₃P were added, and the mixture was heated for 4 h under reflux. Toluene was replaced by *n*-pentane, excess selenium filtered off, and *n*-pentane was removed. Crystallization of the residue from a minimum amount of toluene at 4 °C afforded yellow crystals of **6** (0.30 g, 81% yield). ¹H NMR (Bruker ARX 500, [D₆]benzene, 25 °C): δ = 0.38 (d, 6H, *J* = 6.3 Hz), 0.43 (d, 6H, *J* = 6.3 Hz), 0.50 (d, 6H, *J* = 6.4 Hz), 0.51 (d, 6H, *J* = 6.4 Hz), 0.62 (d, 6H, *J* = 6.6 Hz), 1.11 (d, 6H, *J* = 6.5 Hz), 1.14 (d, 6H, *J* = 6.6 Hz), 1.15 (d, 12H, *J* = 6.9 Hz), 1.18 (d, 6H, *J* = 6.9 Hz), 1.28 (d, 6H, *J* = 6.9 Hz), 1.29 (d, 6H, *J* = 6.7 Hz), 1.35 (d, 6H, *J* = 6.7 Hz), 1.40 (d, 6H, *J* = 6.5 Hz), 1.46 (d, 6H, *J* = 6.4 Hz), 1.47 (d, 6H, *J* = 6.5 Hz), 1.55 (d, 6H, *J* = 6.6 Hz), 1.88 (d, 6H, *J* = 6.6 Hz), 2.68 (sept, 4H), 2.82 (sept, 2H), 3.15 (sept, 2H), 3.66 (sept, 4H), 3.79 (sept, 2H), 3.99 (sept, 2H), 4.74 (sept, 2H), 6.83 (d, 2H), 7.00 (d, 2H), 7.03 (d, 2H), 7.06 (d, 2H), 7.17 (d, 2H), 7.18 (d, 2H), ⁴*J* = 1.5 Hz; ¹³C NMR (125 MHz, [D₆]benzene, 25 °C): δ = 21.80, 23.73, 23.94, 23.98, 24.04, 24.13, 24.30, 24.47, 24.65, 25.26, 26.23, 26.36, 26.67, 27.15, 28.59, 34.51, 34.54, 34.59, 37.99, 121.96, 122.05, 122.76, 122.94, 123.47, 124.07, 127.81, 130.27, 134.30, 142.34, 148.93, 149.78, 149.99, 151.16, 154.69, 154.91, 154.97, 156.15, 156.52; elemental analysis (%): calcd. for C₉₀H₁₃₈SeSi₄: C 76.59, H 9.85, Se 5.59; found: C 76.47, H 9.72, Se 5.68.

7: To a solution of **4** (0.31 g, 0.23 mmol) in toluene (30 mL), an excess of tellurium (1.0 g) and a few drops of Et₃P were added, and the mixture was heated for 16 h under reflux. Toluene was replaced by *n*-pentane, excess tellurium filtered off, and *n*-pentane was removed. Crystallization of the residue from a minimum amount of toluene at 4 °C yielded orange crystals of **7** (0.28 g, 82% yield). ¹H NMR (Bruker ARX 500, [D₆]benzene, 25 °C): δ = 0.31 (d, 6H, *J* = 6.4 Hz), 0.36 (d, 6H, *J* = 6.3 Hz), 0.42 (d, 6H, *J* = 6.4 Hz), 0.45 (d, 6H, *J* = 6.3 Hz), 0.53 (d, 6H, *J* = 6.6 Hz), 1.10 (d, 6H, *J* = 6.8 Hz), 1.12 (d, 6H, *J* = 7 Hz), 1.14 (d, 6H, *J* = 7 Hz), 1.15 (d, 6H, *J* = 6.8 Hz), 1.17 (d, 12H, *J* = 6.7 Hz), 1.29 (d, 6H, *J* = 6.4 Hz), 1.42 (d, 6H, *J* = 6.5 Hz), 1.44 (d, 6H, *J* = 6.7 Hz), 1.46 (d, 6H, *J* = 6.6 Hz), 1.53 (d, 6H, *J* = 6.3 Hz), 1.55 (d, 6H, *J* = 6.6 Hz), 1.90 (d, 6H, *J* = 6.6 Hz), 2.66 (sept, 2H), 2.67 (sept, 2H), 2.77 (sept, 2H), 3.14 (sept, 2H), 3.59 (sept, 4H), 3.81 (sept, 2H), 3.99 (sept, 2H), 5.07 (sept, 2H), 6.79 (d, 2H), 6.97 (d, 2H), 6.98 (d, 2H), 7.03 (d, 2H), 7.17 (d, 2H), 7.18 (d, 2H), ⁴*J* = 1.5 Hz; ¹³C NMR (125 MHz, [D₆]benzene, 25 °C): δ = 22.21, 23.86, 24.03, 24.83, 25.42, 25.71, 26.28, 26.71, 27.37, 28.58, 29.43, 33.36, 34.36, 34.50, 34.58, 35.97, 36.24, 37.51, 37.81, 38.29, 121.52, 122.28, 122.97, 123.29, 123.52, 124.32, 127.81, 131.78, 133.46, 143.19, 148.93, 149.34, 149.91, 150.87, 154.46, 155.29, 156.10, 156.25. On account of the light sensitivity of **7** no satisfactory elemental analysis could be obtained.

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- [9] Crystal structure analyses: STOE-IPDS area detector, Mo $\text{K}\alpha$ radiation, graphite monochromator, $T = 193(2)$ K. The structures were solved by direct methods and refined by full-matrix least-squares techniques against F^2 .^[10] Hydrogen atoms were placed in calculated positions and refined with anisotropic temperature factors; all other atoms were refined anisotropically. In all structures one of the *para*-isopropyl groups was disordered and was refined on two positions with the occupancy factors of 0.5 each. **5**: $\text{C}_{90}\text{H}_{138}\text{SSi}_4 \cdot 2\text{C}_7\text{H}_8$, triclinic, space group $P\bar{1}$, $a = 1421.59(6)$, $b = 1672.47(12)$, $c = 2291.20(15)$ pm, $\alpha = 69.142(8)^\circ$, $\beta = 72.451(7)^\circ$, $\gamma = 81.476(7)^\circ$, $Z = 2$, $V = 4848.5(5) \times 10^6 \text{ pm}^3$, $\rho_{\text{calcd}} = 1.061 \text{ g cm}^{-3}$, $2\theta_{\text{max}} = 52^\circ$; of 59638 measured reflections, 17716 were independent and 11548 observed with $I > 2\sigma(I)$, $R1 = 0.063$, $wR2$ (all data) = 0.1671 for 817 parameters. **6**: $\text{C}_{90}\text{H}_{138}\text{SeSi}_4 \cdot 2\text{C}_7\text{H}_8$, triclinic, space group $P\bar{1}$, $a = 1433.48(6)$, $b = 1674.36(10)$, $c = 2300.24(12)$ pm, $\alpha = 68.812(6)^\circ$, $\beta = 71.954(5)^\circ$, $\gamma = 81.111(6)^\circ$, $Z = 2$, $V = 4888.9(4) \times 10^6 \text{ pm}^3$, $\rho_{\text{calcd}} = 1.084 \text{ g cm}^{-3}$, $2\theta_{\text{max}} = 52^\circ$; of 60128 measured reflections, 17891 were independent and 12099 observed with $I > 2\sigma(I)$. $R1 = 0.0526$, $wR2$ (all data) = 0.1459 for 817 parameters. **7**: $\text{C}_{90}\text{H}_{138}\text{Si}_4\text{Te} \cdot 2\text{C}_7\text{H}_8$, triclinic, space group $P\bar{1}$, $a = 1453.30(8)$, $b = 1697.00(9)$, $c = 2029.62(15)$ pm, $\alpha = 95.390(8)^\circ$, $\beta = 90.089(8)^\circ$, $\gamma = 100.912(6)^\circ$, $Z = 2$, $V = 4892.4(5) \times 10^6 \text{ pm}^3$, $\rho_{\text{calcd}} = 1.116 \text{ g cm}^{-3}$, $2\theta_{\text{max}} = 52^\circ$; of 60004 measured reflections, 17885 were independent and 8548 observed with $I > 2\sigma(I)$. $R1 = 0.0548$, $wR2$ (all data) = 0.0985 for 879 parameters. 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-116 227 (**5**), CCDC-116 228 (**6**), and CCDC-116 229 (**7**) 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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Regio- and Enantioselective Cyclization of Epoxy Alcohols Catalyzed by a [Co^{III}(salen)] Complex**

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The construction of substituted oxygen heterocycles stands as a significant target for synthetic methodology, mainly as a consequence of the abundance of interesting natural products that contain this structural unit.^[1] One of the most important approaches to cyclic ether synthesis involves the intramolecular cyclization of epoxy alcohols, whereby stereospecific epoxide opening offers a stereocontrolled route to these targets.^[2] An inherent challenge to this strategy is the control of the regioselectivity of the ring opening of epoxy alcohols. Our own interest in intermolecular asymmetric ring opening (ARO) reactions^[3] led us to the question of whether chiral catalysts might influence the regiochemical outcome of intramolecular cyclizations of epoxy alcohol. Such regiocontrol would be rendered even more powerful if accompanied by enantiocontrol, so that racemic or prochiral substrates could be cyclized to produce enantioenriched oxygen heterocycles. Herein we disclose the first intramolecular ARO reaction catalyzed by a chiral [Co^{III}(salen)] complex, with the successful attainment of both regio- and stereochemical elements of control ($\text{H}_2\text{salen} = \text{bis(salicylidene)ethylenediamine}$).

To date, terminal epoxides represent the most successful substrate class identified in Co-catalyzed ARO reactions. While products arising from *exo* attack are favored under either acidic or basic conditions for epoxides such as 4,5-epoxypentanol (**2**)^[4], we were quite surprised to observe that (*R,R*)-**1** catalyzed the *endo*-selective ring closure of **2** to afford the tetrahydropyranol **5** as the major cyclization product (12:1) in 46% yield (by GC). Equally impressive was the remarkable enantiodiscrimination displayed in this reaction. Racemic **2** underwent efficient kinetic resolution to afford the *endo* product in 95% *ee*,^[5] which was isolated in 41% yield following benzoylation of the crude reaction mixture and subsequent purification by flash chromatography (Table 1). The [Co^{III}(salen)] complex **1** also catalyzed the *endo*-selective kinetic resolution of racemic 5,6-epoxyhexan-1-ol (**3**) to afford the hydroxyoxepane **6** in 47% yield (39% yield of isolated product) and 94% *ee*. A dramatic decrease in the reaction rate was observed with the homologous substrate **4** containing seven carbon atoms; no detectable cyclization products were observed after 48 hours. Nonetheless, the ability of (*R,R*)-**1** to override the inherent stereoelectronic preference for *exo* attack in the ring opening of epoxides **2** and

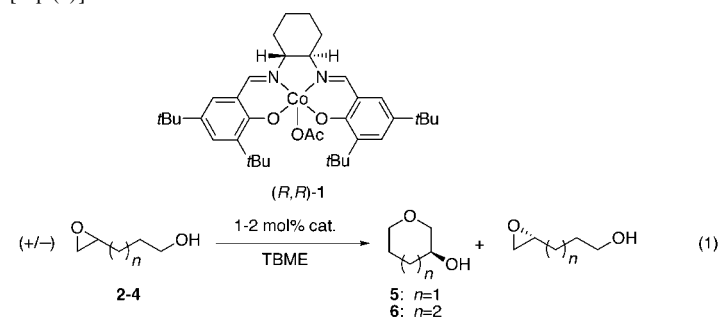
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Table 1. Intramolecular kinetic resolution of epoxy alcohols catalyzed by (*R,R*)-**1** [Eq. (1)].



Epoxide	<i>T</i> [°C]	<i>t</i> [h]	<i>ee</i> (product) ^[a] [%]	Yield ^[b] [%]	<i>ee</i> (epoxide) ^[c] [%]	Yield ^[b] [%]
2 (<i>n</i> = 1)	0	1	95	46 (41)	93	50
3 (<i>n</i> = 2)	0	7	94	47 (39)	98	50
4 (<i>n</i> = 3)	23	48	n.r. ^[d]	n.r.	n.r.	n.r.

[a] Determined by chiral HPLC (Chiracel AD) of the benzoate esters. [b] GC yields. The yields of the isolated benzoate esters of **5** and **6** are given in parenthesis. [c] The

3^[6] coupled with the high enantioselectivity associated with this mode of cyclization was noteworthy.

Having established the viability of intramolecular cyclization^[7] to effect the kinetic resolution of terminal epoxy alcohols, we next turned our attention to the enantioselective cyclization of *meso* substrates. Despite the fact that disubstituted epoxides have proven generally unreactive under hydrolytic conditions with catalyst **1**, good reactivity was observed in the cyclization of 1,2-disubstituted epoxy diols (Table 2). For example, epoxy alcohol **7** cleanly cyclized under

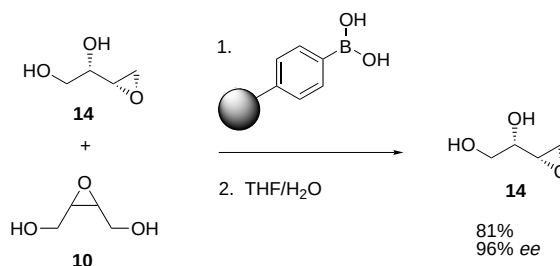
Table 2. Intramolecular desymmetrization of *meso* epoxy alcohols catalyzed by (*R,R*)-**1**.

Substrate	Product	<i>ee</i> [%] ^[a]	Yield [%] ^[b]
		98	96
7	11		
		95	86
8	12		
		99	45 ^[c]
9	13		
		96 ^[d]	81
10	14		

[a] Determined by chiral GC or HPLC. See the supporting information for details. [b] Yields of isolated product. [c] Isolated as the mono-TIPS ether (TIPS = triisopropylsilyl). [d] Absolute configuration established by comparison of optical rotation to literature values. All other assignments are made by analogy.

the influence of Co catalysis to afford the desymmetrized bicyclic ether **11** in 96 % yield and 98 % *ee*. Similarly, the *gem*-bis-hydroxymethylcyclopentene oxide **8**^[8] also underwent highly enantioselective ring closure to afford the bicyclic ring system **12** in 86 % yield and 95 % *ee*. An interesting regiochemical issue was presented by the *meso* epoxy diol **9**, which can potentially cyclize in either a 4-*exo* or 5-*endo* fashion to yield novel bicyclic structures. In the event, substrate **9** underwent exclusive 4-*exo* ring closure to afford oxetane **13** as the sole product, which was isolated from the recovered starting material (50 %, GC) in 45 % yield and 99 % *ee*.^[9]

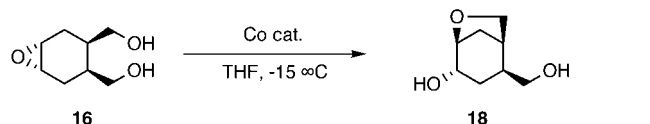
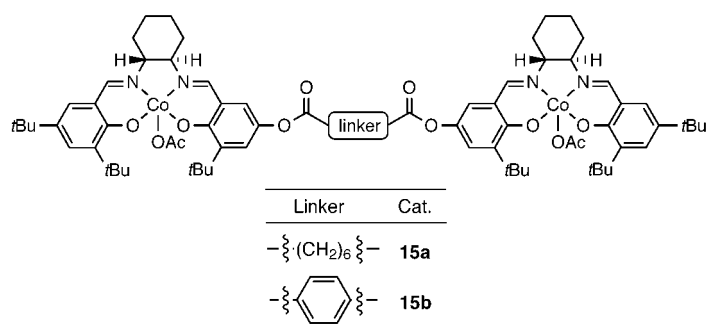
The *meso* epoxy diol **10**, readily prepared as a white crystalline solid by epoxidation of *cis*-2-buten-1,4-diol with *m*-chloroperbenzoic acid (*m*CPBA), was a particularly interesting substrate in the intramolecular ring opening reaction. Diol **10** underwent [Co(salen)]-catalyzed Payne rearrangement^[10] to afford the 1,2-anhydrothreitol product **14**.^[11] GC analysis of this reaction indicated incomplete conversion of **10**, and efforts to drive the rearrangement to completion were unsuccessful. In order to isolate the desired 1,2-diol product from the unchanged 1,4-diol **10**, a resin capture strategy utilizing polystyryl boronic acid^[12] was successfully applied.^[13] Following hydrolysis of the immobilized boronate ester the epoxy diol **14** was isolated in 81 % yield and 96 % *ee* (Scheme 1).



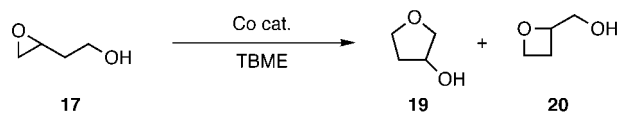
Scheme 1. Resin capture strategy for isolation of **14**.

Preliminary evidence suggests a preferred bimetallic cyclization step for these ring-closure reactions of epoxy alcohols wherein Co^{III} complexes serve dual roles of activating both epoxide and alcohol.^[14] In the ring closure of **3**, for instance, a dramatic rate enhancement is observed with the dimeric [Co^{III}(salen)] catalyst **15a** in which two [Co(salen)] units are covalently tethered through an aliphatic diester chain.^[15] Moreover, results obtained in the cyclization of two substrates which proved problematic with catalyst **1** provided additional support for a favored bimetallic pathway.

The outcome of the enantioselective desymmetrization of **16** was significantly improved when dimeric catalyst **15a** was used in place of monomeric catalyst **1**. Cooperative reactivity of the [Co(salen)] units not only increased the reaction rate relative to that with the monomeric catalyst, but also improved enantioselection in the formation of **18** from 60 % *ee* (with **1**) to 84 % *ee* (Scheme 2). It appears likely that the higher enantioselectivity with **15a** might originate from enhanced stereochemical communication between the two tethered chiral Co catalysts relative to a less-selective mono-



Cat.	Conversion [%]	ee [%]
2 mol% 1	22	60
1 mol% 15a	100	84



Cat.	endo:exo
2 mol% 1	only exo
1 mol% 15b	4.3 : 1

Scheme 2. Cyclization with dimeric [Co(salen)] complexes. TBME = *tert*-butyl methyl ether.

metallic pathway that is more easily accessed with monomeric catalyst **1**.

The effect of enhanced cooperative catalysis was manifested in a dramatic way in the regioselectivity of the cyclization of epoxy alcohol **17**. With monomeric catalyst **1** only the *exo* cyclization product **20** was obtained, whereas cyclization under the influence of dimeric catalyst **15b** led to preferential formation of the *endo* tetrahydrofuran product **19**. A mono-metallic Lewis acid mechanism might explain the exclusive *exo* selectivity shown by catalyst **1**, while dual activation of both the epoxide and hydroxyl groups by dimeric **15b** may create a sterically more-demanding transition state assembly that now favors the *endo* product **19** as the major cyclic product.

The [Co(salen)]-catalyzed intramolecular ARO reaction thus allows the synthesis of novel cyclic and bicyclic ethers ranging from three- to seven-membered rings in good yields and high enantiopurity. This methodology can be applied effectively to the kinetic resolution of racemic epoxy alcohols as well as to the desymmetrization of *meso* substrates, in which the [Co^{III}(salen)] catalyst exhibits exceptional regio- and enantiocontrol. Exploration of the synthetic utility of the cyclization products as well as elucidation of the reaction mechanisms of these [Co(salen)]-catalyzed processes remain important goals in our research.

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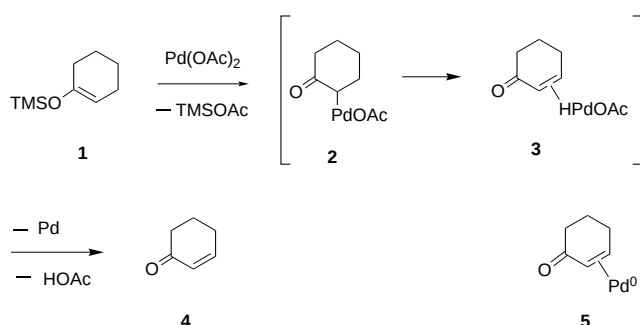
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Insight into the Mechanism of the Saegusa Oxidation: Isolation of a Novel Palladium(0)–Tetraolefin Complex

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*Dedicated to Dr. Gerd Sauer
on the occasion of his 60th birthday*

The Saegusa oxidation^[1] is a standard method for the conversion of a silyl enol ether (**1**) into the corresponding α,β -enone **4**. The oxidizing agent palladium(II) acetate is added in stoichiometric amounts. In analogy to the Wacker oxidation^[2] the formation of a palladium adduct (**2**) is postulated, from which HPdOAc is eliminated, via complex **3**, the enone **4** (Scheme 1). We now provide evidence that this

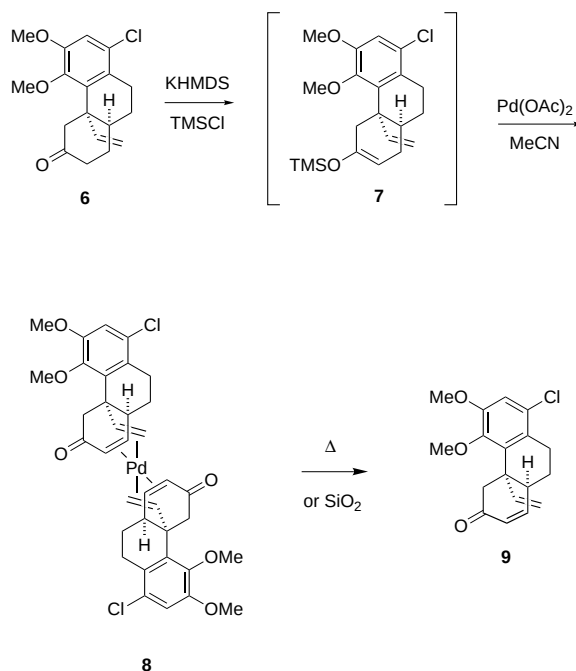


Scheme 1. Postulated mechanism of the Saegusa oxidation. TMS = trimethylsilyl.

assumption may not be true in general, and that in suitable cases the palladium is retained in the oxidized substrate as a stable Pd⁰–olefin complex (**5**). For the first time, such an intermediate was isolated and characterized as a novel d¹⁰ Pd⁰–tetraolefin complex (**8**).

In connection with synthetic studies on hasubanan-type alkaloids,^[3] ketone **6** was converted into the silyl enol ether **7** and submitted to the conditions of the Saegusa oxidation (Scheme 2). A brown solid was precipitated from the mixture, which was crystallized and characterized as the palladium complex **8** by NMR and IR spectroscopy (see the Experimental Section), and single-crystal X-ray diffraction^[4] (Figure 1). Remarkably, only the C₂-symmetrical diastereomer of **8** was formed. On heating or on treatment with silica gel complex **8** was decomposed to furnish enone **9**, as expected.

A comparison with other d¹⁰ Pd⁰–alkene complexes shows that **8** is highly unusual. Normally such species are unstable and undergo rapid ligand dissociation and oxidation to the cor-



Scheme 2. Formation of complex **8** during the Saegusa oxidation of **7**.

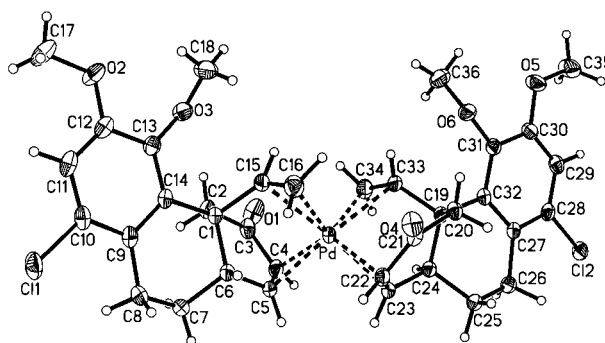


Figure 1. Structure of **8** in the crystal.

responding Pd^{II} compounds.^[5] Accordingly, stable Pd⁰–alkene complexes require electron-deficient alkene ligands with strong d– π backbonding such as dibenzylideneacetone, benzoquinone, or maleic anhydride. The number of coordinated C–C double bonds in these compounds rarely exceeds three, and the coordination geometry is normally trigonal or square planar. Few η^8 Pd⁰ complexes have been described (e.g. the unstable [Pd(cod)₂]^[6] or [Pd(benzoquinone)(cod)]^[7]; cod = cyclooctadiene), and none of these has been characterized by single-crystal X-ray diffraction. Therefore, **8** is the first example of a stable tetrahedral Pd⁰–tetraolefin complex whose solid-state geometry has been fully elucidated (Figure 1).

The coordination geometry of **8** is best described as a distorted tetrahedron, the centers of the double bond moieties forming the corners of the tetrahedron. Remarkably, the Pd–C(vinyl) distances are 150–200 pm longer than the Pd–C(enone) distances (Table 1). The length of the enone C–C double bonds are 139.5 and 139.6 pm in **8** and 133.4 pm in the free ligand **9**,^[4] which indicates that significant d– π backbonding takes place in **8** from the metal to the enone. Accordingly $\tilde{\nu}(\text{CO})$ is 1629 cm^{−1} for **8** and 1675 cm^{−1} for **9**.

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Table 1. Palladium–carbon bond lengths [Å] in **8**.^[4]

Pd–C(4): 2.233(2)	Pd–C(22): 2.242(1)
Pd–C(5): 2.153(2)	Pd–C(23): 2.166(1)
Pd–C(15): 2.400(2)	Pd–C(33): 2.345(2)
Pd–C(16): 2.399(2)	Pd–C(34): 2.352(2)

The superposition of the structures (Figure 2) shows that both molecules have similar conformations, especially with respect to the cyclohexenone segment, so that the palladium is trapped by the ligand as soon as it is liberated from the intermediate. In other words, enone **9** is conformationally preorganized to act as a host for the palladium(0) atom. This

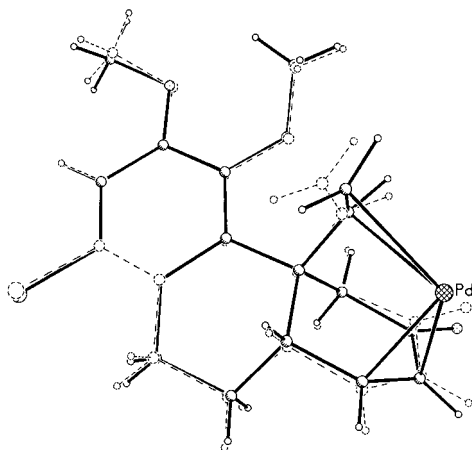


Figure 2. Superposition of the structures of **8** and **9**.

suggests that the present case is a rather special one, which, however, does provide new information on the mechanism of the Saegusa oxidation, in particular with respect to the decomposition of complexes such as **3**.

Experimental Section

To a solution of ketone **6** (500 mg, 1.56 mmol) in THF (15 mL) was added KHMDS (4 mL of a 0.5 M solution in toluene, 2.34 mmol) at -78°C . After the mixture was stirred at -78°C for 1 h, TMSCl (0.4 mL, 3.12 mmol) was added. The mixture was warmed to room temperature and stirred for an additional hour. Then triethylamine (0.65 mL, 4.68 mmol) was added at 0°C , followed by diethyl ether (20 mL) and aqueous phosphate buffer (pH 7, 50 mL). The organic layer was washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was dissolved in acetonitrile (15 mL), and palladium(II) acetate (420 mg, 1.87 mmol) was added. The mixture was stirred at 50°C for 2 h and at room temperature for 12 h. The black suspension was filtered, and the precipitate was washed with dichloromethane. On concentration of the filtrate a brown solid was precipitated, which was collected by filtration and recrystallized from chloroform to give pale yellow needles of **8** (464 mg, 80%). The excess palladium acetate remained in the mother liquor.

8: $\text{C}_{36}\text{H}_{38}\text{Cl}_2\text{O}_6\text{Pd}$, $M = 744.02\text{ g mol}^{-1}$, $R_f = 0.10$ (hexanes:EtOAc 1:1), m.p. $160\text{--}163^{\circ}\text{C}$ (decomp.); elemental analysis calcd: C 58.12, H 5.15; found: C 58.06, H 5.19; ^1H NMR (400 MHz, CDCl_3): the numbering of the H atoms does not correspond to the numbering of the C atoms in Figure 1, but is based on the phenanthrene framework): $\delta = 1.69$ (m, 1H; H-10), 1.94 (m, 1H; H-10), 2.60 (m, 1H; H-9), 2.44 (d, 1H; $J = 17.9\text{ Hz}$, H-4), 2.98 (m, 1H; H-9), 2.91 (d, 1H; $J = 17.9\text{ Hz}$, H-4), 3.66 (d, 1H, $J = 9.1\text{ Hz}$; H-12), 3.79 (s, 6H; OMe), 4.28 (d, 1H, $J = 15.7\text{ Hz}$; H-12), 4.45 (br. s, 1H; H-1), 4.79 (d, 1H, $J = 7.4\text{ Hz}$; H-2), 5.56 (dd, 1H, $J = 15.5, 9.8\text{ Hz}$; H-11), 6.84 (s, 1H; H-7); ^{13}C NMR (62.9 MHz, CDCl_3): $\delta = 25.69, 29.25, 38.88, 42.46, 45.29, 55.91, 60.43, 74.69, 82.71, 91.27, 112.72, 118.29, 125.43, 128.62, 138.10,$

146.05, 151.30, 195.80; IR (KBr): $\tilde{\nu} = 2936$ (m), 1629 (vs), 1467 (s), 1245 (s), 1216 (m) cm^{-1} ; MS (MALDI, sinapinic acid, buffered with ammonium citrate): m/z (%) = 744 (5) [M^+], 424 (15) [Pd+enone], 357 (38) [enone+ K^+], 318 (51) [enone], 254 (100), 108 (55) [Pd].

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- [4] 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-111931 (*rac-8*) and CCDC-114053 (*rac-9*). 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). Crystal data: *rac-8*: $\text{C}_{36}\text{H}_{38}\text{Cl}_2\text{O}_6\text{Pd} \cdot 3\text{CDCl}_3$, triclinic, space group $P\bar{1}$, $a = 11.431(1)$, $b = 14.244(3)$, $c = 14.519(2)$ Å, $\alpha = 76.895(8)$, $\beta = 88.470(8)$, $\gamma = 78.4181^{\circ}$, $V = 2255.0(7)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.623\text{ g cm}^{-3}$ at 173 K. A crystal of dimensions $0.28 \times 0.50 \times 0.85$ mm was measured on a SIEMENS SMART diffractometer with $\text{MoK}\alpha$ radiation. An empirical absorption correction using program SADABS gave an effective transmission range from 0.76 to 1.00. Of 35023 reflections measured up to $2\theta = 61^{\circ}$, 11388 were independent with $R(I)_{\text{int}} = 0.015$; 10991 reflections with $I > 0$ were used. The structure was determined by direct methods using the program SHELXS. The hydrogen atoms were taken from difference Fourier syntheses and were refined with isotropic thermal parameters. $R(F) = 0.031$, $wR(F) = 0.036$, $S = 1.38$ for 679 refined parameters. The final difference density was between -0.10 and $+0.84\text{ e Å}^{-3}$. *rac-9*: $\text{C}_{18}\text{H}_{19}\text{ClO}_3$, a crystal with approximate dimensions of $0.35 \times 0.25 \times 0.20$ mm was investigated on a NONIUS Kappa CCD diffractometer ($\text{MoK}\alpha$ radiation) at temperatures between 293 and 100 K. The crystal structure was determined by direct methods (SHELXS-97) and subsequent Fourier and difference Fourier syntheses. The hydrogen atoms were refined with isotropic thermal parameters. Refinements were done with SHELXL-97, 276 parameters were refined. The discussion in the text is based on the data for the measurement at 293 K. 293 K: triclinic, space group $P\bar{1}$, $a = 6.794(10)$, $b = 10.359(2)$, $c = 11.332(2)$ Å, $\alpha = 98.60(3)$, $\beta = 93.86(3)$, $\gamma = 96.89(3)^{\circ}$, $V = 779.9(2)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.357\text{ g cm}^{-3}$. Of 5799 reflections measured up to $2\theta = 53^{\circ}$, 2921 were independent and 2406 with $F_o > 4\sigma(F_o)$, $R(I)_{\text{int}} = 0.015$, were used. $R(F) = 0.040$, $wR(F^2) = 0.114$, $S = 1.052$. The final difference electron density is between 0.16 and -0.32 e Å^{-3} . 100 K: triclinic, space group $P\bar{1}$, $a = 6.721(1)$, $b = 10.035(2)$, $c = 11.421(2)$ Å, $\alpha = 98.62(3)$, $\beta = 94.53(3)$, $\gamma = 96.98(1)^{\circ}$, $V = 752.3(2)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.407\text{ g cm}^{-3}$. Of 6068 reflections measured up to $2\theta = 57^{\circ}$, 3065 were independent and 2747 reflections with $F_o > 4\sigma(F_o)$, $R(I)_{\text{int}} = 0.015$, were used. $R(F) = 0.032$, $wR(F^2) = 0.090$, $S = 1.096$. The final difference electron density is between 0.33 and -0.22 e Å^{-3} .
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The Origin of the Distorted Close-Packed Elemental Structure of Indium**

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The unique structure of indium belongs to a small group of metallic structures that deviate from the close-packed trend usually displayed by metallic elements. Its body-centered tetragonal (bct) unit cell, with lattice parameters $a = 3.2525$ and $c = 4.9465$ Å,^[1] contains the two equivalent atoms at the corners and the center of the cell. The structure is easily visualized as a slightly distorted close packed cubic (fcc) structure with a c/a ratio of 1.5208 (Figure 1), compared to $\sqrt{2}$ for ideal fcc. Thus the environment of the 12 nearest neighbors in the fcc structure is split into two sets separated by distances d_1 and d_2 , as indicated in Figure 1.

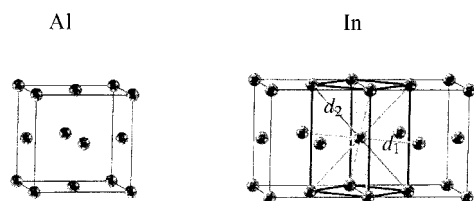


Figure 1. The structure of tetragonal In compared to the close-packed fcc structure of Al.

Why does In adopt this more open-packed structure of lower symmetry, whereas the lighter homologue Al crystallizes in the highly symmetric fcc structure? In the early 1970s Heine and Weaire^[2] and later Hafner and Heine^[3] addressed this question in the context of a beautiful work, where they related the crystal structures of the elements on the common ground of pseudopotential theory. For In it was found that the changes in the size of the ionic core, compared to that of Al, are responsible for the instability of the fcc structure. However, the pseudopotential results are rather qualitative, and the success of this theory is astonishing in the case of In because—as we will see later—the stabilizing energy which In gains by distortion of the fcc structure is extremely small.

The systematic exploration of the electronic reasons behind the “unusual”, low-symmetry metal structures is highly challenging, and reliable quantitative results have not been accessible until recently with the advent of high-accuracy full-

potential techniques in the framework of density functional theory.^[4] Here we treat the question of the origin of the low-symmetry In structure and the high-symmetry Al structure on such a quantitative ground by means of ab initio full-potential calculations (see the computational methods) and relate the results to the differences in the atomic properties of these two elements.

We started our investigation by calculating the change in the total energy of Al and In upon altering the c/a ratio from the ideal value of $\sqrt{2}$ for the fcc structure to lower and higher values, while keeping the volume constant and equal to the experimental ground-state volumes of the elements. Figure 2 depicts the results. For Al, where the fcc structure is the

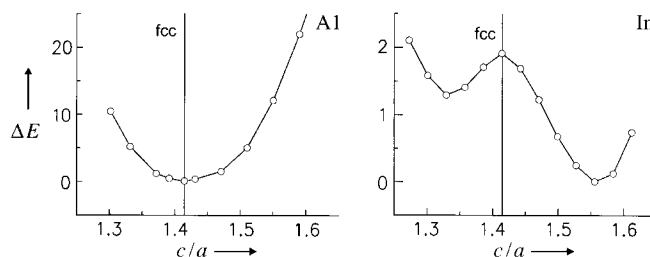


Figure 2. Variations in total energy (in meV per atom) as a function of c/a at the respective experimental ground-state volumes V_0 for Al and In. Note the different energy scales.

ground-state structure, the tetragonal distortion leads to a parabolic change in total energy with respect to c/a , where the minimum of the curve corresponds to the c/a ratio of the fcc structure. For In, however, a very remarkable result is obtained: The total energy curve exhibits a double-well behavior with two minima and a local maximum exactly at a c/a ratio corresponding to the ideal fcc structure. This behavior is reminiscent of a Peierls distortion in solids or a Jahn–Teller distortion in molecular compounds. The deeper minimum at a c/a ratio of 1.55 (at 0 K) is in agreement with the observed ground-state structure of In and compares very well with the experimental value of 1.521 (at 298 K). The stabilizing energy of the ground-state structure with respect to the fcc structure turns out to be extremely small. It is below 2 meV per atom, which corresponds to a temperature of about 20 K. The stabilization of the possible metastable In structure with a c/a ratio of approximately 1.33 is only one-third of this value. Thus the energy changes observed along the tetragonal distortion path are an order of magnitude smaller for In than for Al.

In this context, it is instructive to divide the total energy into the band energy, which represents the sum over the occupied states and thus is most transparent from a chemical point of view, and a part consisting of the remaining contributions (the electrostatic interaction and the exchange–correlation energy). Figure 3 shows the result of this partition. In both cases the trend in the changes of the band energy coincides with that of the total energy: For Al we find a relative minimum and for In a relative maximum at a c/a ratio of $\sqrt{2}$. The sum of the remaining parts of the total energy displays exactly the opposite tendency, favoring a distorted structure for Al and the

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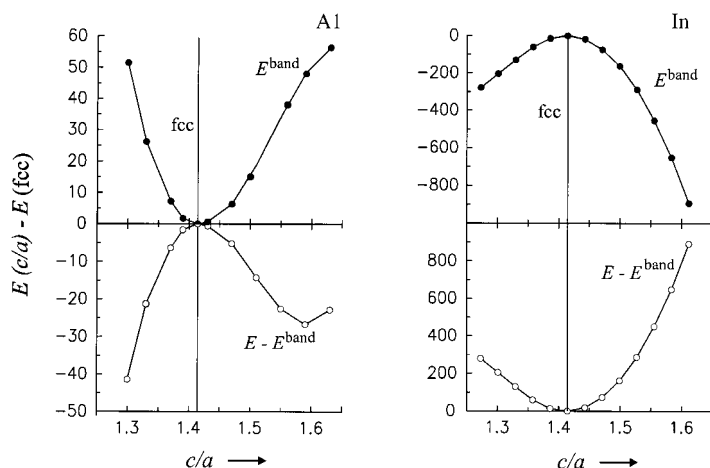


Figure 3. Partition of the total energy (in meV per atom) into the band energy term E^{band} (closed circles) and the sum of the remaining contributions $E - E^{\text{band}}$ (open circles) as a function of c/a at the respective experimental ground-state volumes V_0 for Al and In.

high-symmetry fcc structure for In. Thus, we may identify the band-energy term as the leading contribution to the stability of the ground-state structures of Al and In. How can we explain the opposite changes in the band energies of Al and In when deforming their crystal structure from a high-symmetry close-packed to a more open-packed atomic arrangement?

Aluminum and indium belong to the triel elements (Group 13), which represent the border between the elements with exclusively close-packed structures that show metal bonding and the tetrel elements (Group 14) crystallizing in the diamond structure that show covalent bonding. Indeed, the two most electronegative representatives of the triels, boron and gallium, exhibit ground-state structures with large covalent bonding contributions. Naturally, as the number of valence electrons increases and the separation of the atomic s and p levels decreases when going from the alkali metals to the tetrels, mixing of the s and p bands becomes a more and more significant ingredient in the bonding for the elemental structures. The s–p mixing is first recognized in the semi-metallic behavior of the alkaline earth metals Ca and Sr, and leads finally to the opening of a band gap at the Fermi level for the tetrel elements.^[6] Interestingly, we find that the ratio between the total number of p and s states (N_p/N_s) calculated inside the muffin-tin spheres of Al and In as a function of c/a (Figure 4) displays a trend that is the reverse of that for the corresponding band energies (Figure 3): The N_p/N_s ratio gains

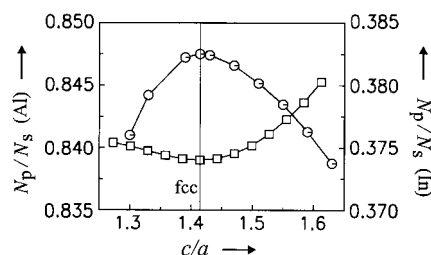


Figure 4. Changes of the ratio of the number of p and s states in the muffin-tin spheres (N_p/N_s) as a function of c/a for Al (circles) and In (squares) at the respective experimental ground-state volumes.

a maximum value for Al and a minimum value for In at $c/a = \sqrt{2}$. Consequently, the apparent tendency for Al and In is to adopt a ground-state structure where s–p mixing is either maximal (Al) or increased by distortion (In). This finding fits nicely to our recent investigation of the stability of α -Ga with respect to other structural alternatives. There we also obtained a maximum in the ratio N_p/N_s for the most stable geometry, which corresponds to the experimental α -Ga structure, and related this result with a situation of “maximum covalency” in α -Ga.^[7]

The analysis of the orbital character of the band structures for fcc-Al and fcc-In (at their experimental ground-state volumes) reveals the reason behind the different distortion behavior of In and Al (Figure 5).^[8] For Al small local band gaps

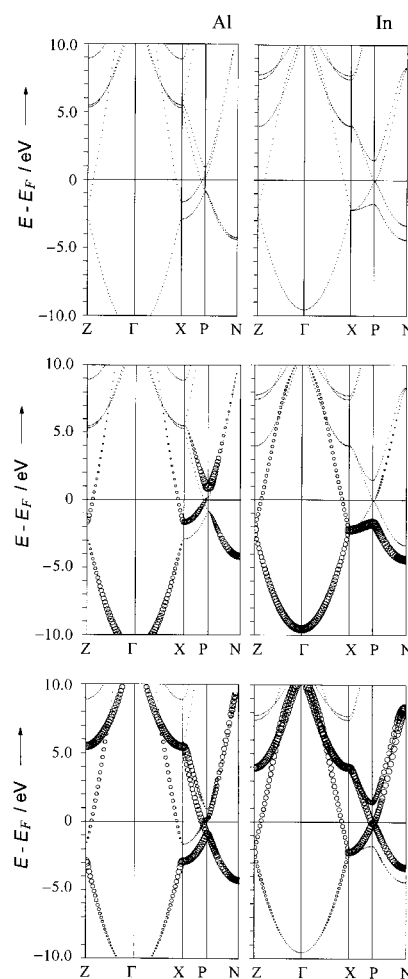


Figure 5. Band structures (top) for fcc-Al and fcc-In at the respective experimental ground-state volumes^[8] as well as the s (middle) and p character of the bands (bottom). The orbital character is proportional to the size of the circles used in the plots.

occur at the high-symmetry k points, which are a consequence of the mixing of the upwards running s band with a downwards running p band. The interaction between s and p bands is especially large along the direction $N \rightarrow P$, resulting in a band gap at P exactly at the Fermi level. In contrast to Al, s–p mixing in In is too weak for opening of local band gaps at the high-symmetry k points. Of course these differences are also reflected in the density of states (Figure 6). At low energies

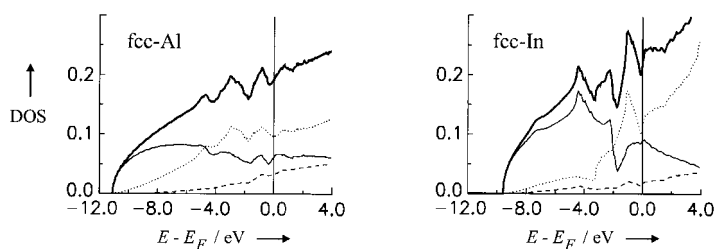


Figure 6. Total density of states (DOS; bold lines) together with the s (solid lines), p (dotted lines), and d orbital contributions (dashed lines) of fcc-Al and fcc-In at their respective experimental ground-state volumes. The unit for the total DOS is the number of electrons per eV.

the density of states shows a parabolic, free-electron-like distribution for both metals. In the case of Al at higher energies additional structuring is seen which is caused by the small band gaps at the high-symmetry points. For In the separation between the 5s and 5p bands is expressed by a spikey behavior with a large p contribution just below the Fermi level, whereas for Al the s and p contribution to the density of states appear much more homogeneously distributed.

The opposite dispersion of the s and p bands along certain symmetry lines is due to the difference in parity of the orbitals. The bands might cross if the size of the s–p separation at the atomic levels is not too large and/or the size of the dispersion of the bands under consideration is sufficiently pronounced.^[9] The energies of the p valence orbitals are almost identical ($\epsilon_{3p}(\text{Al}) = -5.984$, $\epsilon_{5p}(\text{In}) = -5.785$ eV^[10]), but the In 5s orbital is comparatively lower in energy ($\epsilon_{5s}(\text{In}) = -10.122$ eV) than the Al 3s orbital ($\epsilon_{3s}(\text{Al}) = -9.582$). The dispersion of the bands is essentially determined by the size of the overlap between the atomic orbitals, which itself is a function of the size of the atomic orbitals in relation to the distance between the atoms. The expectation values of the valence orbitals are $\langle r \rangle_{3s} = 1.37$ and $\langle r \rangle_{3p} = 1.82$ Å for Al, and $\langle r \rangle_{5s} = 1.44$ and $\langle r \rangle_{5p} = 1.99$ Å for In.^[11] When taking into account the nearest neighbor distances in fcc-Al and fcc-In at the experimental ground-state volumes ($d_{\text{NN}}(\text{Al}) = 2.86$, $d_{\text{NN}}(\text{In}) = 3.33$ Å), it becomes immediately evident that especially the s–s overlap is considerably smaller in In. This leads to a much smaller dispersion of the s band compared to the situation for Al. These two factors, the lower lying s valence orbital energy and the smaller dispersion of the s band, prevent substantial s–p mixing for In in the fcc structure.

The stabilizing nature of s–p mixing was investigated by Burdett^[9] on a model system consisting of a one-dimensional chain of atoms with an s and an appropriate p orbital. The result is summarized schematically in Figure 7. With strong s–p mixing (hybridization), the top of the lower band drops in energy and alters its exclusively s–s antibonding character into a p–p bonding one, while the upper band becomes completely antibonding. Thus the one-dimensional chain is maximally stabilized with an electron count corresponding to a completely filled lower band.

This simple picture is of great importance for the understanding of the structural stability of the metallic triels when assuming the band energy to be the structure-determining factor, and thus bonding is largely influenced by the low-lying s valence band. Imagine a close-packed arrangement with

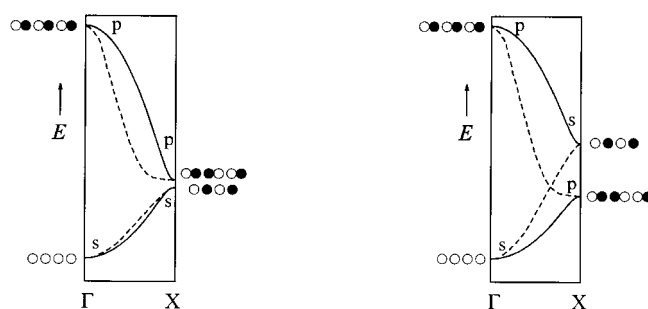


Figure 7. Schematic band structure for a linear chain of atoms with an s and a p orbital only for strong s–p mixing (right) and weak s–p mixing (left; based on ref. [9]). The dashed lines approximate the dispersion of the unhybridized bands. The appropriate orbital combinations at the high-symmetry k points are indicated.

unhybridized bands. Then for many directions in the Brillouin zone the electron count of these elements will produce a completely occupied s valence band with a large antibonding contribution (at the top of the band). This bonding situation is not especially favorable, and instead the triels try to obtain a structure where s–s antibonding states are raised above the Fermi level for the largest possible part of the Brillouin zone, or equivalently where the number of p states below the Fermi level is maximized (i.e., the ratio N_p/N_s is maximal). Aluminum condenses at a volume where strong s–p mixing is possible for the fcc structure, and, even more, is maximal for the close-packed arrangement with respect to the tetragonal distortion. Indium, however, condenses at a volume where s–p mixing is relatively weak. A distortion to a low-symmetry structure can now improve this situation, as some atoms move closer to each other and thus increase the interaction between s and p bands. This hypothesis is corroborated by a computer experiment performed by us, where we expanded Al to a volume of about $V/V_0 = 1.3$. At this volume s–p mixing is considerably suppressed for the fcc structure because the dispersion of the bands is diminished. To improve its bonding situation and take advantage of hybridized bands, Al could be expected to distort to a structure of lower symmetry. Indeed, we found the α -Ga structure to be more stable at this expanded volume than the fcc structure.^[12]

Computational Methods

We calculated the change in the total energy of Al and In along the tetragonal distortion path of the fcc structure at constant volume (experimental ground-state volume $V_0(\text{Al}) = 16.617$, $V_0(\text{In}) = 26.164$ Å³ per atom), and compared the results of the (scalar relativistic) full-potential linearized augmented plane wave (FLAPW) method^[5a] with those of the (scalar relativistic) full-potential linear muffin-tin orbital (FP-LMTO) method.^[5b] In full-potential techniques within the local-density approximation basis functions, electron densities, and potentials are calculated without any shape approximation.

In the case of the FLAPW calculations, a cut-off parameter $R_{\text{m}}K_{\text{max}} = 10.0$ was used, yielding about 250 plane waves for In and 170 for Al. For Al a [Ne] configuration was considered as the core state, whereas for In the [Kr] configuration was used and the 4d states were treated as local orbitals. The exchange–correlation potential was parametrized according to Perdew and Wang.^[5c] The reciprocal space integrations in the irreducible tetragonal Brillouin zone (BZ) were performed with the tetrahedron method.

In the case of the FP-LMTO calculations, we made use of valence band s, p, and d basis functions for both Al and In. For In pseudocore 4d states were included in the basis as well; that is, the resulting basis always formed a single, fully hybridizing basis set. The exchange–correlation potential was parametrized according to Hedin and Lundqvist.^[5d] For sampling the irreducible wedge of the BZ we used the special k points with a Gaussian smearing of width 20 mRy.

To ensure convergency several standard tests were performed for both methods, such as increasing the number of k points used in the summation over the BZ. The calculated energy differences ($E_{\text{c/a}} - E_{\text{fcc}}$) and band structures were found to be virtually identical for the two full-potential techniques. The presented energy differences and density of states were obtained from FP-LMTO calculations, the presented band structures from FLAPW calculations.

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Design and Development of the First Peptide-Chelating Bisphosphane Bioconjugate from a Novel Functionalized Phosphorus(III) Hydride Synthon**

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*Dedicated to Professor S. S. Krishnamurthy
on the occasion of his 60th birthday*

The incorporation of metal-chelating units into peptide backbones (and related biomolecules) has become an area of intense interest because of the potential applications to catalysis^[1] and biomedicine.^[2] Among various ligands available, phosphanes are unique because they display versatile coordination chemistry with transition metals.^[3] The secondary and tertiary structures of peptides to which they are attached may subsequently help in controlling the reactivity of phosphane-coordinated transition metals. Specifically, the chirality and related important stereospecific characteristics associated with biomolecules (e.g. peptides or proteins) may be transferred to the transition metals if peptides are immobilized with chelating units that are capable of coordinating with transition metals.^[4] This approach of conjugating catalytically active transition metals to chiral biomolecules provides a straightforward route to chiral compounds with potential applications in enantioselective catalysis.^[4] The incorporation of phosphanes into peptides (and proteins) will also help to engineer metal binding sites, which may eventually provide conformational integrity, biospecificity, and enhanced enzymatic activities.^[5]

Bioconjugation of cytotoxic transition metals to receptor-active peptides may eventually provide effective vehicles for delivering cytotoxic moieties to specific tumors through receptor-mediated agonist or antagonist interactions.^[6] In this context, peptides (or receptor-binding biomolecules) containing phosphane substituents are important in the design and development of tumor-specific radiopharmaceuticals.^[7, 8] Despite significant catalytic and biomedical applications offered by such peptides (and proteins), synthetic strategies for producing such bioconjugates are still in their infancy. The elegant work by Gilbertson and co-workers on the incorporation of aryl- and cyclohexylphosphanes into specific peptides has provided impetus to this potentially burgeoning field of chemical and biomedical sciences.^[9]

As part of our ongoing research on the development of functionalized phosphanes for biomedical and catalytic ap-

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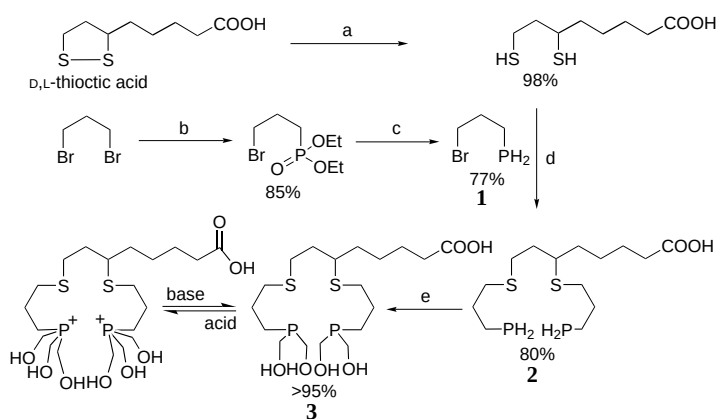
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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

plications,^[10] we have recently developed a novel strategy which allows bisphosphanes with heteroatoms and chelating functionalities to be directly incorporated into peptide backbones, under mild conditions, to produce the first example of a biomolecule functionalized with hydrophilic phosphane chelating units. We discuss here the synthetic utility of 3-bromopropylphosphane (**1**) in the development of a functionalized, water-soluble phosphane based on the dithio-bisphosphane (P_2S_2) framework of 6,8-bis[(3-phosphanylpropyl)thio]octanoic acid (**2**). We also demonstrate that the COOH group in **2** can be efficiently conjugated to peptides while retaining the phosphane moieties.

Our approach to the synthesis of ligand framework **2** is shown in Scheme 1. It involves the use of **1** as a key synthon which was recently obtained in our laboratory by the



Scheme 1. a) $NaBH_4$ (3.0 equiv), H_2O , EtOH, 25 °C, 5 h; b) $P(OEt)_3$ (0.2 equiv), Δ , 1 h; c) $LiAlH_4$ (1.5 equiv), $AlCl_3$ (4.6 equiv), tetraglyme, 0 $\rightarrow$ 25 °C, 12 h; d) NaH (4.1 equiv), THF, Δ , 12 h; e) HCHO (4.0 equiv), EtOH, 25 °C, 15 min.

reduction of diethyl 3-bromopropylphosphonate with dichloroaluminum hydride.^[11] Reaction of **1** with the dianion of 6,8-dithiooctanoic acid produced the COOH-functionalized P_2S_2 framework **2**. The proton-coupled ^{31}P NMR of **2** showed two merged triplets ($J_{PH} = 193.2$ Hz), which confirmed the presence of two different phosphanyl groups. The thermally stable P^{III} hydride **2** was then formylated with 37 % formaldehyde in ethanol to produce the corresponding water-soluble phosphane framework **3** in greater than 95 % yields (Scheme 1). Both **2** and **3** were characterized by ^1H , ^{13}C , and ^{31}P NMR spectroscopy, and HR-FAB mass spectrometry.

The co-existence of phosphanyl and carboxyl groups within the same molecule is difficult to achieve because the reaction conditions that are used to reduce a $P(O)(OEt)_2$ group to a PH_2 group can also reduce COOH groups. Thus, **1** can be used as a versatile synthon to produce compounds in which a chemical functionality that is susceptible to reduction (e.g. a carboxyl or amide group) and a highly reduced phosphanyl group coexist.

In general, primary phosphanes tend to be unstable towards oxidation. For example, PH_3 and RPH_2 ($R = Me, Et$) are oxidized instantaneously upon contact with atmospheric oxygen and water or during reactions in various organic solvents. In contrast, the new primary phosphane **2** exhibited a much improved stability profile under an even wider range of chemical conditions. Furthermore, the reactivity of the PH_2

groups in these compounds toward molecules containing a wide spectrum of functional groups (including carboxylic acids, amines, thiols, and proteins) was remarkably low or nonmeasurable. To our knowledge, this is the first report of compounds containing phosphanyl groups which exhibit such a degree of oxidative stability and lack of reactivity toward solvents and chemical functionalities. Preliminary AM1 calculations carried out with the MOPAC program^[12a] on **2** and related molecules suggest there are atomic orbital contributions from the heteroatom (e.g. S in **2**) to the frontier molecular orbitals. It is conceivable, therefore, that there is negative hyperconjugation involving specific orbitals of S and the P^{III} centers in **2**. This electronic effect may explain the unusual stability towards oxidation of **2** and related molecules with heteroatoms on their backbone.^[12b] More detailed calculations are underway. This electronic effect appears to operate even in the hydroxymethylated phosphanes **3**. For example, **3** is stable towards oxidation in aqueous media over several days (Figure 1). Such stability of alkyl-substituted phosphanes is remarkable.

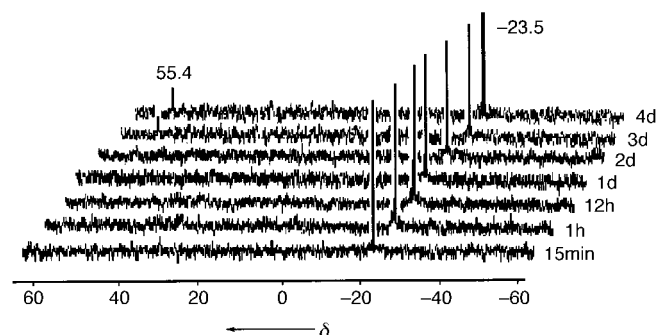
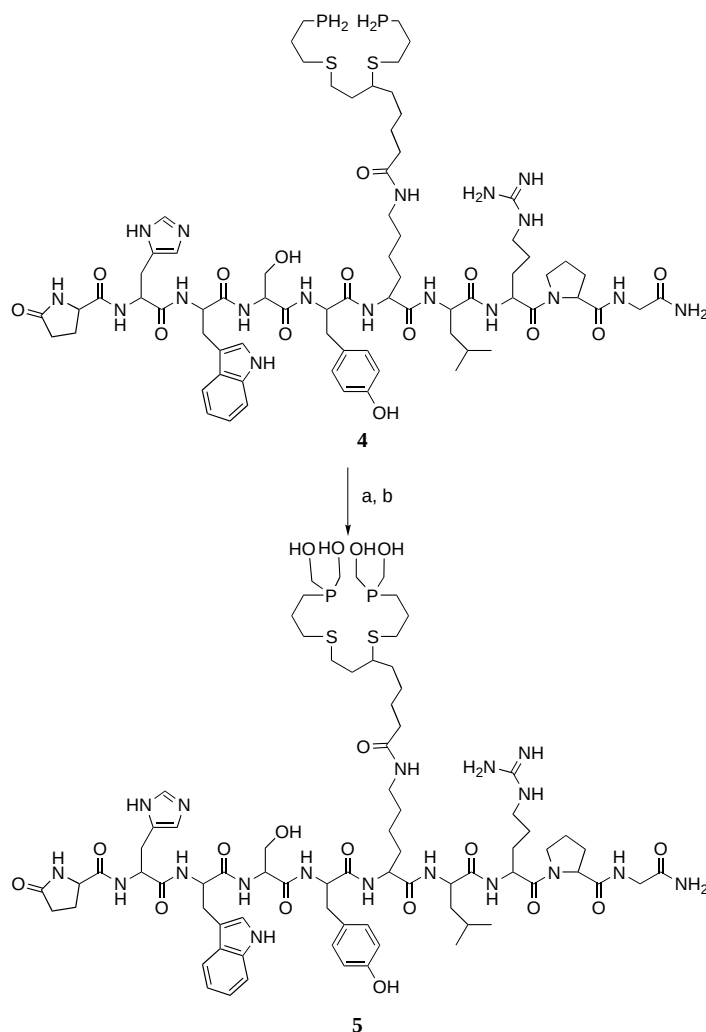


Figure 1. ^{31}P NMR spectra of **3** (aqueous sodium bicarbonate solution, pH 8.5) at various timepoints. Peaks at $\delta = -23.5$ and 55.4 refer to **3** and the corresponding phosphane oxide, respectively.

To determine the feasibility of linking compounds containing PH_2 groups to biomolecules, a P_2S_2 -D-Lys conjugate was prepared and then used to synthesize a P_2S_2 conjugate of a luteinizing hormone releasing hormone peptide, the D-Lys⁶-LHRH conjugate **4**, by automated solid-phase peptide synthesis (SPPS; Scheme 2). This method involved repeated use of a variety of chemicals in high concentrations (including trifluoroacetate (TFA) for cleavage of the peptide from the resin. Peptide **4** was purified by HPLC and analyzed by ^{31}P NMR spectroscopy and mass spectrometry (Figure 2). These data demonstrate that the peptide conjugate **4** was formed in high yields with no modification of the PH_2 groups. These results also confirm that the PH_2 groups of **2** are resistant to oxidation and are unreactive towards other functional groups in the LHRH peptide and the reagents used in SPPS. The synthesis of such biomolecules which contain PH_2 groups allows their conversion into hydrophilic alkylphosphanes. Thus, formaldehyde reacted rapidly with the PH_2 groups of **4** to produce the peptide-functionalized phosphane **5** (Scheme 2) now containing additional P–C bonds. Either PH_2 groups or their disubstituted analogues PR_2 may be used as a part of the chelator framework of biomolecules to form well-defined metalated conjugates by complexation with transition metals.



Scheme 2. a) 37% aq. HCHO, 0.1N HCl, 25°C, 5 min; b) 1M aq. NaHCO₃, 25°C, 5 min.

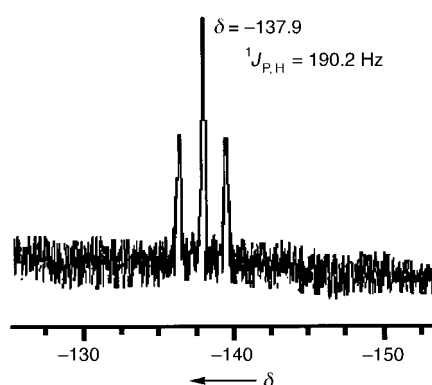


Figure 2. Proton-coupled ³¹P NMR spectrum of peptide–phosphane conjugate **4**.

The chemistry described herein demonstrates for the first time that primary phosphanes containing thioether groups possess high oxidative and thermal stabilities. Furthermore, the COOH group of such primary phosphanes provides an elegant opportunity for their incorporation into peptide backbones. Metalated analogues of **5** and related biomolecules may open up new avenues in the design of chiral

transition metal compounds for catalytic applications. Moreover, the metalation of functionalized peptides with cytotoxic metals, for example Pt^{II}, will also enable the design and development of site-specific drugs for delivery of cytotoxic agents to specific cancer sites through receptor-mediated agonist–antagonist interactions. Therefore, the new bioconjugate chemistry reported herein has implications in both catalysis and biomedicine fields.

Experimental Section

Diethyl 3-bromopropylphosphonate was synthesized by the Arbuzov reaction.^[13] Typically, triethyl phosphite in a tenfold excess of 1,3-dibromopropane was heated at reflux for 1 h to yield diethyl 3-bromopropylphosphonate in 95% yield after distillation under reduced pressure (85°C, 2 mm Hg). D,L-6,8-dithiooctanoic acid was synthesized according to the literature procedure.^[14]

1: To a well-stirred cold (0°C) suspension of LiAlH₄ powder (5 g, 135 mmol) in tetraglyme (100 mL) was added anhydrous AlCl₃ powder (55 g, 412 mmol) over 15 min. After the reaction mixture was stirred for 30 min at 0°C, diethyl 3-bromopropylphosphonate (23 g, 89 mmol) was added dropwise over 20 min. Stirring was continued at 25°C for 12 h, and then the reaction mixture was distilled under reduced pressure (50°C, 2.5 mm of Hg) to afford **1** (12 g, 77%), which was collected in a cooled receiver at –77°C. ¹H NMR (300 MHz, CDCl₃, 25°C): δ = 1.99 (br m, 2H), 2.32 (br m, 2H), 2.98 (br m, 2H), 3.32 (m, 2H); ¹³C NMR (75 MHz, CDCl₃): δ = 12.59 (d, ³J_{PC} = 9.2 Hz), 33.72 (s), 35.81 (s); ³¹P NMR (121 MHz, CDCl₃): δ = –138.37 (s).

2: D,L-6,8-dithiooctanoic acid (7.8 g, 37.8 mmol) in dry tetrahydrofuran (25 mL) was added dropwise to a well-stirred suspension of 60% NaH (6.2 g, 155 mmol) in dry tetrahydrofuran (200 mL) at 0°C. After the reaction mixture was stirred for 20 min at 0°C, a solution of **1** (11.8 g, 76.1 mmol) in dry tetrahydrofuran (25 mL) was added. The reaction mixture was then heated at reflux under nitrogen for 12 h. Excess NaH was quenched with a minimum of 10% HCl, the mixture was filtered through silica gel, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (3:2) to give pure **2** (10.8 g, 80%) as a colorless viscous oil. ¹H NMR (300 MHz, CDCl₃): δ = 1.44–1.61 (m, 6H), 1.69–1.78 (m, 6H), 2.29–2.34 (m, 2H), 2.46–2.66 (m, 12H), 2.93–2.99 (m, 4H), 11.45 (brs, 1H); ¹³C NMR (75 MHz, CDCl₃): δ = 13.46 (t, ³J_{PC} = 13.8 Hz), 24.9 (s), 26.62 (s), 29.72 (s), 31.23 (d, ³J_{PC} = 8.8 Hz), 33.05 (m), 33.38 (d, ³J_{PC} = 3 Hz), 34.34 (s), 35.02 (s), 35.08 (s), 44.1 (s), 180.34 (s); ³¹P NMR (121 MHz, CDCl₃): δ = –136.38 (s), –136.34 (s); ³¹P NMR (121 MHz, proton coupled, CDCl₃): δ = –136.29 (t, ¹J_{PH} = 193.2 Hz), –136.24 (t, ¹J_{PH} = 193.2 Hz); IR (NaCl): ν̄ = 2294 (P–H), 1723 (C=O) cm^{–1}; HR-MS (FAB): *m/z* calcd for [*M*⁺+H]: 357.1240; found: 357.1237.

3: To a solution of **2** (1 g, 2.8 mmol) in degassed ethanol (5 mL) at room temperature (25°C) was added 37% aqueous formaldehyde solution (0.92 mL, 11.3 mmol), and the reaction mixture was stirred for 1 h under nitrogen. Removal of the solvent under reduced pressure afforded **3** (1.3 g, 97%) as a colorless viscous oil. ³¹P NMR (121 MHz, D₂O): δ = –25.16 (s), –25.03 (s). For characterization and all other studies **3** was converted into its bisphosphonium chloride salt by the addition of 5N hydrochloric acid (0.5 mL) and 37% aqueous formaldehyde (0.5 mL, 5.6 mmol) to a solution of **3** (1.3 g, 2.7 mmol) in degassed ethanol (5 mL) at room temperature (25°C). After removal of solvent under reduced pressure the crude product was purified by chromatography on a C-18 Sep-Pak reverse phase column with water/methanol (3:2) to afford a pure bisphosphonium chloride (1.6 g, 94%) as a colorless viscous oil. ¹H NMR (300 MHz, D₂O): δ = 1.18–1.23 (m, 2H), 1.31–1.36 (m, 4H), 1.50–1.63 (m, 2H), 1.65–1.80 (m, 4H), 2.10–2.31 (m, 6H), 2.42–2.46 (m, 6H), 2.55–2.60 (m, 1H), 4.32 (brs, 12H); ¹³C NMR (75 MHz, D₂O): δ = 12.0 (d, ³J_{PC} = 10.56 Hz), 12.54 (d, ³J_{PC} = 10.5 Hz), 20.52 (s), 20.98 (s), 23.62 (s), 25.2 (s), 27.91 (s), 29.76 (d, ¹J_{PC} = 15.7 Hz), 31.32 (d, ³J_{PC} = 15.8 Hz), 33.36 (s), 43.66 (s), 49.64 (d, ¹J_{PC} = 54.4 Hz), 178.27 (s); ³¹P NMR (121 MHz, D₂O): δ = 28.98 (s), 28.99 (s); HR-MS (FAB): *m/z* calcd for [*M*⁺–Cl]: 573.1641; found: 573.1649.

4: Conjugate **4** was synthesized on a solid-phase peptide synthesizer. Standard fmoc-protected amino acids (fmoc = 9-fluorenylmethoxycarbonyl) were used for the synthesis. Conjugate **4** was purified by HPLC and analyzed with ^{31}P NMR spectroscopy and mass spectrometry (Figure 1). ^{31}P NMR (121 MHz, D_2O): $\delta = -137.91$ (s). ^{31}P NMR (121 MHz, proton coupled, D_2O) $\delta = -137.90$ (t, $J_{\text{PH}} = 190.2$ Hz). LR-MS (FAB) m/z calcd for $[M^+ + \text{H}]$: 1591.9; found: 1592.0.

5: To a solution of **4** (0.5 mg) in ethanol (400 μL) and DMF (100 μL) were added 0.1N HCl (25 μL) and 37 % aqueous formaldehyde (25 μL), and the reaction mixture was stirred at room temperature (25 °C) for 5 min. The formation of $\text{P}_2\text{S}_2\text{-D-Lys}^6\text{-LHRH}$ hydroxymethylphosphonium chloride was confirmed by the ^{31}P NMR signal at $\delta = 31.39$ (s). The $\text{P}_2\text{S}_2\text{-D-Lys}^6\text{-LHRH}$ hydroxymethylphosphonium chloride was converted into **5** by the addition of 1M aqueous sodium bicarbonate (30 μL) in near quantitative yields as demonstrated by the ^{31}P NMR chemical shift at $\delta = -24.23$.

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Modeling a Nitrogenase Key Reaction: The N_2 -Dependent HD Formation by D_2/H^+ Exchange**

Dieter Sellmann* and Anja Fürsattel

Dedicated to Professor Helmut Werner
on the occasion of his 65th birthday

Biological N_2 fixation is one of the fundamental natural synthetic processes and is catalyzed by FeMo, FeV, or FeFe nitrogenases.^[1] X-ray structure analyses have revealed the molecular structure of FeMo nitrogenase and its active centers, in particular the structure of the FeMo cofactors (FeMoco).^[2] However, the intimate molecular mechanism of biological N_2 reduction and the concomitant “obligatory dihydrogen evolution” (OHE) has remained a mystery. The OHE is an integral part of enzymatic N_2 reduction and cannot

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be suppressed even under high pressures of N₂. Equation (1) gives the (probably) limiting stoichiometry for FeMo nitrogenase.^[1, 2] The key feature of this OHE is the N₂-dependent formation of HD in the presence of D₂ [Eq. (2)], first

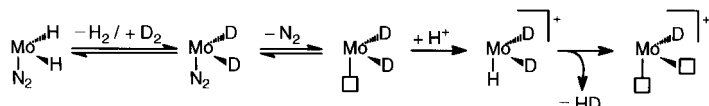


observed back in 1960.^[3] The HD must result from D₂ and protons derived from H₂O. The dependence on N₂ is stringent, and N₂ cannot be replaced by any other nitrogenase substrate, for example C₂H₂, N₃[−], or N₂O. Electron balance studies prove that one electron is required per each molecule HD formed.^[4]

The stringent N₂ dependence of HD formation proves the intimate coupling of OHE and N₂ reduction, and it compellingly demands the occurrence of a reduction intermediate that only is able to form with N₂. Elucidation of its molecular mechanism can therefore be expected to shed light also on the mechanistic details of N₂ reduction and the function of the FeMo cofactors.

Two mechanisms have been postulated in order to explain the N₂-dependent HD formation. Both the “trihydride” and the “diazene” mechanism suggest that nitrogenase (as isolated in the dithionite reduced state) must first be reduced before HD formation can occur, explaining that NH₃, H₂ as well as HD formation are electron-requiring processes.

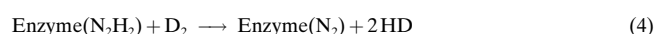
The “trihydride” mechanism is based on the kinetic Lowe–Thorneley scheme of nitrogenase and model reactions of dinitrogen(phosphane)molybdenum complexes such as [Mo(N₂)₂(PR₃)₄].^[5] Successive electronations and protonations transfer nitrogenase into a stage in which the Mo center of FeMoco binds N₂ and hydride ligands (Scheme 1). Exchange



Scheme 1. “Trihydride” mechanism of the N₂-dependent HD formation.

of the hydride ligands with D₂, loss of N₂, and protonation of the resulting vacant coordination site (□) gives a [Mo(H)(D)₂] species which liberates HD. A direct interaction between the N₂ and hydrogen/deuterium ligands occurs at no stage. In the decisive step of HD formation, N₂ does not bind to the Mo center, and N₂ essentially acts as “stand-in” ligand only. This raises the question why N₂ is indispensable for HD formation and cannot be replaced by other nitrogenase substrates known to function likewise as two-electron ligands, for example, N₃[−] or N₂O.

The “diazene” mechanism avoids this problem by proposing N₂H₂ as enzyme-bound pivotal N₂ reduction intermediate formed by Equation (3).^[3, 4] D₂ attack upon the N₂H₂ intermediate is postulated to result in formation of HD and enzyme-bound N₂ according to Equation (4). Equation (4)



sums up to a “D₂-catalyzed N₂H₂ decomposition”.^[4] The “diazene” mechanism is supported by the electron balance (1e[−] per HD), but raises the question why N₂ should first get reduced in order to be subsequently reoxidized by D₂(!). The intimate molecular mechanism remained speculative because a chemical equivalent could not be found that catalyzed the H/D exchange between molecular D₂ and N₂H₂ whose protons ultimately had to come from water. Here we want to report the first example for such a reaction.

The ruthenium complex [(μ-N₂H₂){Ru(PCy₃)(‘S₄’)}₂] (**1a**) was synthesized by the method described for the homologous [(μ-N₂H₂){Fe(PPr₃)(‘S₄’)}₂] (H₂‘S₄’ = 1,2-bis(2-sulfanylphe-nylthio)ethane).^[6] N₂H₂ was generated from K₂N₂(CO₂)₂ and acetic acid and trapped by [Ru(dmso)(PCy₃)(‘S₄’)] in THF. Acetolysis of K₂N₂(CO₂)₂ with CH₃COOD yielded the deuterated derivative [(μ-N₂D₂){Ru(PCy₃)(‘S₄’)}₂] (**1b**).^[7] The spectroscopic properties of **1a/1b** (see Figure 2a and 3a) and X-ray structure determinations of analogous [(μ-N₂H₂){Ru(PR₃)(‘S₄’)}₂] complexes (R = Ph, *i*Pr)^[8] support the structure indicated in Figure 1 for **1a**. A *trans*-diazene

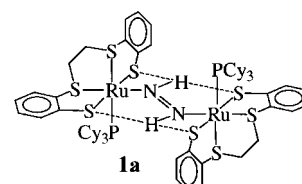


Figure 1. Schematic representation of the structure of **1a**; only one of the two diastereomers existing in solution at room temperature is shown.

ligand bridges two enantiomeric [Ru(PCy₃)(‘S₄’)] fragments and gives rise to two (unsymmetrical) bifurcated N–H⋯(S)₂ bridges. Complex **1a**, like most complexes of this type, forms two diastereomers in solution. Both are centrosymmetric and each diastereomer gives rise to one ¹H NMR diazene signal (Figure 2a). The two “hydrogen bridge diastereomers” differ only in the positioning of the N–H⋯(S)₂ bridges.^[8b]

Under standard conditions (1 bar, 25 °C) treatment of **1a** with molecular D₂ gave **1b** and HD [Eq. (5a)]. Higher pressures resulted in accordingly faster turnovers. Likewise, **1b** reacted with H₂ to give **1a** and HD [Eq. (5b)]. (Absolutely



anhydrous conditions were observed and gases were dried with Na/K alloy in order to exclude any interference with the reactions according to Equation (6) (below)). Formation of

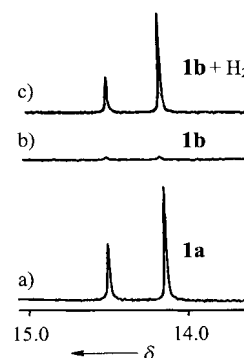


Figure 2. Diazene region of the ¹H NMR spectra (CD₂Cl₂) of a) **1a**, b) **1b**, and c) **1b** after complete reaction with H₂.

either **1a** or **1b** was monitored by their characteristic $^1\text{H}/^2\text{D}$ NMR diazene signals in the region 14–15 ppm (shown in Figure 2b, c for reaction (5b)). Formation of HD was confirmed by mass spectrometry and ^1H NMR spectroscopy. In the reaction of **1b** with H_2 (35 bar) in a NMR pressure tube, in addition to the signal for **1a**, a 1:1:1 triplet ($J(\text{H},\text{D}) = 42.51\text{ Hz}$) was detected at $\delta = 4.57$ for the resulting HD (Figure 3b). Because the Equations (1), (2), and (3), imply

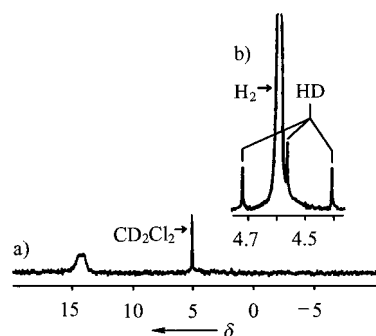


Figure 3. a) ^1H NMR spectrum of **1b** in CH_2Cl_2 ; b) H_2/HD region of the ^1H NMR spectrum of **1b** in CD_2Cl_2 after reaction with 35 bar of H_2 .

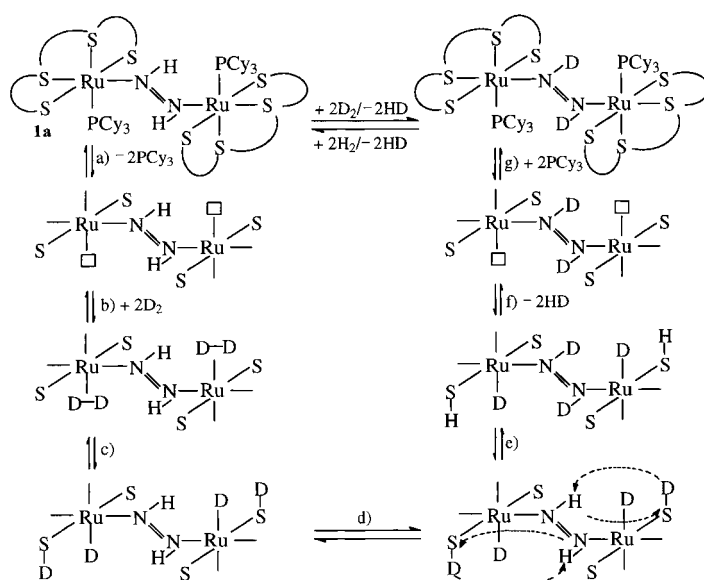
that the protons of reductively formed N_2H_2 must derive from H_2O , also the H^+/D^+ exchange of **1a/1b** was probed according to Equation (6). As in the case of the isoelectronic Fe complex



$[(\mu\text{-N}_2\text{H}_2)\{\text{Fe}(\text{PPr}_3)(\text{S}_4')\}_2]^{[9]}$ a reversible H^+/D^+ exchange was observed. It is noteworthy that this H^+/D^+ exchange took place about seven times slower than the D_2/H^+ exchange according to Equation (5). Combining reactions (5) and (6) thus proves that HD formation results from D_2 exchange with N_2H_2 whose protons can derive from water.

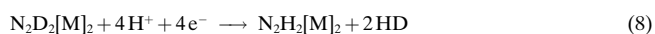
Insight into the D_2/H^+ exchange mechanism of reaction (5a) results from previous findings: 1) The complexes $[\text{Rh}(\text{H}_2\text{O})(\text{PCy}_3)(\text{S}_4')]\text{BF}_4$ and $[\text{Ru}(\text{dmsO})(\text{PCy}_3)(\text{S}_4')]$, which have labile H_2O and dmsO ligands and are closely related to **1**, catalyze the heterolytic cleavage of H_2 (or D_2) via $[\text{M}(\eta^2\text{-H}_2)]$ and $[\text{M}(\text{H})(\text{SH})]$ hydride–thiol intermediates. They also catalyze the scrambling of hydride ligands and thiol protons.^[10] 2) The N_2H_2 complex **1a** exchanges its PCy_3 for $\text{P}i\text{Pr}_3$ ligands under retention of the $[\mu\text{-N}_2\text{H}_2[\text{Ru}(\text{S}_4')]]_2$ entity. This indicates that, through dissociation of PCy_3 , **1a** can provide vacant Ru sites for the addition and heterolytic cleavage of H_2 .^[7b, 10]

Combination of these results and Equations (5) suggests the mechanism given in Scheme 2 for the NH/D_2 or ND/H_2 exchange of **1**. Essential core atoms of **1** are the Ru centers, thiolate donors, and diazene atoms. Dissociation of PCy_3 (step a) yields vacant sites ($\square$) to which D_2 adds (step b) that is cleaved heterolytically into D^- and D^+ by the concerted action of the Lewis acidic Ru centers and Brønsted basic thiolate donors (step c). Intramolecular scrambling of the acidic diazene protons and thiol deuterons (steps d and e) gives the N_2D_2 species, which releases HD (step f). Readdition of PCy_3 yields **1b** (step g).



Scheme 2. D_2/NH exchange of **1a**. The arrows in the lower right formula should only be considered to indicate intramolecular H/D exchange which is possible following D_2 heterolysis; it should not be considered to imply simultaneous H/D migration.

These results thus support a “diazene” mechanism that explains the N_2 dependence of nitrogenase-catalyzed HD formation. They need, however, further discussion, because Equations (5) evidently contrast with Equation (4). Equations (5) show that H_2 or D_2 attack upon bound diazene does not decompose N_2H_2 to give N_2 . They rather suggest that the N_2 -dependent HD formation takes place on the diazene level. In other words: The diazene reduction stage stays preserved in the course of HD formation and does not switch back to the N_2 level. This necessitates an important conclusion with regard to the overall mechanism of the N_2 -dependent HD formation. Reaction (5a), rewritten as Equation (7), utilizes only one half of the D_2 for HD formation, binds the other half in the diazene, and does *not* require electrons. In order to utilize also the diazene-bound deuterium and to make the HD formation catalytic, the reaction according to Equation (8) must take place. Adding Equation (7) to Equation (8) gives Equation (9). Equation (9) fulfills the experimentally established stoichiometry of nitrogenase-catalyzed N_2 -dependent HD formation (1e^- per HD).



These considerations show that even on the diazene level two distinguishable pathways of HD formation have to be taken into account. However, in nitrogenase the H^+/e^- flow must be assumed to continue when the diazene stage has been reached. In order to achieve reaction (8), a scheme can be suggested which is quite similar to Scheme 2. As additional elementary reaction, it contains a H^+ reduction for which chemical precedents at iron–sulfur centers are known.^[11]

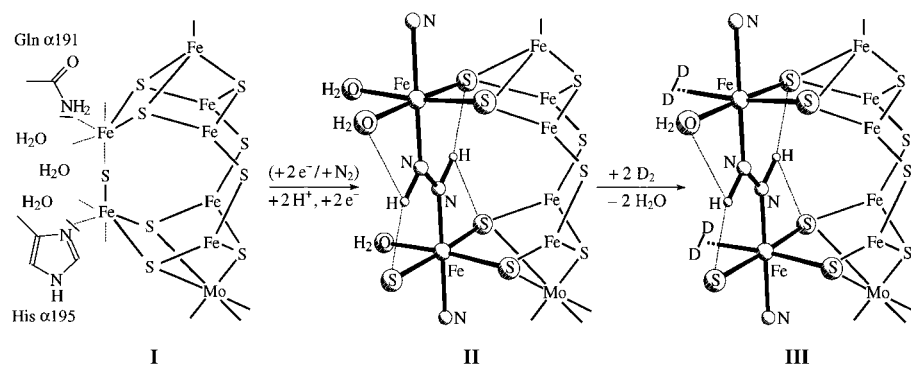
With regard to the FeMo cofactors, the results reported here are plausibly compatible with and support the recently

proposed concept of FeMoco functioning.^[12] According to this concept reduction of nitrogenase for turnover leads to opening the FeMoco cluster. One Fe-S-Fe bridge dissociates and vicinal amino acid donors (from Gln α 191 and His α 195) and H₂O molecules add such that two unique five-coordinate low-spin Fe^{II} centers result. The (variable) space between these Fe centers can accommodate N₂ as well as its reduction products N₂H₂, N₂H₄, and two NH₃. Scheme 3 depicts the N₂H₂ stage. Like PCy₃ dissociation in **1**, dissociation of the H₂O ligands in Scheme 3 can generate vacant sites at the two

under 1 bar, 35 bar, and 120 bar. The reaction with H₂ at 35 bar was monitored by using a high-pressure NMR tube purchased from Firma Wilmad (528-PV-1, inner diameter 2.2 mm). H⁺/D⁺ exchange of **1b**: An approximate 70-fold excess of H₂O was added to a saturated solution of **1b** in CD₂Cl₂. NMR spectra were recorded after 25 h.

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Scheme 3. Opening the FeMoco when changing from the resting (**I**) to the turnover state (**II**), and catalysis of the D₂/H⁺ exchange (**III**).

unique Fe centers rendering possible D₂ addition and D₂/H⁺ exchange. It is pointed out that the labile H₂O binding sites also permit addition of CO or other *non-competitive* N₂ reduction inhibitors.

Experimental Section

Unless noted otherwise, all manipulations were carried out in absolute solvents under nitrogen at room temperature. Reactant gases were dried with Na/K alloy. Reaction rates were estimated by means of the NH/CH intensity ratio of diazene protons and aromatic protons of the 'S₄' ligands. Smaller line widths and greater sensitivity make ¹H NMR spectra better suited for this purpose than ²H NMR spectra, which, however, were also recorded.

1a, b: Acetic acid (14 mL, 2.80 mmol, 0.2 M in H₂O) was added dropwise to a yellow-green suspension of K₂N₂C₂O₄ (560 mg, 2.88 mmol) and [Ru(dmsO)-(PCy₃)('S₄')] (552 mg, 0.72 mmol) in THF (20 mL). Gas evolved and the suspension turned into a dark green solution, from which dark green microcrystals precipitated. Removal of the aqueous phase and dropwise addition of MeOH (10 mL) to the THF phase completed the crystallization. The precipitated microcrystals were separated after 1 h, washed with MeOH (10 mL), and recrystallized from CH₂Cl₂/MeOH (–30 °C). Compound **1a** (455 mg, 88%) crystallizes in diastereomeric pure form at this temperature. Correct elemental analyses. ¹H NMR (269.6 MHz, CD₂Cl₂, –30 °C): δ = 14.15 (s, 1H, N₂H₂), 7.55–6.75 (m, 8H, C₆H₄), 2.85–2.60 (m, 2H, C₂H₄), 2.10–0.85 (m, 35H, C₂H₄, P(C₆H₁₁)₃); ¹³C{¹H} NMR (67.7 MHz, CD₂Cl₂, –30 °C): δ = 158.80 (d), 158.00, 134.20, 134.00, 132.80, 131.60, 130.80, 130.30, 128.50, 128.20, 122.30, 121.10 (C₆H₄), 44.80 (d), 38.30 (C₂H₄), 37.60, 30.00, 28.30 (d), 26.70 [P(C₆H₁₁)₃]; ³¹P{¹H} NMR (109.38 MHz, CD₂Cl₂, –30 °C): δ = 28.00 (s); UV/Vis (CH₂Cl₂): $\lambda_{\max}$ (nm) ($\epsilon \times 10^{-4}$) = 500 (0.67), 630 (0.40). Compound **1b**: Using CH₃COOD/D₂O and CH₃OD and recrystallization from CH₂Cl₂/MeOD (–30 °C) yielded **1b** in an analogous way.

D₂/NH exchange of **1a** at standard pressure: NMR spectra were recorded of a solution of **1a** in CH₂Cl₂ that was stirred under D₂. The N₂D₂ signals of **1b** were observed after 48 h. H₂/ND exchange of **1b**: ¹H NMR spectra were recorded of saturated solutions of **1b** in CD₂Cl₂ which were treated with H₂

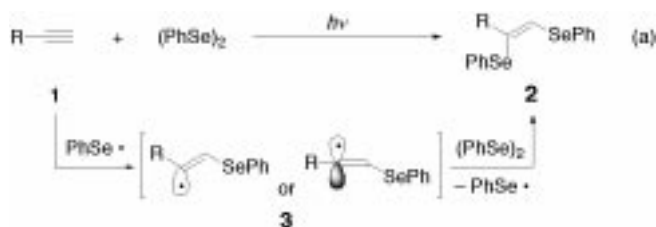
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Highly Selective Three-Component Coupling of Ethyl Propiolate, Alkenes, and Diphenyl Diselenide under Visible-Light Irradiation**

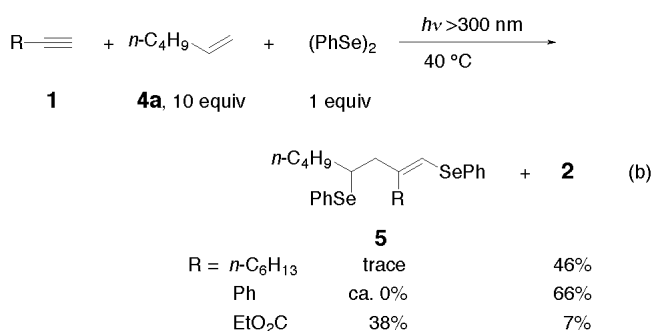
Akiya Ogawa,* Mikio Doi, Ikuko Ogawa, and Toshikazu Hirao*

Diphenyl diselenide has its absorption maximum in the near-UV region, and therefore irradiation with light of wavelength greater than 300 nm induces homolytic cleavage of the selenium–selenium bond to generate a phenylseleno radical as a labile species.^[1] Compared with the corresponding thiyl radical, the reactivity of the phenylseleno radical toward unsaturated carbon–carbon bonds is lower by a factor of 10–50.^[2] However, (PhSe)₂ is an excellent trap for carbon radicals and is about 160 times more effective than (PhS)₂.^[3] Owing to the lower reactivity of PhSe•, the free-radical addition of (PhSe)₂ to alkenes does not proceed efficiently.^[4] Recently, we found that (PhSe)₂ smoothly adds to alkynes with high efficiency at a high initial concentration of the reactants [Eq. (a)].^[5] In this reaction, β -(phenylseleno)vinyl radicals **3**



appear to be formed as a key intermediate, so if the photoinitiated reaction of (PhSe)₂ with alkynes is performed in the presence of alkenes, the sequential addition of the vinyl radical intermediate **3** to alkenes can be expected. The intramolecular coupling reaction between an alkyne, an alkene, and a radical precursor (tin hydride, thiol, sulfonyl chloride, etc.) is well documented;^[6] however, their selective intermolecular coupling has remained largely unexplored.^[7]

We began our study of this sequential addition reaction by examining the combination of alkyne and alkene. With 1-hexene as standard alkene, three alkynes were tested for the sequential addition of (PhSe)₂ [Eq. (b)]. Neither 1-octyne nor phenylacetylene, which generate vinyl σ and π radicals,^[8] respectively, by the attack of PhSe• at the terminal carbon atom, provided the desired alkyne–alkene coupling products



5. Instead, 1,2-bis(phenylseleno)-1-alkenes (**2**) were obtained as the major products. When the electron-deficient acetylene ethyl propiolate^[9] was employed, however, the desired three-component coupling reaction with 1-hexene and (PhSe)₂ took place and gave 1,4-bis(phenylseleno)-2-(ethoxycarbonyl)-1-octene (**5**; R = EtO₂C, *E/Z* = 21/79) in moderate yield with good selectivity. Accordingly, the reaction proceeds via a vinyl radical **3a**, which is formed by selective attack of PhSe• on the alkyne,^[10] and subsequent addition of **3a** to 1-hexene to generate the homoallylic radical **6a**. These results suggest that a combination of radicals bearing an electron-withdrawing substituent at the radical center and alkenes with an electron-donating substituent is favorable for diselenide-mediated sequential addition to alkynes and alkenes.

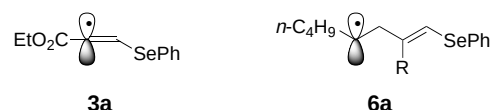


Table 1 lists the results of the addition of (PhSe)₂ to ethyl propiolate and various alkenes. As expected, increasing the electron density of the alkene dramatically improved the yield of the sequential addition product **5** (entries 1–3). In the case of butyl vinyl ether (**4b**), for example, the desired three-component coupling product **5b** was obtained in 89% yield, and the formation of ethyl 2,3-bis(phenylseleno)acrylate (**2**, R = EtO₂C) was suppressed to 2–3% (entry 1). The sequential addition products **5a–g** were obtained as mixtures of stereoisomers with a predominance of the *Z* isomers. With 2,3-dihydrofuran (**4d**), the stereochemistry at the furan ring of the product **5d** was exclusively *trans* (entry 3). Similar reactions can be performed with the enol silyl ether **4e**, allyl alcohol (**4f**), and the acetal **4g**, which give the corresponding coupling products **5e–g** in good yields (entries 4–6). Furthermore, sequential alkyne–diene coupling was demonstrated (entries 7 and 8). In both cases, conjugate addition products **5h**, **5i**, and **5i'** were obtained selectively.

The coupling products have two kinds of seleno group, that is, an alkylseleno group and a vinylseleno group, and are potentially useful synthetic intermediates. The alkylseleno group can be removed reductively,^[11] whereas oxidation of the alkylseleno group can lead to olefin formation by *syn* elimination of selenoxide.^[12] Furthermore, vinyl selenides are regarded as both carbonyl equivalents (by hydrolysis)^[13] and vinyl-transfer reagents (by transition metal catalyzed cross-coupling reactions).^[14] We investigated the alkylation of the sequential addition products with organocopper re-

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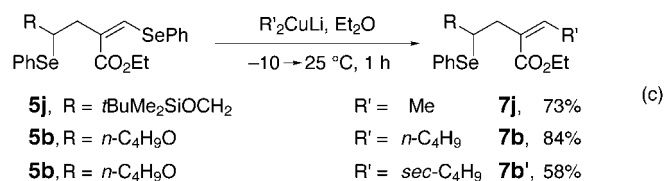
Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

Table 1. Three-component coupling of ethyl propiolate, alkenes, and (PhSe)₂.^[a]

Entry	Alkene	Product	Yield [%] ^[b]	E/Z
1			89	10:90
2			78	7:93
3			70	15:85
4 ^[d]			71	6:94
5			59	12:88
6			57	8:92
7 ^[f]			56 (72)	67:33 ^[e]
8 ^[f]			55	94:6 ^[e]
			23	84:16 ^[e]

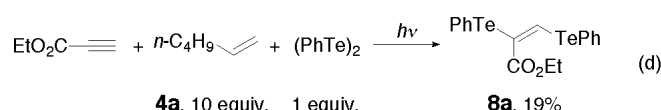
[a] Reaction conditions: ethyl propiolate (0.16 mmol), alkene (15 equiv), (PhSe)₂ (1 equiv, added over 1.5 h in several portions), 15 °C, 2 h, *hν* > 300 nm (tungsten lamp, 500 W, Pyrex). [b] Yield of isolated product (NMR). [c] The stereochemistry at the furan ring is exclusively *trans*. [d] Enol silyl ether **4e** (40 equiv). [e] 4*E*/4*Z* ratio; 1*E*/1*Z* = 0/100. [f] Diene (6 equiv).

agents.^[15] Treatment of the coupling product **5j** with lithium dimethylcuprate led to chemoselective methylation to give **7j** with retention of stereochemistry [Eq. (c)]. Similarly, **5b**

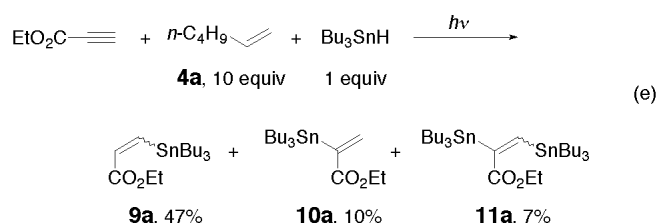


underwent site-selective butylation on treatment with *n*Bu₂CuLi. The introduction of a secondary alkyl group such as *sec*-butyl requires the use of an excess of *s*Bu₂Cu(CN)Li₂ (5 equiv) in the presence of BF₃·Et₂O (THF, −78 to ca. 25 °C, 12 h).^[16]

In summary, we have developed a highly selective sequential addition of (PhSe)₂ to ethyl propiolate and alkenes. Note that the use of diphenyl disulfide instead of (PhSe)₂ for the sequential addition reaction resulted in the polymerization of the unsaturated compounds and no formation of the desired three-component coupling products. This is most probably because the ability of (PhS)₂ to capture carbon radicals was insufficient to suppress the polymerization reaction.^[17] In contrast, the attempted sequential reaction with diphenyl ditelluride resulted only in the formation of the product of (PhTe)₂ addition to the alkyne, **8a**, probably due to effective trapping of the carbon radicals [Eq. (d)].^[17]



Although modern, radical-based synthetic methods often utilize tin hydrides,^[18] the attempted tin hydride mediated reaction of ethyl propiolate with 1-hexene under similar reaction conditions gave only the products of addition to the alkyne, **9a**, **10a**, and **11a** [Eq. (e)]. These observations suggest



that the present sequential three-component coupling reaction with (PhSe)₂ is a kinetically controlled system in which (PhSe)₂ facilitates selective sequential addition and inhibits the polymerization of the unsaturated compounds.

Experimental Section

5b: In a Pyrex glass tube (5 mm × 18 cm) under argon atmosphere, ethyl propiolate (30.2 mg, 0.3 mmol), *n*-butyl vinyl ether (760 mg, 7.6 mmol), and diphenyl diselenide (9.4 mg, 0.03 mmol) were irradiated with a tungsten lamp (500 W) at 15 °C. During irradiation, diphenyl diselenide (84.3 mg, 0.27 mmol) was added in nine portions over 1.5 h. After the reaction was complete, the volatile materials were evaporated, and the residue was purified by recycling preparative HPLC (Japan Analytical Industry, Model LC-908), equipped with JAIGEL-1H and -2H columns (GPC) with CHCl₃ as eluent to give 136.3 mg (89%) of **5b** as a mixture of stereoisomers (*E/Z* = 10/90). The stereochemistry of the products was determined by NOE experiments.

5b: Pale yellow oil. ¹H NMR (400 MHz, CDCl₃, 25 °C, TMS): *Z* isomer: δ = 0.87 (t, ³*J*(H,H) = 7.3 Hz, 3H, CH₃(CH₂)₃), 1.28 (t, ³*J*(H,H) = 7.1 Hz, 3H, CH₃CH₂O), 1.25–1.38 (m, 2H, CH₃CH₂(CH₂)₂), 1.51 (quint, ³*J*(H,H) = 7.0 Hz, 2H, CH₃CH₂CH₂CH₂), 2.85 (dd, ³*J*(H,H) = 7.8, ²*J*(H,H) = 14.6 Hz, 1H, CH₂C=C), 2.98 (dd, ³*J*(H,H) = 4.9, ²*J*(H,H) = 14.6 Hz, 1H, CH₂C=C), 3.31 (dt, ³*J*(H,H) = 6.5, ³*J*(H,H) = 9.3 Hz, 1H, CH₂CH₂O), 3.88 (dt, ³*J*(H,H) = 6.4, ²*J*(H,H) = 9.3 Hz, 1H, CH₂CH₂O), 4.23 (q, ³*J*(H,H) = 7.2 Hz, 2H, CH₃CH₂O), 5.07 (dd, ³*J*(H,H) = 4.9, 8.3 Hz, 1H, CH=SePh), 7.43 (s, 1H, CH=C), 7.19–7.64 (m, 10H, Ph); *E* isomer: δ = 0.89 (t, ³*J*(H,H) = 7.3 Hz, 3H, CH₃(CH₂)₃), 1.23 (t, ³*J*(H,H) = 7.1 Hz, 3H, CH₃CH₂O), 1.25–1.38 (m, 2H, CH₃CH₂(CH₂)₂), 1.55 (quint, ³*J*(H,H) = 7.0 Hz, 2H, CH₃CH₂CH₂CH₂), 3.01 (dd, ³*J*(H,H) = 7.8, ²*J*(H,H) = 14.6 Hz, 1H, CH₂C=C), 3.10 (dd, ³*J*(H,H) = 4.9, ²*J*(H,H) = 14.6 Hz, 1H, CH₂C=C), 3.31 (m, 1H, CH₂CH₂O), 3.87 (m, 1H, CH₂CH₂O), 4.15 (q, ³*J*(H,H) =

6.6 Hz, 2H, CH₃CH₂O), 5.21 (dd, ³J(H,H) = 4.8, 8.4 Hz, 1H, CH–SePh), 7.19–7.64 (m, 10H, Ph), 8.05 (s, 1H, CH=C); ¹³C NMR (100 MHz, CDCl₃): Z isomer: δ = 13.77, 14.17, 19.26, 31.17, 39.68, 60.62, 69.79, 85.91, 124.32, 127.46, 127.95, 128.77, 129.15, 133.24, 135.39, 147.51, 167.03; E isomer: δ = 12.93, 14.24, 19.29, 31.10, 41.69, 60.78, 69.72, 86.38, 127.39, 127.95, 128.54, 129.33, 132.93, 133.20, 135.26, 143.39, 164.79; IR (NaCl): $\tilde{\nu}$ = 1694 (C=O), 1574 cm⁻¹ (C=C); MS (CI): m/z (%): 439 (70) [M⁺ – BuO] or [M⁺ – CO₂Et]; elemental analysis calcd for C₂₃H₂₈O₃Se₂: C 54.13, H 5.53; found: C 54.24, H 5.66.

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Keywords: alkenes • alkynes • C–C coupling • radical reactions • selenium

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The First Determination of Eu–H Distances by Neutron Diffraction on the Novel Hydrides EuMg₂H₆ and EuMgH₄**

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The complete crystal structure analysis of metal hydrides usually requires neutron diffraction data. Some elements, however, show excessively high absorption cross sections, σ_a , for neutrons, thus making this technique seemingly impractical. Natural europium (^{nat}Eu) consists of nearly equal amounts of the isotopes ¹⁵¹Eu and ¹⁵³Eu. Its absorption cross section for thermal neutrons (σ_a = 4530 barns at λ = 179.8 pm) is higher than that of the shielding material cadmium.^[1] Pure ¹⁵³Eu has a lower σ_a but is extremely expensive. Thus no complete, refined crystal structure data are known for europium hydrides, that is no reliable value exists for the distance Eu–H.^[2]

Fortunately, the neutron absorption cross section of natural europium is strongly wavelength dependent and shows a minimum at λ = 72 pm (Figure 1). This prompted us to perform the first neutron diffraction experiment at this

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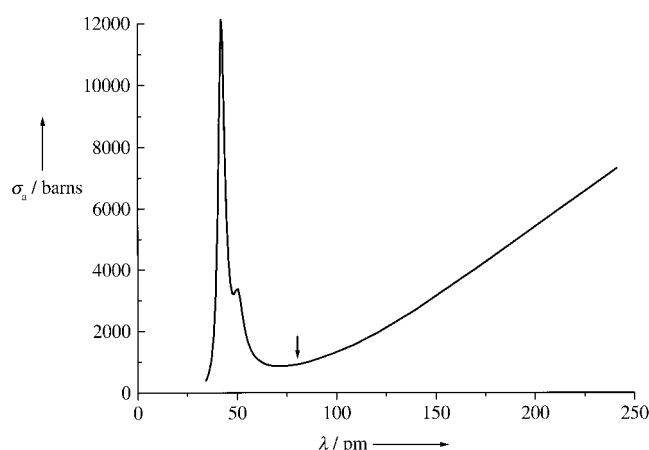


Figure 1. The absorption cross section of natural europium for neutrons as a function of the wavelength, calculated from $\sigma_a = 4\pi/k b_c''$ (k = norm of the neutron radiation wave vector; b_c'' = imaginary part of the coherent scattering length according to Lynn^[3]). The wavelength used for the neutron diffraction experiment ($\lambda = 80.45$ pm) is marked by an arrow.

wavelength on $^{\text{nat}}\text{Eu}$ -containing hydrides. The intensively colored carmine-red EuMg_2H_6 and brown EuMgH_4 , which are the first ternary hydrides known in the Eu-Mg-H system, appeared to be well suited for this purpose (see Experimental Section). Since single crystals were not available, the structures were solved from powder diffraction data. The X-ray pattern of EuMg_2H_6 was indexed to a tetragonal unit cell^[4] and the EuMg_2 substructure solved ab initio. The patterns of EuMgH_4 suggested an isotopic structure to orthorhombic SrMgH_4 (noncentrosymmetric) or BaMgH_4 (centrosymmetric),^[4, 5] but did not allow to differentiate between these two models. Neutron diffraction data of two deuterated, multiphase samples were collected on the diffractometer D20 equipped with a position-sensitive detector at the high-flux reactor at the Institut Laue-Langevin (Grenoble) at $\lambda = 80.45$ pm (Figure 2). All deuterium positions could be located and refined. Owing to its higher transmission the EuMg_2D_6 sample yielded more precise data than the EuMgD_4 sample (calculated: $T = e^{-\mu x} = 0.45$ and 0.08 ; Tables 1 and 2).

EuMg_2H_6 crystallizes in a novel AB_2X_6 type structure which can be described either as a stuffed variant of the ReO_3 type (BX_3), or as an ordered vacancy variant of the cubic perovskite type (ABX_3). Hydrogen occupies the X, magnesium the B, and europium half of the A sites. Its structural relationship to the cubic perovskite type can be illustrated by means of group-subgroup relationships (Figures 3a and 4). The doubling of the c axis in the second step of the symmetry reduction causes a splitting of the A cation position in two symmetry-independent sites, from which only one is occupied by europium. The niobium and tantalum bronzes AB_3O_9 ($=\text{A}_{2/3}\text{B}_2\text{O}_6$; $\text{A} = \text{La-Nd}$; $\text{B} = \text{Nb, Ta}$) and $\text{ANb}_4\text{O}_{12}$ ($=\text{A}_{1/2}\text{Nb}_2\text{O}_6$; $\text{A} = \text{Th, U}$)^[9] can be described as defect variants of the EuMg_2H_6 type, which are orthorhombically distorted for $\text{B} = \text{Nb}$.

Europium has a nearly cuboctahedral, and magnesium a nearly octahedral deuterium configuration in EuMg_2D_6 . The metal coordination of deuterium is quadratic-bipyramidal

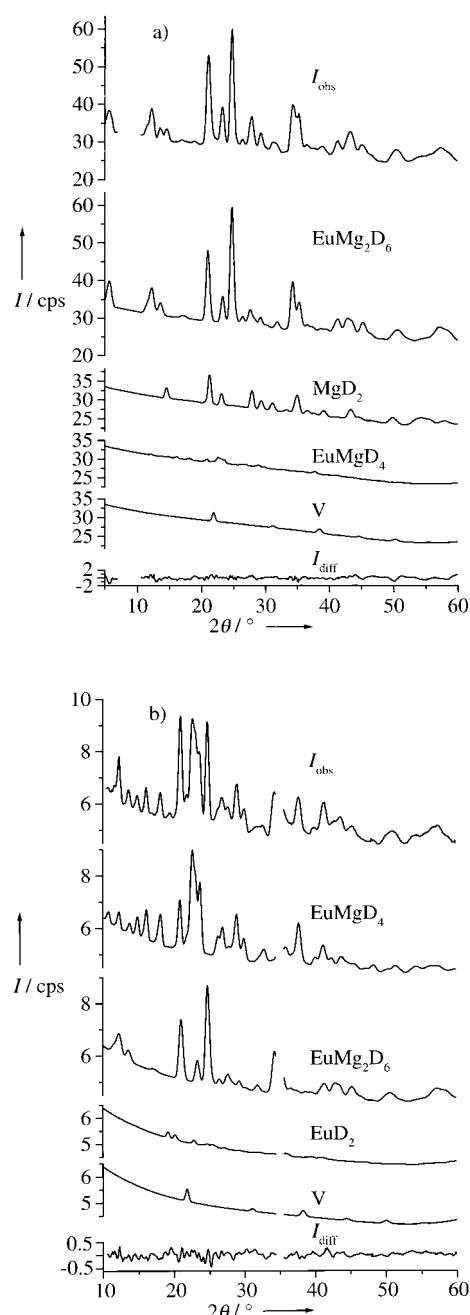


Figure 2. Graphical representation of the Rietveld refinement of the crystal structures of EuMg_2D_6 and EuMgD_4 . From top to bottom: a, b) observed neutron diffraction pattern of the EuMg_2D_6 sample and of the EuMgD_4 sample, respectively, calculated patterns of EuMg_2D_6 and of EuMgD_4 , respectively, of the by-products MgD_2 and EuMgD_4 and EuMg_2D_6 and EuD_2 , respectively, as well as of vanadium (sample holder) and the difference pattern. Intensity in counts per second; refinement using the program FULLPROF^[6] neglecting the angular dependence of the absorption ((a) ca. 5%, (b) ca. 30%). Data collection: diffractometer D20, Institut Laue-Langevin, Grenoble (France); $\lambda = 80.45$ pm; $T = 293$ K; calculated linear absorption coefficients $\mu = 8.09$ cm⁻¹ (a), $\mu = 12.53$ cm⁻¹ (b); 1.5 g each in double-walled vanadium cylinder with $d_i = 13.5$ mm (a), 7 mm (b); $d_o = 14.5$ mm (a), 9 mm (b); data collection time 7 h (a), 13.5 h (b); 515 (a), 485 data points (b); blanks correspond to excluded regions due to detector failure.

(D2: 4 Eu + 2 Mg), saddlelike (D1: 2 Eu + 2 Mg) or linear (D3: 2 Mg). The latter geometry is remarkable insofar as linear Mg-H-Mg units have never been observed in saltlike hydrides.

Table 1. Crystal structure data of EuMg_2D_6 refined from neutron powder diffraction data ($P4/mmm$, $a = 376.57(5)$, $c = 799.2(2)$ pm, $T = 293$ K, $R_p = 0.075$, $R_{wp} = 0.063$, $R_{Bragg} = 0.039$). Temperature factor $T = \exp(-B_{\text{iso}}(\sin \theta/\lambda)^2)$.

Atom	Site	Symmetry	x/a	y/b	z/c	$B_{\text{iso}} [10^4 \text{ pm}^2]$
Eu	1a	4/mmm	0	0	0	1.3(1)
Mg	2h	4mm	$\frac{1}{2}$	$\frac{1}{2}$	0.2779(8)	0.05(9)
D1	4i	2mm	0	$\frac{1}{2}$	0.2132(4)	1.80(7)
D2	1c	4/mmm	$\frac{1}{2}$	$\frac{1}{2}$	0	$B_{\text{iso}}(\text{D1})$
D3	1d	4/mmm	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$B_{\text{iso}}(\text{D1})$

Table 2. Crystal structure data of EuMgD_4 refined from neutron powder diffraction data ($Cmc2_1$, $a = 392.97(5)$,^[a] $b = 1346.8(2)$,^[a] $c = 553.86(7)$ pm,^[a] $T = 293$ K, $R_p = 0.096$, $R_{wp} = 0.078$, $R_{Bragg} = 0.069$). Temperature factor as in Table 1.

Atom	Site	Symmetry	x/a	y/b	z/c	$B_{\text{iso}} [10^4 \text{ pm}^2]$
Eu	4a	$m..$	0	0.1523(4) ^[a]	0.25(–)	2.5(4)
Mg	4a	$m..$	0	0.405(2)	0.193(5)	3.9(5)
D1	4a	$m..$	0	0.332(2)	0.464(5)	2.3(2)
D2	4a	$m..$	0	0.292(2)	0.990(5)	$B_{\text{iso}}(\text{D1})$
D3	4a	$m..$	0	0.069(2)	0.662(3)	$B_{\text{iso}}(\text{D1})$
D4	4a	$m..$	0	0.540(2)	0.342(3)	$B_{\text{iso}}(\text{D1})$

[a] Refined from X-ray powder diffraction data, fixed in the refinement on the neutron data.

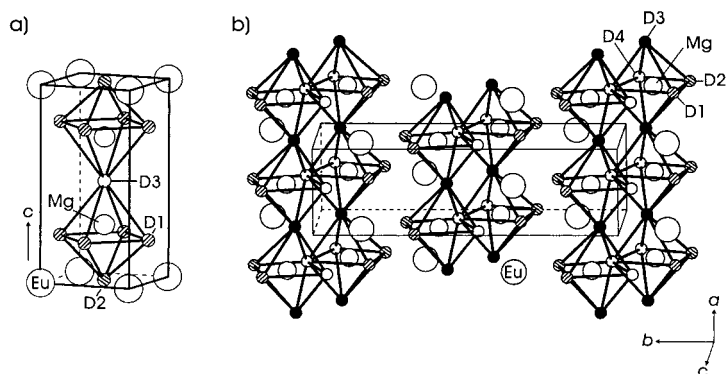


Figure 3. Crystal structure of a) EuMg_2D_6 (distances [pm]: Eu–8D1 253.9(2), Eu–4D2 266.21(1), Mg–D3 177.4(7), Mg–4D1 195.2(2), Mg–D2 222.0(7))^[7] and b) EuMgD_4 (distances [pm] Eu–D2 237(2), Eu–2D2 249(2), Eu–2D1 253(2), Eu–2D4 254(1), Eu–D3 254(3), Eu–D1 270(2), Mg–D1 179(4), Mg–D2 189(4), Mg–D4 199(4), Mg–2D3 200.3(8), Mg–D4 208(3)).

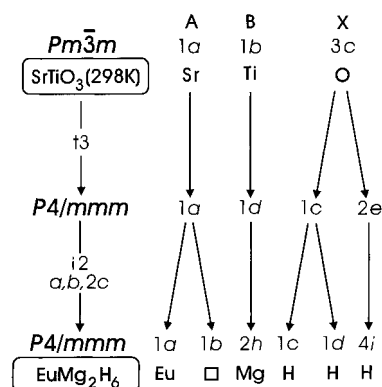


Figure 4. Crystallographic group–subgroup relationships according to Bärnighausen^[8] between the crystal structure types of cubic perovskite SrTiO_3 and EuMg_2H_6 .

Hence, the Mg–D3 distance is very short (177 pm), the shortest ever reported in magnesium-containing metal hydrides. The shortest D–D contacts are 253.9(2) pm.

EuMgH_4 (Figure 3b) crystallizes in the orthorhombic BaZnF_4 type (space group $Cmc2_1$) and has similar atomic parameters to SrMgH_4 .^[5a] The EuMg sublattice displays a pseudo-symmetry which is the reason why the X-ray data could be well modeled with the centrosymmetric BaMgH_4 structure (space group $Cmcm$).^[5b] Europium is surrounded by nine deuterium atoms in a distorted tricapped trigonal-prismatic configuration, and magnesium has a distorted octahedral deuterium coordination. As in EuMg_2D_6 the MgD_6 octahedra are linked through common corners. However, unlike EuMg_2D_6 in which the MgD_6 octahedra form a three-dimensional network, the octahedra in EuMgD_4 are linked in two directions only, thus forming two-dimensional layers in accordance with the higher D:Mg ratio (Figure 3). Deuterium is threefold (D3) and fourfold (D1, D2, D4) coordinated by metal atoms. The shortest D–D distance is 241(3) pm.

Studies of the magnetism (Figure 5) indicated ferromagnetic order for both compounds. The ordering temperatures T_C as extrapolated from the linear part of the plots of χ^{-1} versus T are 27 K for EuMg_2D_6 and 19 K for EuMgD_4 . The

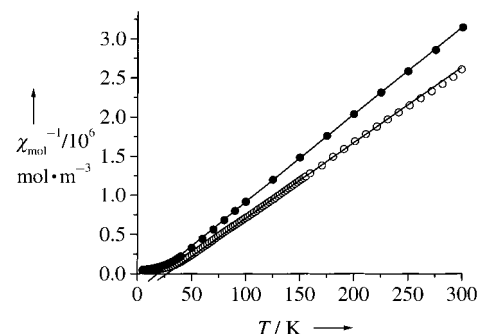


Figure 5. The reciprocal molar magnetic susceptibility of EuMg_2D_6 (open circles) and EuMgD_4 (filled circles) as a function of temperature (SQUID magnetometer, powder pellets, $B = 2$ T). The content of by-products (EuMgD_4 in the EuMg_2D_6 sample and vice versa) was estimated from a phase analysis by X-ray diffraction and considered in the calculation of magnetic moments. The fit of the linear part leads to the values $T_C = 27.2(5)$ K, $\mu_{\text{eff}} = 8.12(2) \mu_B$ for EuMg_2D_6 , and $T_C = 18.7(5)$ K, $\mu_{\text{eff}} = 7.54(2) \mu_B$ for EuMgD_4 .

calculated magnetic moments (8.1 and $7.5 \mu_B$) confirm the presence of Eu^{II} (theoretical values for free ions: $7.95 \mu_B$ for Eu^{2+} , $0 \mu_B$ for Eu^{3+}). The deviations from the theoretical value might be explained by crystal field effects and the presence of impurities. Thus EuMg_2H_6 and EuMgH_4 can be considered as nonmetallic, ionic solids, which is consistent with their red and brown colors, respectively.

Divalent lanthanides such as Eu^{II} and Yb^{II} can usually be substituted in solid-state compounds by alkaline earth elements such as Sr and Ca, respectively. This is particularly true for metal hydrides. All Eu- and Yb-containing hydrides known crystallize with structures isotypic to their Sr and Ca analogues, respectively. The $\text{EuMgH}_4/\text{SrMgH}_4$ pair is a further example. EuMg_2H_6 , however, appears to be an exception as

no Sr analogue is known. Hydrogenation of SrMg_2 yields the phase $\text{Sr}_2\text{Mg}_3\text{H}_{10}$ ^[10] to which no corresponding compound in the Eu-Mg-H system could be found (see Experimental Section).

The structure data of EuMg_2D_6 and EuMgD_4 provide the first refined Eu–D distances. The values for the various coordination numbers are 266 (Eu[12]–D[6]), 254 (Eu[12]–D[4]), and 252 pm (Eu[9]–D[4]). The collection of these data became possible through the development of advanced neutron diffractometers and detectors with a high flux neutron source. This underlines the importance of instrumental developments on such sources for solid-state research.

Experimental Section

EuMg_2 and EuMg , prepared by arc-melting of the elements (Eu 99.9%, Mg 99.95%) at the nominal composition 1:3 and 1:1.3, respectively, were hydrogenated (deuterated) in an autoclave at 600 K and 50 bar $\text{H}(\text{D})_2$ pressure. The excess of Mg compensated the sublimation losses in the arc furnace. Owing to their moisture sensitivity EuMg_2H_6 and EuMgH_4 were handled in an argon-filled glove box. Both hydrides decompose at 800 K and 5 bar H_2 pressure into EuH_2 and Mg metal. Variations of the sample composition and synthesis conditions gave no evidence for the existence of further ternary phases in the Eu-Mg-H system.

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Design, Synthesis, and Evaluation of Novel Modular Bisubstrate Analogue Inhibitors of Farnesyltransferase**

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The ras signal transduction pathway plays a decisive role in cell growth and differentiation. Point mutations in the *ras* gene can lead to *ras* proteins of constitutive activity, that is, they are unable to return to an inactive form after activation. These mutant variants deliver constant signals to the nucleus, which result in growth stimulation. Such *ras* mutations have been found in nearly 30 % of all tumors; the incidence can be as high as 90 % in certain tumor types.^[1] *Ras* proteins must be post-translationally modified for localization at the inner cell membrane. The first such modification, crucial to the function of both normal and mutant *ras* proteins, is catalyzed by farnesyltransferase (FTase), which transfers a farnesyl residue from farnesylpyrophosphate (FPP) to the side chain SH group of cysteine in the C-terminal sequence CAAX (C: cysteine, A: aliphatic amino acid, X: C-terminal methionine or serine).^[1] Both FPP and the CAAX tetrapeptide serve as suitable templates for FTase inhibitors. Bisubstrate analogue inhibitors, which contain elements from both peptide and farnesyl moieties, represent a further class of FTase inhibitors.^[1a, 2] Such bisubstrate inhibitors are of particular interest as they can circumvent the need for the free SH function present in almost all CAAX peptidomimetics.^[3] Such free SH groups are unfavorable both as a result of their inherent chemical sensitivity and, more importantly, because they are a source of serious side effects as seen, for example, with the antihypertensive drug Captopril.^[4] In addition, recent kinetic studies show that FTase has an unusually high affinity for its product, the farnesylated *ras* protein, which is only released upon binding of a further FPP molecule.^[5]

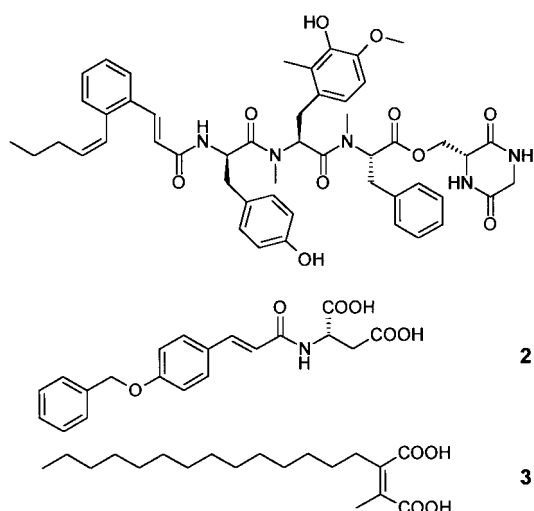
Bisubstrate inhibitors, which represent product analogues, should therefore be particularly good FTase inhibitors.^[5] Few bisubstrate or product analogues have been reported to date. Recently, Waldmann et al. synthesized the natural product peptidocinnamin E (**1**, Scheme 1) in multiple step reaction sequence and showed it to be a suitable model for bisubstrate FTase inhibitors.^[6]

Herein we present a novel class of fully synthetic modular bisubstrate inhibitors. Our aim was to develop a bisubstrate inhibitor containing a peptidomimetic (corresponding to CAAX) and a non-prenyl lipophilic group (corresponding to farnesyl). In a series of *N*-acylaspartates, for example, **2** (Scheme 1) we have identified the benzyloxycinnamoyl moiety as a suitable farnesyl mimetic.^[7] The FPP analogue,

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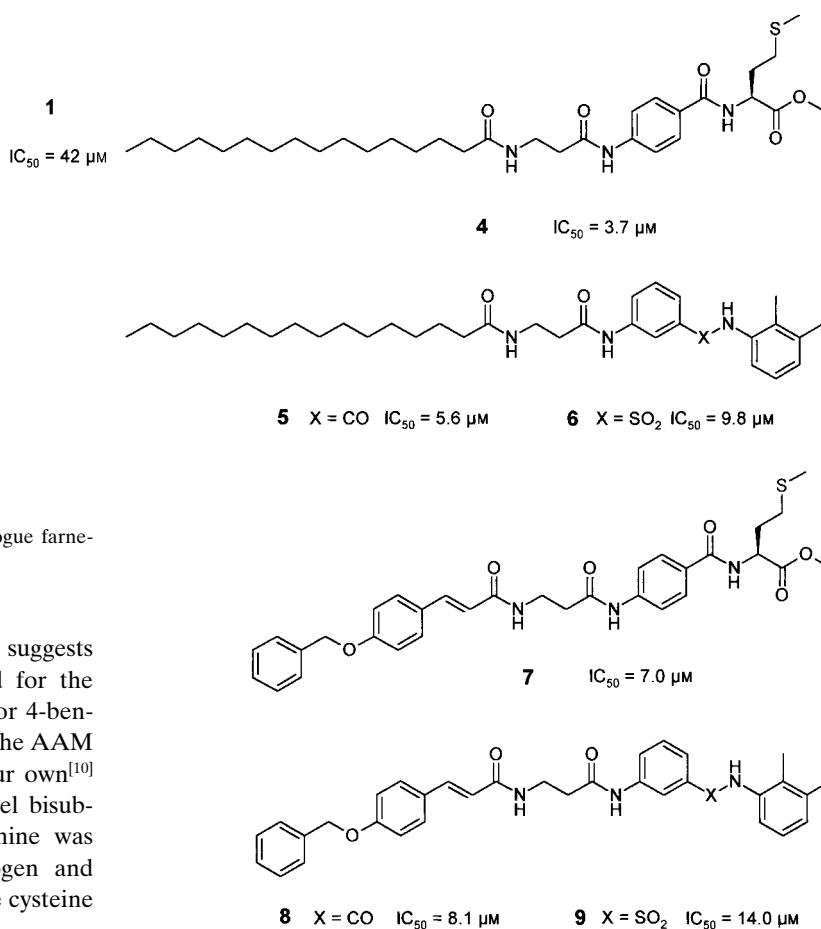
Scheme 1. Peptidocinnamin E (**1**) and the non-prenyl FPP-analogue farne-syltransferase inhibitors **2** and **3**.

natural product chaetomelic acid (**3**, Scheme 1),^[8] suggests that long-chain alkyl residues may be substituted for the farnesyl group. We have therefore fused palmitic- or 4-benzyloxycinnaminic acid through a β -alanine linker to the AAM or AA-mimetic partial structures of known^[9] or our own^[10] CAAX-analogue FTase inhibitors to form the novel bisubstrate product analogues **4–9** (Scheme 2). β -Alanine was chosen as the distance between its amide nitrogen and carbonyl carbon atoms is closest to that between the cysteine SH group and the C-1 atom.

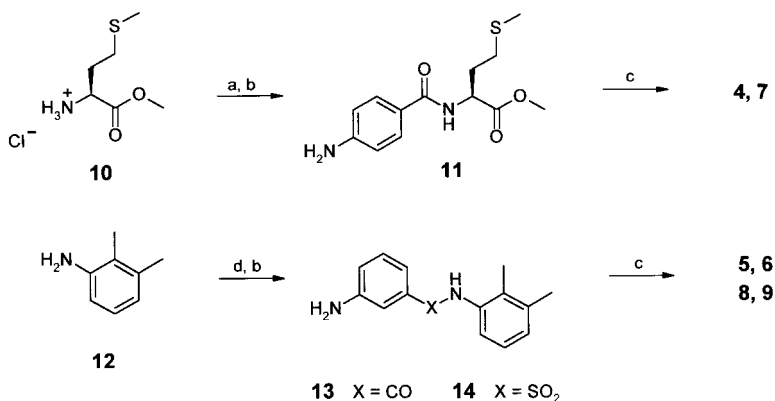
Synthesis of compounds **4–9** was achieved by reaction of methionine methyl ester **10** with 4-nitrobenzoyl chloride or 2,3-dimethylaniline (**12**) with 3-nitrobenzoyl chloride or 3-nitrobenzenesulfonyl chloride. The nitro group of the resulting amide was reduced to an amino group with zinc(II) chloride.^[11] The intermediates **11**, **13**, and **14** were linked through *N*-acyl- β -alanine, obtained by reaction of β -alanine with carboxylic acid chloride in aqueous alkaline solution,^[12] to form the final products **4–9** (Scheme 3).

The inhibitory potency of the compounds was measured by using the same fluorescence enhancement assay used by Waldmann et al.^[6] to determine the activity of **1**. The FTase reaction rate was measured at different concentrations of test substance through the increase in fluorescence at 505 nm that occurs upon farnesylation of a dansyl-labeled pentapeptide (Ds-GlyCysValIleSer).^[13] Recombinant *Saccharomyces cerevisiae* FTase was expressed as a GST-fusion protein in *E. coli* and isolated by GST-affinity chromatography (GST = glutathione-S-transferase).^[14]

The novel bisubstrate (product) analogues (Scheme 2) inhibit FTase at a lower concentration (IC_{50}) than the natural product peptidocinnamin E (**1**, Scheme 1). In addition to their lack of labile prenyl or peptidyl substructures, these synthetic bisubstrate inhibitors are particularly attractive for their modular construction. The synthesis and linkage of the three building blocks—AA(X) mimetic, linker, and farnesyl mim-



Scheme 2. Modular non-prenyl and non-peptidyl bisubstrate or product-analogue FTase inhibitors **4–9**.



Scheme 3. Synthesis of **4–9**. a) 4-Nitrobenzoyl chloride, CH₂Cl₂, *N*-methylmorpholine (NMM), 12 h, 0°C, 90%; b) SnCl₂·2 H₂O, EtOAc, 2 h, reflux, **11** 88%, **13** 81%, **14** 80%; c) *N*-palmitoyl- or *N*-(4-benzyloxycinnamoyl)- β -alanine, *i*BuOCOCl, NMM, DMF, -15°C → RT, 16 h, **4** 78%, **5** 82%, **6** 68%, **7** 53%, **8** 77%, **9** 13%; d) 3-nitrobenzoyl chloride or 3-nitrobenzenesulfonyl chloride, CH₂Cl₂, NMM, 12 h, 0°C, 86% and 95%, respectively.

etic—is a relatively straightforward procedure. This facilitates the synthesis of structural variants with advantageous pharmacological profiles, as well as the production of tailored “chemical” tools to further investigate the ras signal transduction pathway.^[6, 15]

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Rb₁₀Mo₃₆S₃₈: A Novel Reduced Molybdenum Sulfide Containing the Highest Nuclearity Metal Transition Cluster in a Solid-State Compound

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Reduced molybdenum chalcogenides are characterized by Mo–Mo bonds that lead to the formation of clusters with different geometries and nuclearities. The most frequently observed cluster is the octahedral Mo₆, which is present in the Chevrel–Sergent ternary compounds MMo₆X₈ (M = Na, K, Ca, Sr, Ba, rare-earth metals, Sn, Pb...).^[1] Clusters with higher nuclearities result essentially from the uniaxial *trans* face sharing of octahedral Mo₆ units. This condensation process is well exemplified by the series of compounds M_{2n–2}Mo_{6n}X_{6n+2} (M = Rb, Cs; X = S, Se, Te)^[2] where *n* ranges between 2 to 5 and infinity. The first member (*n* = 1) corresponds to the

binary Mo₆X₈,^[3] which constitutes the host structure of the MMo₆X₈ compounds. The final member is the one-dimensional compound M₂Mo₆X₆,^[4] containing infinite chains of *trans*-face-sharing Mo₆ octahedra |Mo_{6/2}|_∞^[1]. In addition to their novel interesting structures, Mo condensed cluster compounds also show unusual physical properties. Indeed, the sulfides and selenides generally present superconducting or metal–insulator transitions at low temperature. Thus, studies of the normal and superconducting states of Cs₂Mo₁₂Se₁₄ (*n* = 2)^[2b] and Rb₄Mo₁₈Se₂₀ (*n* = 3)^[2c] by measuring the conductivity and magnetization of single crystals and powder samples have shown that these compounds can be classified among the “exotic” superconductors.^[5] The quasi-one-dimensional superconductor Ti₂Mo₆Se₆ presents extreme type II and non-Bardeen–Cooper–Schrieffer behavior. On the other hand, the anisotropy of the electronic properties in the latter compound is one of the largest ever observed in a superconductor, with a ratio of the conductivities parallel and perpendicular to the infinite chains ($\sigma_{\parallel}/\sigma_{\perp}$) of about 1000, and a ratio of the upper critical fields ($H_{c2\parallel}/H_{c2\perp}$) of about 26.^[5]

We present here the crystal structure of the sixth member of the M_{2n–2}Mo_{6n}X_{6n+2} family: Rb₁₀Mo₃₆S₃₈. The Mo₃₆ cluster present in this new compound constitutes the largest observed in solid-state chemistry to date.

The new Mo₃₆ cluster is shown in Figure 1 with the 44 sulfur atoms of its environment, the whole forming a Mo₃₆S₄₄ cluster unit. Of these 44 sulfur atoms, 38 are inner ligands and the remaining 6 are outer ligands. The local symmetry of the Mo₃₆S₄₄ cluster unit is the same ($\bar{3}$ or S₆) as that of the monomeric Mo₆S₈ unit. The Mo₃₆ core can be seen as the result of the uniaxial *trans* face sharing of 11 octahedral Mo₆ clusters.^[6] It can alternatively be described as a stack of 12 staggered Mo₃ triangles.

The metal–metal distances lie between 2.637(1) and 2.744(1) Å, and the metal–chalcogen distances between 2.394(2) and 2.602(2) Å. As noticed previously for all phases containing Mo_{6n}S_{6n+2} entities,^[2] Mo–Mo bonds between Mo₃ triangles spread over a larger range (2.662(1)–2.744(1) Å) than those within triangles (2.637(1)–2.662(1) Å). In average, both types of distances tend to those found in the infinite chains of the M₂Mo₆S₆ sulfides. This trend is also reflected by the average distance between the Mo₃ triangles of 2.22 Å in Rb₁₀Mo₃₆S₃₈, compared to 2.402 and 2.213 Å for the first member Mo₆S₈ and the final member M₂Mo₆S₆, respectively.

Each cluster shares six outer sulfur atoms (*a* type) with six neighboring clusters to form the three-dimen-

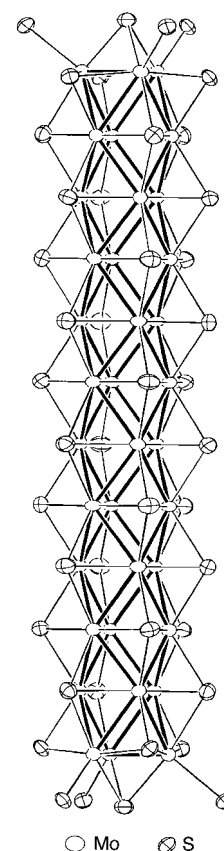


Figure 1. Schematic representation of the Mo₃₆S₄₄ cluster unit (ORTEP drawing).

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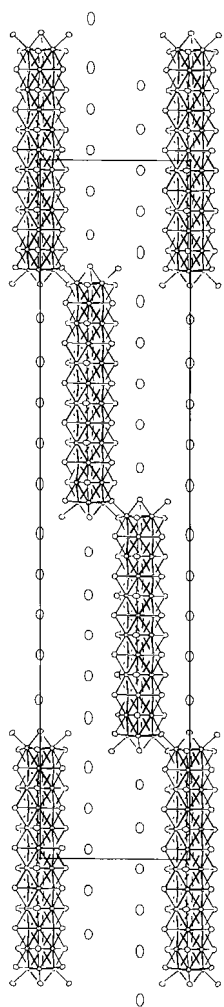


Figure 2. Projection of the crystal structure of $\text{Rb}_{10}\text{Mo}_{36}\text{S}_{38}$ onto the hexagonal (110) plane.

sional Mo–S network with the following connective formula $\text{Mo}_{36}\text{S}_{32}^i\text{S}_{6/2}^{i-g}\text{S}_{6/2}^{a-i}$ in the notation of Schäfer and von Schnering.^[7] This classical interunit linkage for reduced Mo chalcogenides leads to a Mo–Mo distance of 3.113(1) Å between adjacent Mo_{36} clusters and consequently to only weak Mo–Mo interactions. The large cavities between the $\text{Mo}_{36}\text{S}_{44}$ cluster units are occupied by the Rb^+ cations which form linear finite chains (Figures 2 and 3). The two terminal Rb^+ cations are surrounded by ten S atoms forming a distorted tetracapped trigonal prism, and the remaining Rb^+ cations are surrounded by nine S atoms in a distorted tricapped trigonal prismatic arrangement. The Rb–S distances range from 3.363(2) to 3.936(2) Å, and the spacing between Rb^+ cations from 4.509(3) to 5.430(3) Å.

Calculation of the number of valence electrons on the Mo atoms for the Mo_{6n} clusters show that the valence electron count (VEC) asymptotically tends towards the value of 4.333 found for the $\text{M}_2\text{Mo}_6\text{X}_6$ compounds with increasing cluster size. The VEC of 4.167 in $\text{Rb}_{10}\text{Mo}_{36}\text{S}_{38}$ indicates that Mo_{6n} clusters with $n > 6$ might be possible electronically. This situation differs from that observed in the reduced molybdenum oxides $\text{M}_{n-x}\text{Mo}_{4n+2}\text{O}_{6n+4}$ ^[8] ($n = 2, x = 0$ for $\text{M} = \text{Ca}, \text{Sr}, \text{Sn}, \text{Pb}, \text{La} - \text{Gd}$; $n = 3, x = 0$ for $\text{M} = \text{K}$; $n = 4$ and $5, x = 1$ for $\text{M} = \text{Ba}$ and $x = -1$ for $\text{M} = \text{In}$) in which *trans*-edge-sharing Mo_{6n} clusters occur. Indeed, members with $n = 5$ have a VEC (3.26) of the same magnitude as those in the infinite chain present in the series $\text{M}_x\text{Mo}_4\text{O}_6$, which range from 3.25 for NaMo_4O_6 ^[9a] to 3.45 for $\text{Sn}_{0.9}\text{Mo}_4\text{O}_6$.^[9b] Consequently, clusters Mo_{4n+2} with $n > 5$ should be unstable with respect to the infinite chain compounds.

The principle of face-sharing condensation of octahedral M_6 clusters is also observed in metal organic compounds with cobalt,^[10] nickel,^[11] and rhenium.^[12] However, in the latter systems, the maximum number of octahedral M_6 clusters involved is four in $\text{Ni}_{15}\text{X}_{15}(\text{PPh}_3)_6$ ($\text{X} = \text{S}, \text{Se}$).

Finally, magnetic susceptibility measurements made on a cold-pressed powder of $\text{Rb}_{10}\text{Mo}_{36}\text{S}_{38}$ between 300 and 2 K did not reveal superconductivity.

Experimental Section

Preparation of $\text{Rb}_{10}\text{Mo}_{36}\text{S}_{38}$: The starting materials MoS_2 , Rb_2MoS_4 , and Mo were all in powder form. Before use, the Mo powder was reduced under H_2 atmosphere at 1000 K over 10 h in order to eliminate any trace of oxygen. The MoS_2 was prepared by the reaction of molten S with reduced

Mo in a ratio of 2:1 in an evacuated (10^{-2} Pa) and sealed silica tube, and heated to 1073 K for 2 d. Rb_2MoS_4 was obtained by treatment of Rb_2MoO_4 with CS_2 gas at 723 K for 2 d under an argon flow. Rb_2MoO_4 was synthesized from an equimolar ratio of MoO_3 and Rb_2CO_3 with heating in an alumina vessel at 1073 K for 2 d. After the synthesis, all reactants were kept and handled in a purified argon-filled glovebox. Single crystals of $\text{Rb}_{10}\text{Mo}_{36}\text{S}_{38}$ were obtained by high-temperature solid-state reaction of MoS_2 , Rb_2MoS_4 , and Mo in the molar ratio 2.3:1:4.5. These powders were mixed, ground together in a mortar, and then cold-pressed using a hand press. The pellet was then loaded in a molybdenum crucible, which was sealed under a low argon pressure using an arc welding system. The crucible was heated at a rate of 300 K h^{-1} to 1800 K and held there for 10 d, then cooled at 100 K h^{-1} to 1300 K and finally allowed to cool in the furnace to room temperature.

Crystal structure data for $\text{Rb}_{10}\text{Mo}_{36}\text{S}_{38}$: trigonal rhombohedral, space group $R\bar{3}$ (no. 148), $a = 9.0968(7)$, $c = 77.37(1)$ Å, $V = 5544.6(1)$ Å³, $Z = 3$, $\rho_{\text{calc}} = 4.966 \text{ Mg m}^{-3}$, $F(000) = 7470$, $\lambda(\text{MoK}\alpha) = 0.71073$ Å, $\mu(\text{MoK}\alpha) = 13.484 \text{ mm}^{-1}$, $T = 298$ K. Of 6270 reflections collected in the θ range 1–35° using the θ – 2θ scan mode on a Nonius CAD-4 diffractometer, 5443 were independent ($R_{\text{int}} = 0.040$). Lorentzian polarization and empirical absorption corrections using the psi-scan technique^[13] ($T_{\text{min}} = 0.794$, $T_{\text{max}} = 1.00$) were applied. The structure was solved and refined against F^2 using SHELXS^[14] and SHELXL97.^[15] The positional and anisotropic displacement parameters for all atoms were refined to the values $R_1 = 0.0394$, $wR_2 = 0.0671$ for 128 parameters and 2659 reflections with $I > 2\sigma(I)$, $w = 1/[\sigma^2(F_o^2) + (0.0132P)^2 + 235.0669P]$ where $P = [\max(F_o^2, 0) + 2F_c^2]/3$; max./min. residual electron density 2.237/–1.976 e Å^{-3} . Further details on the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666, on quoting the depository number CSD-410865).

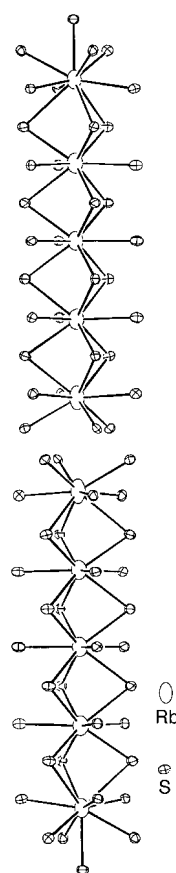


Figure 3. Distribution of the Rb^+ cations within the inter $\text{Mo}_{36}\text{S}_{44}$ cluster unit cavities.

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Non-Oxide Sol–Gel Chemistry: Preparation from Tris(dialkylamino)silazanes of a Carbon-Free, Porous, Silicon Diimide Gel

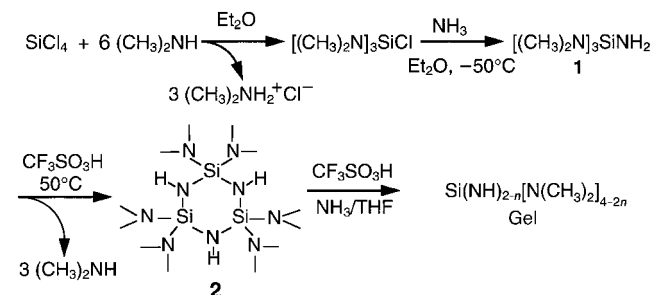
Riccardo Rovai, Christian W. Lehmann, and John S. Bradley*

Sol–gel chemistry plays an important role in the preparation and processing of oxide materials, including microporous oxides and dense ceramics.^[1, 2] There are few examples of non-oxide sol–gel techniques,^[3–5] and the only reported sol–gel preparation in silicon–nitrogen chemistry is the synthesis of a Si/C/N solid by the reaction of methyltrichlorosilane with bistrimethylsilylcarbodiimide to form a nonporous gel in which the structural framework contains only SiNCNSi units.^[6, 7] We are attempting to apply the chemistry of silicon amide compounds to the development of a “chimie douce” methodology for carbon-free SiM(NH)_x gels (M = Si or metal), to give a sol–gel route to non-oxide ceramics and provide an alternative, mild synthetic route to nitridosilicates.^[8–10] Such gels would be the azo analogues of silica-based amorphous oxide gels, and the nitridosilicate analogue to

silica gel would be a gel of silicon diimide (Si(NH)₂). A putative imide/nitride pathway can be envisioned in which dialkylamido compounds of silicon are subjected to ammonolysis and condensation to an imide.

The reactions of Si(NR₂)₄ compounds, and of bimetallic analogues, with ammonia have been reported to give amorphous powders^[11–16] and, in OMCVD experiments, silicon nitride films.^[17] However, silicon diimide gels have not been reported from these or any other starting materials. We report here the preparation of a silicon imide gel by acid-catalyzed ammonolysis of the hitherto unreported tris(dimethylamino)silylamine [(CH₃)₂N]₃SiNH₂, **1**, which we have prepared from silicon tetrachloride in high yield and purity. The probable first step in this process is the acid-catalyzed self-condensation of **1** to the cyclic trimer [((CH₃)₂N)₂SiNH]₃ (**2**), for which we report the X-ray crystal structure. The acid-catalyzed ammonolysis of **2**, or the equivalent sequential self-condensation and ammonolysis of **1**, under mild conditions, yields a semirigid translucent gel. On drying under mild conditions in an ammonia atmosphere this non-oxide gel yields a high surface area silicon diimide xerogel, the first example of a porous non-oxide silicate gel.

Compound **1** was prepared in high yield from silicon tetrachloride (Scheme 1) as a colorless liquid, and characterized by elemental analysis, IR and ¹H, ¹³C, and ²⁹Si NMR spectroscopies, and mass spectrometry. It can be distilled



Scheme 1. Synthesis of **1** and **2** as well as the translucent gel of the composition Si(NH)_{2–n}[N(CH₃)₂]_n.

under atmospheric pressure without detectable decomposition. Compound **1** is the simplest member of the series (R₂N)_nSi(NH₂)_(4–n) (R = Me, n = 3), and is one of very few examples of a tris(amido)silylamine yet reported.^[18] We have also prepared tris(morpholino)silylamine as a crystalline solid analogue to **1**. Details of the synthesis and structure of this compound will be published separately.

When **1** is heated in the presence of excess ammonia under autogenous pressure (100 bar) at 110 °C, a white powder is obtained, which is shown by IR spectroscopy to be Si(NH)₂.^[19] The material is of low surface area (< 50 m² g^{–1}), and is presumably identical to the powder prepared by Union Carbide workers by the acid-catalyzed ammonolysis of (Me₂N)₄Si.^[11] However, when **1**, either neat or in THF, is treated at 50 °C with a catalytic amount of trifluoromethanesulfonic acid, self-transamination occurs with loss of dimethylamine to give predominantly the cyclic trimer **2** (80% according to MS, 61% after recrystallization; Scheme 1). Compound **2** has been previously described as a product of the reaction of (Cl₂SiNH)₃ (a by-product (3% yield) of the

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reaction of ammonia with silicon tetrachloride) with dimethylamine, but neither its structure nor reaction chemistry have been reported.^[20, 21] The molecular structure of **2** was determined by X-ray diffraction (Figure 1). The compound is

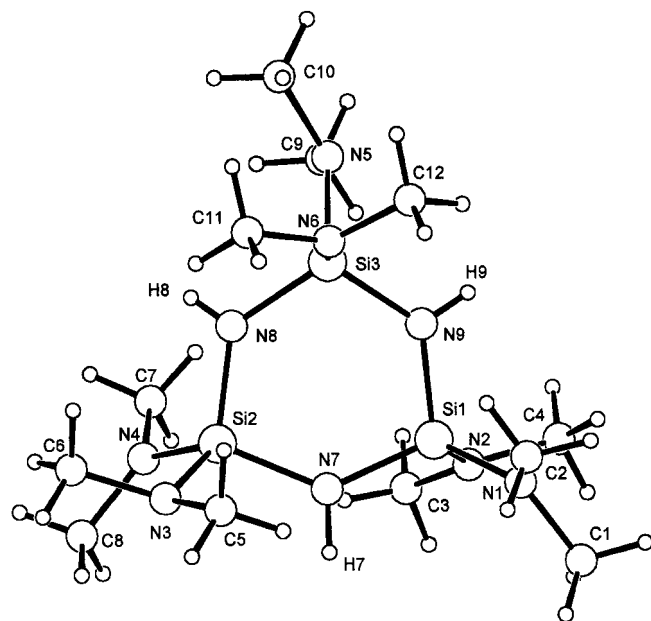


Figure 1. Crystal structure of **2**. Selected bond lengths [nm] and angles [°]: Si1–N1 0.1717(2), Si1–N2 0.1716(2), Si1–N7 0.1722(1), Si1–N9 0.1723(2), Si2–N3 0.1736(2), Si2–N4 0.1718(2), Si2–N7 0.1718(2), Si2–N8 0.1715(2), Si3–N5 0.1730(2), Si3–N6 0.1713(2), Si3–N8 0.1717(2), Si3–N9 0.1710(2), N7–H7 0.091(2), N8–H8 0.073(2), N9–H9 0.081(2); N9–Si1–N7 106.2(1), N8–Si2–N7 105.3(1), N9–Si3–N8 107.1(1), Si2–N7–H7 113.6(13), Si1–N7–H7 113.1(13), Si2–N8–H8 116.3(19), Si3–N8–H8 112.8(19), Si1–N9–H9 116.4(16), Si3–N9–H9 112.8(16).

made up of a six-membered Si_3N_3 ring in which each of the three Si atoms is bound to two exocyclic nitrogen atoms of dimethylamino groups and two endocyclic NH groups to provide an SiN_4 environment. The mean deviation of Si and N atoms from a plane through the six-membered ring is 0.105 Å. The sums of the interatomic angles at the three endocyclic nitrogen atoms are 360.0° (N7), 360.1° (N8), and 359.9° (N9), which reflect the essential planarity of these nitrogen atoms.

When the mixture obtained by acid-catalyzed self-condensation of **1** in THF is exposed to ammonia, gelation occurs with further loss of dimethylamine to give a semirigid, dimensionally stable, translucent gel. The intermediacy of **2** in the ammonolysis of **1** is demonstrated by rapid gel formation on exposure to ammonia of a solution of pure **2** in THF containing 1 mol% of trifluoromethanesulfonic acid. It seems that four equivalents of ammonia are needed to cause rapid gelation, instead of the three equivalents which the stoichiometry of the ammonolysis reaction would require. When a gel obtained in this manner in a cylindrical glass mould was dried slowly over a period of several days under an atmosphere of argon that was constantly replaced, it shrunk from the walls of the mould but retained its cylindrical form before cracking after about 50% linear shrinkage. We have as yet made no attempt to optimize the drying procedure to obtain fully dried monoliths, and the effect of changes in

gelation conditions, and of variation of the alkyl substituent in the tris(dialkylamido)silylamine starting material, are under investigation.

The xerogel produced by drying under reduced pressure, which has a Brunauer–Emmett–Teller (BET) surface area of about 1000 m² g^{−1}, contains residual dimethylamino groups, as shown by the presence of $\nu(\text{CH})$ bands in the IR spectrum of the dried material. A low-intensity shoulder (3475 cm^{−1}) on the principal $\nu(\text{NH})$ band (3362 cm^{−1}) and a low-intensity band at 1551 cm^{−1} in the IR spectrum of the dried gels are assigned to the $\nu(\text{NH}_2)$ and $\delta(\text{NH}_2)$ bands of SiNH_2 groups. The presence of these bands suggests that the condensation reaction was not yet complete by the time rigid gel formation was observed. Thermogravimetric analysis in combination with mass spectrometry (TGA/MS; argon stream, 5 °C min^{−1}, 25–900 °C) of the gels shows only loss of dimethylamine and ammonia, which are expected to be eliminated as the condensation reaction goes to completion. Treatment of the gel under flowing ammonia at 50 °C for 15 h removes all dimethylamino groups. The IR spectrum of the resulting solid shows only $\nu(\text{NH})$, $\nu(\text{NH}_2)$, $\delta(\text{NH}_2)$, and Si–N absorbances, and thus identifies the resulting white amorphous solid (surface area 500 m² g^{−1}) as a porous, carbon-free silicon diimide gel containing terminal NH_2 groups.

The imide chemistry described here is a direct analogue to the hydrolytic sol–gel chemistry of tetraalkylorthosilicates for the preparation of silica gels, and represents the first example of ammonolytic sol–gel synthesis of a porous silicon diimide gel. The chemistry of **1** is amenable to the synthesis of other single-source molecular precursors for non-oxide gels, and in future papers we will report on the preparation of Si–Al, Si–B and Si–Ti compounds and imide gels derived from them.

Experimental Section

1: Anhydrous dimethylamine (45.3 g, 1.86 mol) was allowed to react with silicon tetrachloride (45 g, 0.27 mol) in diethyl ether (1.5 L) at −40 °C over 15 h. The resulting dimethylammonium chloride was removed by filtration and washed with diethyl ether (2 × 200 mL). The combined solutions of $[(\text{CH}_3)_2\text{N}]_3\text{SiCl}$ in diethyl ether were cooled to −50 °C, and anhydrous liquid ammonia (70 g, 4.0 mol) was added in two aliquots. After 15 h the precipitated ammonium chloride was removed by filtration at room temperature, and the filtrate concentrated under reduced pressure to provide **1** as a colorless liquid (99.2% purity according to GC/MS). Yield 35.1 g (0.20 mol, 74% based on SiCl_4); b.p. 186 °C (760 torr); elemental analysis calcd: C 40.9, H 11.4, N 31.8, Si 15.9; found: C 40.8, H 10.9, N 31.8, Si 15.8; ¹H NMR (300 MHz, C_6D_6): δ = 2.3 (s, 18 H, $[\text{Si}[(\text{CH}_3)_2]_3]$), 0.3 (brs, 2 H, SiNH_2); ¹³C NMR (75 MHz, C_6D_6): δ = 37.89 (s, $[\text{Si}[(\text{CH}_3)_2]_3]$); ²⁹Si NMR (79.5 MHz, C_6D_6): δ = −21.73 (s, $[\text{Si}[(\text{CH}_3)_2]_3]$); ¹⁵N NMR (60.8 MHz, C_6D_6 , external CH_3NO_2): δ = −376.1 (NH_2), −375.4 ($\text{N}(\text{CH}_3)_2$); IR (liquid film): $\tilde{\nu}$ = 3475 (w), 3406 (w; 2 × $\nu(\text{NH}_2)$), 2972 (s), 2835 (s), 2788 (s; 3 × $\nu(\text{CH}_3)$), 1552 cm^{−1} (m) ($\delta(\text{NH}_2)$); MS (EI): m/z (%): 176 (100) [M^+], 132 (90) [$M^+ - \text{N}(\text{CH}_3)_2$].

2: To a sample of **1** (2.5 g, 14.2 mmol) was added trifluoromethanesulfonic acid (12 μL , 0.142 mmol, 1 mol%) under argon, and the mixture heated to 100 °C, resulting in evolution of gaseous dimethylamine. After about 4 h the mixture was cooled to room temperature where it solidified to a colorless crystalline mass. Extraction into pentane (5 mL) and cooling to −80 °C gave colorless crystalline **2**. Yield 1.1 g (61%). Elemental analysis calcd: C 36.6, H 9.90, N 32.0, Si 21.3; found: C 36.9, H 9.86, N 32.0, Si 21.3; ¹H NMR (300 MHz, C_6D_6): δ = 2.43 (s, 12 H, $[\text{Si}[(\text{CH}_3)_2]_3]$), 0.0 (brs, 3 H, SiNH); ¹³C NMR (75 MHz, C_6D_6): δ = 36.61 (s, $[\text{Si}[(\text{CH}_3)_2]_3]$); ²⁹Si NMR (79.5 MHz, C_6D_6): δ = −31.5 (s, $[\text{Si}[(\text{CH}_3)_2]_3]$); IR (KBr): $\tilde{\nu}$ = 3392 (m),

($\nu(\text{NH})$), 2971 (s), 2865 (s, sh), 2855 (s, sh), 2834 (s), 2786 (s; $5 \times \nu(\text{CH}_3)$), 1171 (s), 987 cm^{-1} (s); MS (EI): m/z (%): 393 (20) [M^+], 349 (20) [$M^+ - \text{N}(\text{CH}_3)_2$], 303 (100) [$M^+ - 2\text{N}(\text{CH}_3)_2$].

Crystal structure analysis of **2**: Crystals of **2** were grown from pentane at -40°C , crystal dimensions $0.63 \times 0.24 \times 0.11$ mm; $\lambda = 0.71073$ Å; crystallographic data at 100 K: triclinic, space group $P\bar{1}$ (no. 2); $a = 832.83(17)$, $b = 1069.0(2)$, $c = 1408.3(3)$ pm; $\alpha = 69.62(3)^\circ$, $\beta = 74.95(3)^\circ$, $\gamma = 69.41(3)^\circ$; $V = 1.0867(4)$ nm³; $Z = 2$; $\rho_{\text{calc}} = 1.203$ Mg m⁻³; $F(000) = 432$; $\mu(\text{MoK}\alpha) = 0.234$ mm⁻¹. Data were collected on a Bruker AXS SMART-CCD-System with ω scans. Of 13602 reflections collected ($4.24^\circ < 2\theta < 55.04^\circ$), 4891 were independent ($R_{\text{int}} = 0.0622$). The structure was solved by direct methods (SHELXS-97^[22]) and refined by full-matrix least squares on F^2 using 241 parameters ($R(F) \geq 4\sigma(F) = 0.0470$, $wR2 = 0.1328$); max./min. residual electron density 0.728/−0.571 e Å⁻³. Hydrogen atoms were located from the difference Fourier map, all methyl hydrogen atoms were constrained to idealized positions, and imido hydrogen atoms were refined freely. 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-112321. 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).

Silicon imide gel preparation by ammonolysis of **1** and **2**: To a solution of **1** (2.5 g, 14.2 mmol) in dry THF (100 mL) in a 250-mL round-bottomed glass flask was added trifluoromethanesulfonic acid (12 μL , 0.14 mmol, 1 mol%). On warming to 50°C evolution of gaseous dimethylamine was observed. After 18 h analysis by IR and MS showed complete conversion of **1** into **2** (ca. 80%) and other unidentified by-products (ca. 20%). A solution of ammonia (42 mmol) in dry THF (40 mL) was added, and the mixture was left quiescent at 50°C for 18 h, at which point a slight opalescence was observed. Addition of further ammonia (14 mmol) resulted in rapid gelation to give, after several minutes, a translucent rigid gel filling the original volume of the reaction mixture. Evaporation of the solvent and dimethylamine in a stream of argon followed by drying under reduced pressure at 50°C for 10 h yielded a translucent white solid. IR (KBr): $\tilde{\nu} = 3475$ (w, sh; $\nu(\text{NH}_2)$), 3362 (m; $\nu(\text{NH})$), 2973 (m), 2863 (m, sh), 2857 (m, sh), 2835 (m), 2787 (m; $\nu(\text{CH}_3)$), 1551 (w; $\delta(\text{NH}_2)$), 1181 (s), 985 cm^{-1} (s).

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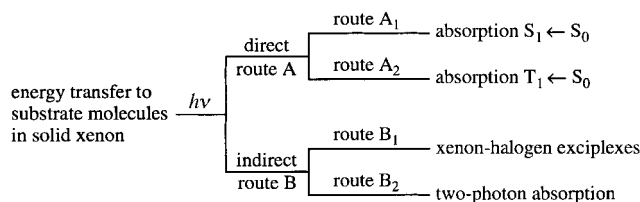
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Solid Xenon: A Medium for Unusual Photoreactions**

Günther Maier* and Christian Lautz

As is shown in this communication, a molecule isolated in a xenon matrix at 10 K can react upon irradiation depending on the conditions by four, in principle different mechanisms (Scheme 1). Of course, as in any other transparent medium a



Scheme 1. Possible photoexcitations of a substrate molecule in a xenon matrix.

substance absorbing in the wavelength region of the irradiating light can be induced to undergo a photoreaction also in a xenon matrix ($S_1 \leftarrow S_0$ absorption; direct route A₁). For instance, matrix irradiation ($\lambda = 313$ nm) of 2-diazo-2H-imidazole in argon as well as in xenon leads to 2H-imidazol-2-ylidene.^[1] In this case the special feature of xenon lies only in its ability to stabilize the generated carbene by complexation.^[1]

Surprisingly, in a xenon matrix even those molecules are accessible for photoreactions, which cannot absorb light at the wavelength employed. This phenomenon can be observed when the xenon matrix is doped with halogen atoms (indirect route B₁).^[2] Hereby the halogen atoms absorb the excitation energy, which is stored in xenon halogen exciplexes, whose

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photophysics is well known.^[3] Subsequently the stored excitation energy is transferred to the substrate molecules embedded in the matrix.

Another variant (indirect route B₂) is the two-photon excitation of the xenon using an excimer laser (KrF; $\lambda = 248$ nm). The excitons Xe_n^{*} generated by this procedure transfer their energy to the matrix-isolated molecules of the starting material resulting in their cleavage into smaller fragments.^[2d, 4, 5]

Until now we have used three- and four-membered ring compounds in our studies on indirect excitation. Thus, it was an open question, whether the ring strain present in these molecules was a prerequisite for the bond cleavage. Propane (**1**) was considered as test molecule, first, because it possesses no strain energy, second, a direct S₁ ← S₀ excitation at a wavelength longer than 170 nm should not be possible, and third, the expected products generated by a C–H or C–C bond cleavage, initiated by the indirect photoexcitation, should easily be identified by comparison with their known matrix spectra. The results reported herein not only prove that indirect photofragmentation following the pathways B₁ and B₂ also occurs in strain-free open-chain hydrocarbons, but also another important observation was made: Propane (**1**) is even fragmented when for the irradiation in undoped xenon the 185 nm line of a low-pressure mercury lamp is used instead of a KrF laser. This direct fragmentation is observed in spite of the fact that under the applied conditions a S₁ ← S₀ transition (route A₁) can be excluded. Thus we assume that in solid xenon an additional reaction channel exists, presumably a direct T₁ ← S₀ excitation (route A₂), which is promoted by the heavy atom effect of xenon and results finally in bond cleavage.

Irradiation of 1 in a Br[•]-doped xenon matrix (route B₁): Propane (**1**) is photostable in an argon matrix doped with bromine atoms when light of the wavelength $\lambda = 254$ nm is employed. However, if a Br[•]-doped xenon matrix is used instead, nearly 50 % of the molecules of **1** have reacted after irradiation for 47 h at the same wavelength. The products of this photolysis are propene (**2**), the allyl radical (**3**), propyne (**4**), allene (**5**), ethene (**6**), methane (**7**), and acetylene (**8**) (Scheme 2, Table 1). In addition the bands of the cation Xe₂H⁺^[6] (730.9, 843.0, and 951.7 cm^{−1}), which is formed from exciplex Xe₂⁺X[−] by addition of a H atom, can be registered.

During the photolysis the products of the primary steps can clearly be differentiated from those of the secondary steps. The first detectable molecules originating from the C–H bond cleavage of **1** are propene (**2**) and the allyl radical (**3**), whose signals appear simultaneously in the spectrum. At a later stage

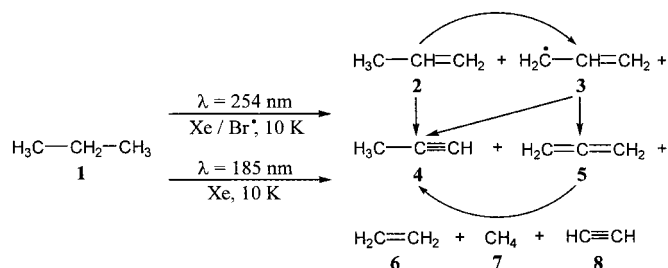
Table 1. Band positions ([cm^{−1}]; relative intensities in parentheses; vw = very weak, w = weak, m = middle, s = strong, vs = very strong) and assignment of the products upon photolysis of **1**. First column: Irradiation in a Br[•]-doped xenon matrix with $\lambda = 254$ nm (route B₁). Second column: Irradiation in a xenon matrix with laser light (KrF; $\lambda = 248$ nm; route B₂). Third column: Irradiation in a xenon matrix with a Hg lamp ($\lambda = 185$ nm; route A₂).

Route B ₁	Route B ₂	Route A ₂	Assignment
626.8 (m)	–	626.8 (m)	4
727.7 (s)	727.7 (m)	727.7 (s)	8
798.5 (s)	798.5 (s)	798.5 (w)	3
834.9 (m)	–	834.9 (vw)	5
ca. 910 (br., s)	ca. 910 (br., s)	905.9 (w)	2
947.9 (m)	947.9 (w)	947.9 (vw)	6
971.0 (m)	971.0 (w)	971.0 (vw)	3
988.4 (m)	988.4 (w)	988.4 (vw)	2
1300.2 (vs)	1300.2 (m)	1300.2 (s)	7
1317.2 (w)	–	1317.2 (w)	8
1432.9 (s)	1432.9 (m)	1432.9 (w)	6
1448.5 (m)	1448.5 (m)	1448.5 (vw)	2
ca. 1955 (br., w)	–	–	5
2978.0 (m)	–	–	6
3007.5 (s)	–	–	7
3094.5 (m)	–	–	6
3265.1 (m)	–	–	8

of the photolysis the secondary products of **2** and **3**, namely propyne (**4**) and allene (**5**), can be detected. This course of the photolysis corresponds with experiments with separately studied samples of **2** and **5** in a halogen-doped xenon matrix: Propene (**2**) yields upon irradiation with light of the wavelength $\lambda = 254$ nm besides **3** also **4** and **5**, whereas **5** slowly isomerizes to the thermodynamically more stable propyne (**4**).^[7]

The primarily detectable products of the simultaneously occurring C–C bond cleavage of **1** are ethene (**6**) and methane (**7**). Practically at the same time also the signals of acetylene (**8**), which is a secondary product of **6**, begin to rise. At no stage of the photolysis are signals of methyl and ethyl radicals^[8] measured. However, this is no surprise since both radicals are formed within the same matrix cage and can undergo either a back-reaction to **1** or secondary reactions such as the disproportionation into **6** and **7**.

This finding indicates that upon irradiation in a Br[•]-doped xenon matrix the fragmentation of **1** can follow two reaction channels, a primary C–H or C–C bond cleavage. Since a direct absorption of the applied energy ($\lambda = 254$ nm; $E = 112.6$ kcal mol^{−1}) by the hydrocarbon **1** (no absorption band in this region) is not possible, the energy of irradiation has to be absorbed by the halogen atoms isolated in the solid xenon and is stored in the already mentioned xenon–halogen exciplexes.^[3] The mechanism of the energy transfer to the substrate molecules and the details of the bond cleavage still need to be verified. Besides a charge-transfer complex with holes in the valence band of solid xenon^[2] also radical cations have to be discussed as potential intermediates. An estimate (Born equation) of the effect of the Br[•]-doped xenon matrix on the ionization energy of **1** gives a strongly reduced value of $I_{\text{matrix}} \approx 5.4$ eV = 124.5 kcal mol^{−1} ($I_{\text{gas phase}} = 10.95$ eV = 252.5 kcal mol^{−1}^[9]). That is, this estimated value for the reduced ionization energy approximates the used irradiation



Scheme 2. Photolysis of **1** in a xenon matrix.

energy. The geometry of the radical cation of **1** is known from ESR experiments.^[10] One of the two C–C bonds and one of the primary C–H bonds are considerably lengthened. The thermal chemistry of the radical cation of **1**, for instance the formation of the 1-propyl radical,^[11] can be explained by this change in geometry. We found no experimental evidence for the intermediacy of the radical cation of **1** in our experiments. Nevertheless, it cannot be excluded that upon irradiation of **1** in a Br-doped xenon matrix the observed bond cleavages occur via the radical cation.^[12]

Irradiation of 1 in a xenon matrix with laser light (route B₂): Propane (**1**) is photostable in an argon matrix upon irradiation with light of the wavelengths $\lambda = 254$ nm and $\lambda = 185$ nm. However, if a xenon matrix is applied in combination with an excimer laser (KrF; $\lambda = 248$ nm) as the light source **1** undergoes a slow photoreaction (10% transformation after 10 h photolysis). The main products of this photolysis, namely propene (**2**) and allyl radical (**3**), originate from the C–H bond cleavage. Propyne (**4**) and allene (**5**) are not formed under these conditions of irradiation (Table 1). The C–C cleavage of **1** plays only a minor role upon laser excitation, as can be documented by the observation that the concentrations of **6–8** are smaller than in the case of the irradiation in a Br-doped xenon matrix. The photolability of **1** upon usage of a laser can be explained by the two-photon mechanism mentioned above.^[2d, 4, 5]

Irradiation of 1 in an undoped xenon matrix with a Hg low-pressure lamp (route A₂): The most astonishing observation is that **1** reacts in a xenon matrix—but not in an argon matrix—even in the absence of halogen atoms with light of the wavelength $\lambda = 185$ nm of a mercury low-pressure lamp (with interference filter). Owing to the low photon density a two-photon process can be excluded under these conditions.

The products **2–6** of this photolysis (Figure 1, Table 1) are the same as those in the experiments in the doped matrix, but the relative concentrations differ. An additional intense signal

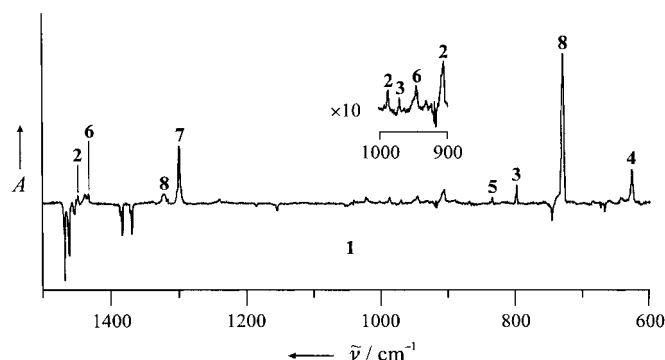


Figure 1. Difference spectrum after 45.5 h photolysis of **1** in a xenon matrix with light of the wavelength $\lambda = 185$ nm. IR bands with negative values diminish, while those with positive values are formed during irradiation.

at $\tilde{\nu} = 2023.9$ cm^{−1} can be assigned to the cumulenec carbon species C₃.^[13] Figure 1 shows the difference spectrum after the photolysis of **1** with light of the wavelength $\lambda = 185$ nm for 45.5 h. After this period of irradiation 20% of the starting material had reacted. During this irradiation the products of the C–C bond cleavage, **7** and **8**, clearly prevail. Presumably the photochemically induced reaction steps are similar

to those for the irradiation in a Br-doped matrix (see above). That means, the primary products are methane (**7**) and ethene (**6**), which is subsequently dehydrogenated to acetylene (**4**).^[14]

Surprising about the fragmentation of **1** in a xenon matrix by irradiation with light of the wavelength $\lambda = 185$ nm is the fact that the reaction occurs at all—after all, **1** is photostable in an argon matrix under the same conditions. A comparison with the gas phase UV spectrum of **1**^[15] and the spectrum of a film of solid **1**^[16] shows that the long wavelength edge of the absorption lies at $\lambda \approx 160$ nm. The observed reaction also cannot originate from a direct S₁ ← S₀ absorption (route A₁). Even if one assumes a bathochromic shift of the absorption cut-off of $\Delta\lambda \approx +10$ nm, which we have observed in other studies for instance for 1,3-butadiene and trimethylamine upon a change of the matrix material from argon to xenon, a direct S₁ ← S₀ absorption is unlikely. In addition one has to realize that under the applied matrix conditions an energy transfer by a triplet sensitizer can be excluded.

Evidently the use of xenon as matrix material causes a change in the absorption behavior. It is known^[17, 18] that due to the heavy atom effect the course of a photochemical reaction can be influenced by the choice of xenon as the matrix material. After direct absorption (S₁ ← S₀) of the irradiating light an intersystem crossing, favored by xenon, can occur, and thus a different mechanism—compared to reactions in usual matrix materials (e. g. argon or nitrogen)—becomes possible.

Xenon also has an influence on the lifetime of the phosphorescence of embedded substrate molecules. For benzene it amounts to 16 s in argon, but to only 0.07 s in xenon.^[19] The external heavy atom effect enhances the rate of the T₁ → S₀ transition. There are even examples for a direct entry to the T₁-hypersurface by absorption of a molecule isolated in a xenon matrix. For instance, the forbidden T₁ ← S₀ transition of α -chloronaphthalene can be measured directly in the presence of xenon under high pressure. This absorption only becomes measurable by the spin-orbit interaction with the external heavy atom xenon.^[20] A direct T₁ ← S₀ absorption can also have chemical consequences: Irradiation of *cis*- or *trans*-1,2-dichloroethene in a xenon matrix with light of the wavelength $\lambda > 300$ nm results in an isomerization of the respective alkene,^[21] in spite of the fact that these molecules show no S₁ ← S₀ absorption in the range of the irradiating light. On the other hand, the T₁ ← S₀ transition of the chloroethenes lies at 300–400 nm (measured in the presence of oxygen^[22]).

We assume that upon irradiation of **1** in a xenon matrix with light of the wavelength $\lambda = 185$ nm a direct T₁ ← S₀ excitation can similarly be achieved by the external heavy atom effect of xenon. Former calculations^[23] on the excited states of **1** indicate that the triplet states should lie not much (0.15–0.30 eV) below the singlet states. In a pure saturated hydrocarbon a $\sigma^* \leftarrow \sigma$ excitation leads to a dissociative Rydberg state. Starting from such an excited state the detected fragmentation reactions can be explained easily.

Conclusion: A bond cleavage by indirect irradiation of the substrate molecule in a halogen-doped xenon matrix (route B₁) is even possible in strain-free open-chain hydrocarbons.

Moreover, the fragmentation of matrix-isolated molecules can be achieved by using the heavy atom effect of xenon (route A₂).

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Organic Quantum-Confined Structures through Molecular Layer Epitaxy**

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Organic multilayered structures have attracted significant attention because they are potential substitutes for regular elemental components in electronic and electrooptic devices as well as excellent objects for experimental studies of basic quantum-mechanical models and principles of solid-state physics.^[1] Novel layered structures of organic light emitting devices (OLEDs) were reported to exhibit three-color emission.^[2] The study of organic field effect transistors (OFETs) fabricated from organic conjugated heterocyclic compounds has shown that an electron mobility as high as 1.3 cm² V⁻¹ s⁻¹ is achievable in molecular electronic devices.^[3] Recently the first OLED- and OFET-integrated optoelectronic devices were reported.^[4]

Multilayered structures such as self-assembled monolayers,^[5] electrostatic polymer assemblies,^[6] and Langmuir–Blodgett films,^[7] can be obtained by solution-derived methods, whereas ultra high vacuum (UHV) deposition,^[8] organic molecular beam epitaxy (OMBE),^[9] and molecular layer deposition^[10] were used for vapor-phase assembly of organic heterostructures. However, there is as yet no straightforward vapor-phase deposition method for assembling organic superlattices that enable epitaxial growth with covalent bonding. Inorganic atomic layer epitaxy (ALE)^[11] is the main vapor-phase method that can be used to build covalently bonded epitaxial multilayers in a layer-by-layer fashion.

We developed a new method for deposition of organic multilayers, molecular layer epitaxy (MLE), which is based on four levels: deposition of a template layer, self-limiting, vapor-phase reactions, covalent (“c axis”) interlayer bonding, and π stacking in the xy plane. A template layer is firstly deposited on an oxide surface, such as Si/SiO₂, quartz, or glass/indium tin oxide (ITO; Figure 1 a, step 1), exposing propylamine functionalities toward the interface and in turn dictating epitaxial growth. We choose the siloxane coupling network to enhanced thermal stability. This coupling reaction on silicon’s native oxide yields an ordered monolayer. However, on rougher surfaces such as ITO this reaction flattens the surface by a less controlled condensation reaction that can result in the assembly of two to three layers. Then discrete pulses of reactants, liquids or solids that undergo self-limiting reactions, are carried to the surface by an inert carrier gas in an MLE deposition reactor

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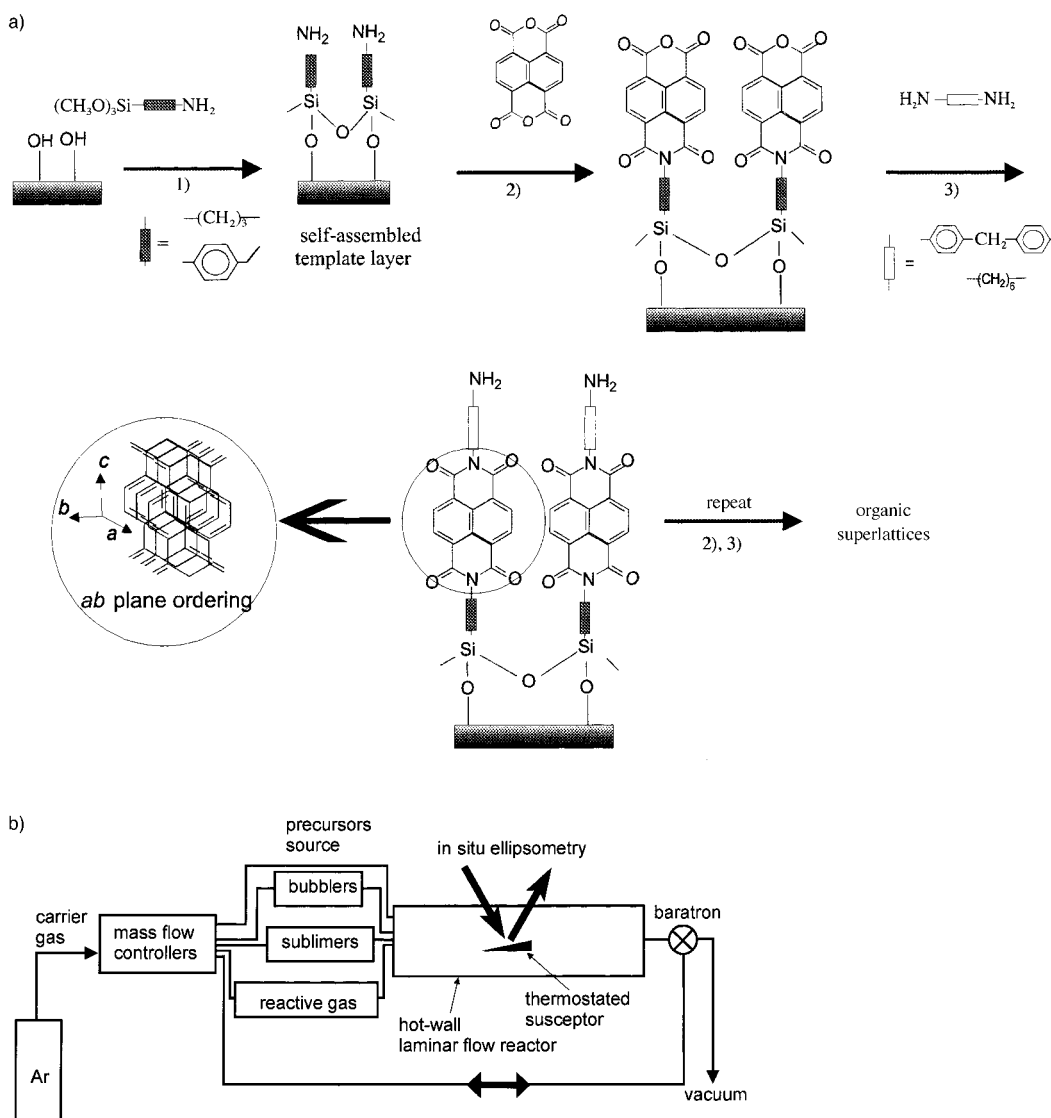


Figure 1. The molecular assembly approach and reactor for molecular layer epitaxy (MLE): a) MLE surface chemistry, b) laminar flow MLE reactor setup.

(Figure 1b). An alkylamine-containing surface is hit with a pulse of 1,8:4,5-naphthalenetetracarboxylic dianhydride (NTCDA) precursor (Figure 1a, step 2), forming imide linkages.^[12] Then a pulse of a vaporized aromatic or aliphatic diamine spacer (Figure 1a, step 3) is added to regenerate amine functionalities on the surface, which can again react with the dianhydride. Repeated cycling through these steps (Figure 1a, steps 2 and 3) leads to the formation of organic superlattices based on 1,8:4,5-naphthalenetetracarboxydiimide (NTCDI).

Those superlattices are organized by two motifs: individual structural units held together by covalent bonds perpendicular to the surface and, parallel to the surface, interplanar π stacking of aromatic groups (see the *ab* plane ordering in Figure 1a). The MLE-derived multilayers are stable with respect to heat (up to 300 °C) and most organic solvents and acids.

The structural regularity of the resulting NTCDI-based multilayers was tested by variable angle spectroscopic ellipsometry (VASE). Figure 2 shows measured and fitted ellipso-

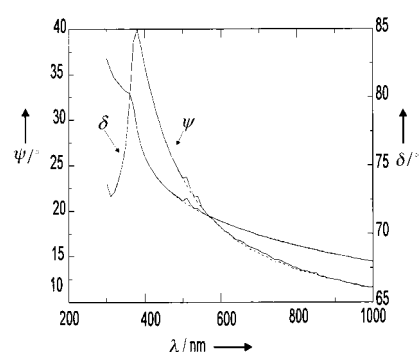


Figure 2. Variable angle spectroscopic ellipsometry (VASE) to determine the structural regularity in MLE-derived organic superlattices: generated (—) and experimental ellipsometric data (---) for nine bilayers of NTCDI-HM. Ψ = real, δ = imaginary part of the complex index of refraction.

metric data for a superlattice containing the aliphatic spacer hexamethylene (HM) with nine bilayers, (NTCDI-HM)₉. Interplanar spacing along the molecular *c* axis are 7.01 ± 0.03 Å for NTCDI and 6.90 ± 0.02 Å for the hexamethylene

spacer. The structural ordering of the system should be viewed as a two-dimensional “smectic-like” liquid crystal, with the confinement of NTCDI building blocks in a single layer without interpenetration of neighboring layers. In this plane ordering NTCDI might have a herring-bone structure,^[13] as is also suggested from the measured tilt angle of 23°. To verify the dimensional parameters of MLE-derived multilayers and the spectroscopic data, we synthesized the two low molecular weight model compounds *N,N'*-dihexyl-NTCDI (**1**) and *N,N'*-diphenyl-NTCDI (**2**).^[14] Model compounds of dialkylated NTCDI (see Experimental Section) exhibited smectic phases at elevated temperatures. Such spontaneous ordering might be induced by the MLE-derived topotactic chemistry.

Excitons play a fundamental role in the optical properties of solids, and are suggested to be responsible for finite size effects in the absorption spectra of organic multilayered structures.^[9] The size-dependent characteristics in NTCDI-based superlattices were investigated by optical absorption signature. The blue shift of the absorption peak and the increase in transition energy ΔE predicted for multilayers of decreased thickness were observed for PTCDA/NTCDA multilayers (PTCDA = 3,4,9,10-perylenetetracarboxylic dianhydride).^[15] The same trend is observed for the peak shift of the lowest energy absorption of NTCDI superlattices. An energy decrease of about 12 meV (Figure 3 a) is measured for the fewer number of layers showing a NTCDI multilayered structure with the aliphatic HM spacer. We considered the organic multiple quantum well (OMQW) energy levels to be consistent with a narrow potential well for NTCDI semiconduction placed in between very high potential barriers (aliphatic spacers). In this OMQW design both the electron and hole were approximated as being confined in the NTDCI layer. Conductivity-type anisotropy of the hole and electron in NTCDI-like molecular solids was established recently.^[16] Thus a model of a confined Wannier exciton in the quantum wells was considered by us.^[17] Minimizing of the binding exciton energy ($E_{1s} = \langle \psi | \mathcal{H} | \psi \rangle$) was applied to fit the blue-shifted absorption peak (marked in the insert of Figure 3 a).

Conjugated polymers or molecular stacks are organic semiconductors by virtue of the delocalization of electrons on the π molecular orbitals in the stacking direction. Such materials frequently exhibit electroluminescence (EL) when incorporated into a device. In an OLED device, excitation is caused by the injection of an electron into the conduction band (LUMO) and a hole into the valence band (HOMO), which recombine with emission of visible light. We fabricated MLE-derived OLEDs as devices that were as small as 4–6 nm thick.^[18] The EL spectra of OLEDs formed by assembling of two and four bilayered NTCDI structures with a diphenylmethane (DPM) spacer in the glass/ITO/OMQW/Al configuration at 8 V are shown in Figure 3b. It is clearly observed that two different centers emit under these conditions. The blue EL is attribute to radiative recombination on a single NTCDI molecule, and the red EL is assigned to radiative recombination within NTCDI molecules. The coexistence of two emitting centers within the same layer is an indication for two different excitation paths, intramolecular

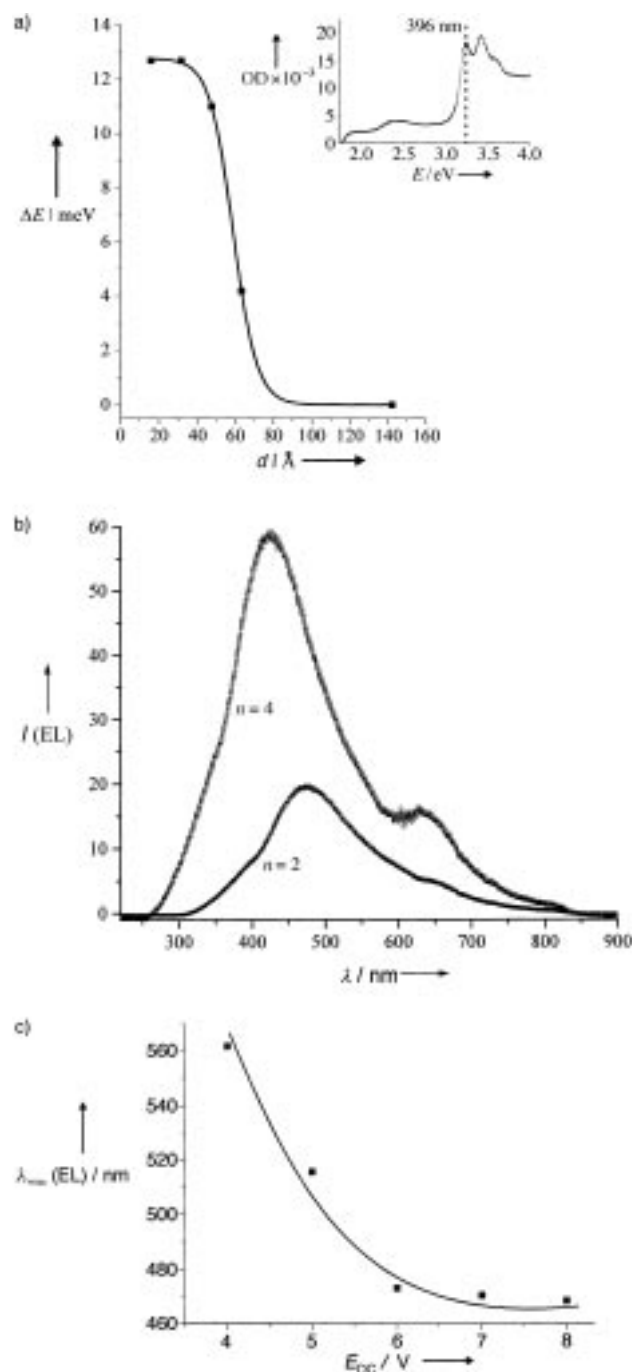


Figure 3. Dependence of effects on size in MLE-derived organic superlattices: a) Energy shift ΔE of the NTCDI-HM superlattice absorption peak (marked in the insert) as a function of thickness d . The symbols ■ correspond to experimental data, and the solid curve is the fit to the model described in the text. Insert: Absorption spectrum of a $(NTCDI-HM)_2$ multilayer sample; OD = optical density. b) Dependence of the EL on thickness in $(NTCDI-DPM)_n$ for $n=2$ and 4 in MLE-based OLEDs at $E_{DC} = 8$ V. c) Dependence of the EL on the DC voltage for $(NTCDI-DPM)_4$ -based OLEDs.

excitons and intermolecular excitons,^[19] and is a sign for homogeneous structure. The same trend for the appearance of a new EL peak due to epitaxial ordering was observed in perylene-based OLEDs^[20] and the single crystal of **1**.^[21]

Another finite size effect was observed for the EL of MLE-derived structures: the dependence of the EL of $(NTCDI-$

DPM)_n on thickness (Figure 3b). The EL maxima at 480 nm for (NTCDI-DPM)₂ was shifted by almost 50 nm upon doubling of the thickness. This blue-shifted EL can be explained by the electric field modulated Stark shift effect^[6] observed in inorganic^[22] and organic low-dimensional structures.^[23] The EL can be shifted to the blue by ramping the applied potential in the same (NTCDI-DPM)_n, OMQW-based devices (Figure 3c). At low voltage the red emission of the stacked NTCDI molecules (intermolecular excitons) dominate the EL spectrum, since these centers have a lower energy gap for excitation and charge recombination. Increasing the applied potential led to charge recombination at the higher energy gap (intramolecular excitons) and to blue-shifted EL. In summary, EL tunability is governed by finite size effects and the direct current (DC) field.

Figure 4a demonstrates the voltage dependence of the EL and current curves of MLE-derived OLEDs with a turn-on voltage of about 0.5 V, which is in contrast to the 5 to 10 V required to turn on most OLEDs. The current–voltage curve of an organic thin film of (NTCDI-DPM)₂ having a thickness

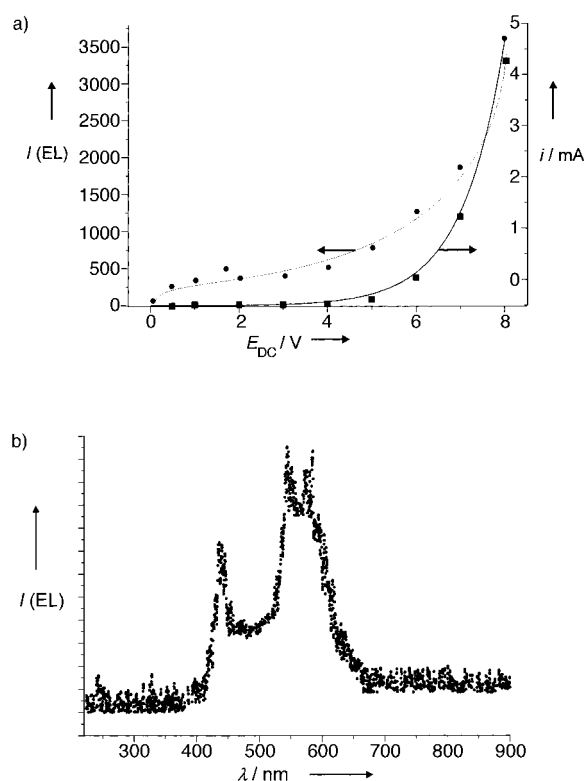


Figure 4. Electrical characteristics and capacitor effects in MLE-derived OLED's: a) Dependence of the EL intensity I (●) and the current i (■) on the voltage for (NTCDI-HM)₄. b) Anomalous emission of (NTCDI-HM)₄ at $E_{DC} = 0$ V.

of about 30 Å exhibits an archetypical semiconductor character with an EL lifetime of more than 100 hours (room temperature, ambient environment, $E_{DC} = 4$ V). Following the first cycle of EL, (NTCDI-HM)_n structures that incorporate aliphatic spacers act like tiny capacitors and emit light for a few hours after the voltage source was turned off; a spectrum is shown in Figure 4b. The EL band at 438 nm is narrower and sharper than for the DC-derived EL (FWHM = 10 nm).

We connect this unusual EL with the barrier structure of OMQW, which can trap the injected charges in the potential well structure and thus slow the radiative recombination. In terms of operation stability the MLE-derived devices are also robust in their ability to operate at a DC field of 3×10^7 V cm⁻¹, which is comparable to the operation values of amorphous silicon and positioned in the high range of dielectric breakdown in organic materials.

In summary, we have introduced a new synthetic route for the assembly of organic superlattices, the MLE approach. The combination of *c*-axis bonding and in-plane stacking led to the formation of densely packed and highly regular organic multilayers. The chemical and temporal stability of these organic structures were proved to be useful in device operation. Finite size effects have a major role in the optical properties of MLE-derived OMQWs. The aliphatic spacers produced heterostructures with capacitor-like behavior, and aromatic spacers led to the formation of highly efficient OLEDs. We expect that the molecular layer epitaxy approach will contribute to the fields of molecular optoelectronics as well as fundamental and applied sciences.

Experimental Section

Substrate cleaning: Quartz and silicon wafers were cleaned in aqueous detergent, rinsed copiously with deionized water, cleaned for 1 h in an ultrasonic bath in a hot (90 °C) "piranha" solution (H₂SO₄/H₂O₂ 70/30 v/v), and then allowed to cool to room temperature over the course of 1 h while sonicating. The cleaned substrates were rinsed then with deionized water. Further cleaning was carried out with H₂O/H₂O₂/NH₃ (5/1/1). After subsequent washing with deionized water, the substrates were immersed for 5 min in pure methanol, then in methanol/toluene (1/1), and finally in dry toluene.

Growth of organic multilayers: The template layer was obtained on precleaned hydroxylated substrates by liquid-phase silanization reaction with 3 mm (3-aminopropyl)trimethoxysilane in dry toluene at 90 °C for 8 h under a N₂ atmosphere. After the template layer was deposited all of the following film growth steps were conducted in the CVD reactor: The NTCDI precursor was vaporized at $T = 200$ – 250 °C to react with the surface-bound amine; 1,6-diaminohexane and 4,4'-methylenedianiline precursors were vaporized at $T = 35$ – 40 °C and 90 – 110 °C, respectively. Imidization in both steps was carried out at a substrate temperature of 280 – 290 °C for 15–25 min at a total pressure of 0.05 Torr. The laminar flow MLE reactor setup (Reynolds number < 250) was equipped with a spectroscopic ellipsometer for in situ process monitoring. Following the assembly of each monolayer, the reaction zone was cleaned by resublimation of unchanged precursors under a N₂ flow for 5 min. The reactor walls were independently heated to 330 °C.

Model compounds **1** and **2** were synthesized according to procedures in reference [24]. The modified synthesis will be reported elsewhere.

UV/Vis spectra were recorded on a Shimadzu UV-Vis-NIR 3101 PC scanning spectrophotometer. Luminescence was measured on a RF-5301 PC spectrofluorimeter. Electroluminescence was observed on a home-made setup consisting of RF-5301 PC and the source measure unit Keithley 236.

The variable angle spectroscopic ellipsometer (Woollam Co.) VASE 32 PC was used for measuring ellipsometry with 5-nm intervals in the range 300–800 nm. The structural model for fitting ex situ ellipsometry data uses the data of three different incident angles; in Figure 2 only the 75° data is shown. The fitting model includes the real (refractive index) and imaginary parts (extinction coefficient) of optical constants and the thickness of the layers. The multilayered structure that we studied consists of two different layers. These layers have a different free volume distribution and electron density: NTCDI is a highly dense layer with heteroatomic units, and the aliphatic spacer is a more diluted layer with properties closer to those of

polyethylene or monolayers containing long alkyl chains. The molar extinction coefficients (ϵ) were obtained from absorption spectra of MLE precursors, and the refractive index (n) was defined by surface plasmon resonance measurements on physisorbed films (50-nm-thick layer of NTCDA precursor). The starting values for the fitting model^[25] were taken from crystallographic data on compounds **1** and **2**, in accordance with literature data.^[13]

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Highly Efficient, Enantioselective Total Synthesis of the Active Anti-Influenza A Virus Indole Alkaloid Hirsutine and Related Compounds by Domino Reactions**

Lutz F. Tietze* and Yifa Zhou

In memory of Ulrich Schöllkopf

Owing to their high bond forming efficiency, domino reactions allow the construction of complex molecules in a few steps from simple substrates.^[1] Within this context, we have developed inter alia a domino Knoevenagel–hetero-Diels–Alder reaction in which a highly reactive 1-oxa-1,3-butadiene is formed by condensation of a 1,3-dicarbonyl compound with an aldehyde and then transformed into a functionalized dihydropyran in a subsequent hetero-Diels–Alder reaction with an enol ether or an alkene.^[2] This three- or four-component reaction^[3] also proceeds on a polymer support and is thus suitable for combinatorial synthesis.^[4] Recently, we reported the preparation of the Vallesiachotamine alkaloid dihydroantirrhine by a domino Knoevenagel/hetero-Diels–Alder reaction.^[5]

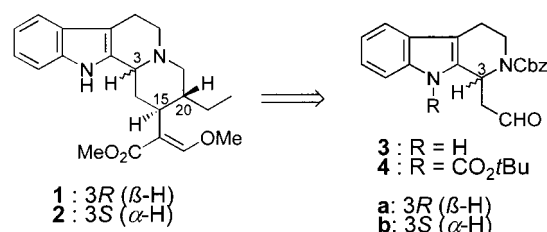
We describe here the application of this method to the highly efficient, enantioselective synthesis of the corynanthe indole alkaloids^[6] hirsutine^[7] (**1**) and dihydrocorynantheine (**2**).^[8] Hirsutine (**1**) and related compounds are currently of great interest as it has been shown that **1** exhibits a strong inhibitory effect against the influenza A virus (sub-type H3N2), with an EC₅₀ value of 0.40–0.57 µg mL⁻¹.^[9] It is

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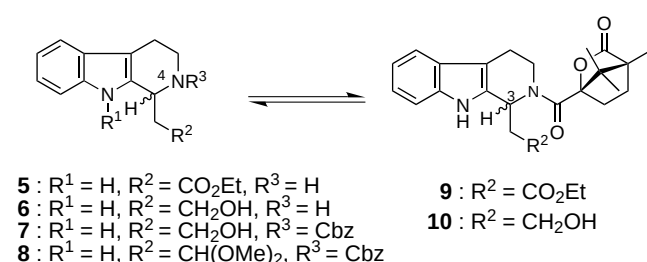
thus about 11 to 12 times more effective than the clinically used ribavirin.^[10]

Enantiomerically pure tetrahydro- β -carboline carbaldehydes **3a** and **4a** as well as **3b** and **4b** were required for the synthesis of **1** and **2** in a domino reaction (Scheme 1). Attempts at a diastereoselective alkylation at C-1 of a chiral



Scheme 1. Retrosynthesis of hirsutine (**1**) and dihydrocorynantheine (**2**). Cbz = benzyloxycarbonyl.

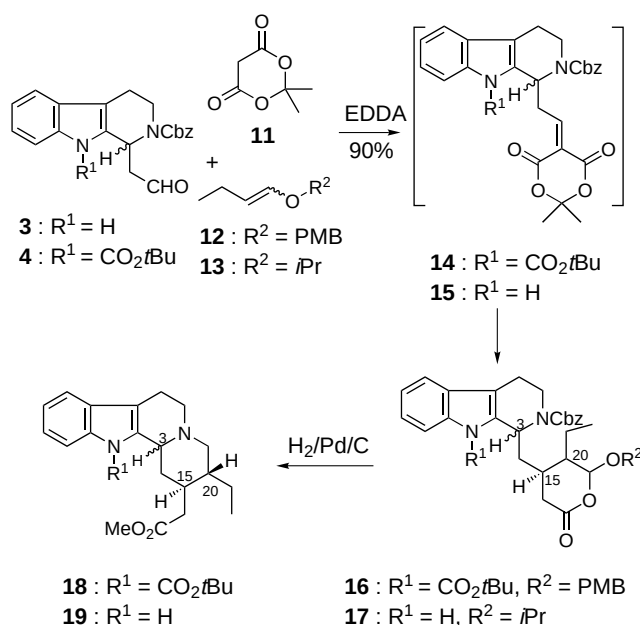
formamidinium derivative of tetrahydro- β -carboline with a silyl ether of bromoethanol according to the method of Meyers et al.^[11] gave **4** with only 50% *ee*.^[12] Consequently, separation of the enantiomers of *rac*-**5**, which is readily prepared by condensation of tryptamine hydrochloride with diethyl oxosuccinate by formation of diastereomeric salts with, for example, dibenzoyl tartaric acid, was investigated;^[13] however, **5** could only be obtained with at most 60% *ee*. Attempts at crystallization and chromatographic separation of the diastereomeric amides from *rac*-**5** and (–)-(1*R*)-menthyl chloroformate as well as (+)-camphor-10-sulfonyl chloride were also unsuccessful. In contrast, the diastereomeric amides **9** from *rac*-**5** and (–)-camphanic acid could be separated by chromatography, albeit with difficulty $\Delta R_f = 0.05$; (Scheme 2).^[14] Finally, the diastereomeric alcohols **10a** and **10b**, obtained by reduction of **9** with LiAlH₄, were separated without problems ($\Delta R_f \approx 0.2$).^[15] The procedure can be carried out on a large scale and allows ready access to the pure enantiomers of **3** and **4**.



Scheme 2. Synthesis of enantiomerically pure (3*R*)-**6** and (3*S*)-**6**. **a**: 3*R* (β -H), **b**: 3*S* (α -H).

Solvolysis of **10a** and **10b** with methanol followed by protection of N-4 with benzoyloxycarbonyl chloride and Dess–Martin oxidation^[16] afforded aldehydes **3a** and **3b** in 60% yield via **6** and **7**. In addition, aldehydes **4a** and **4b** with a *tert*-butoxycarbonyl group at the indole nitrogen atom were prepared in an overall yield of 55% by conversion of **3a** and **3b** into the acetals **8a** and **8b** and subsequent reaction with di-*tert*-butyl dicarbonate and cleavage of the acetal.

Condensation of **4a** with Meldrum's acid (**11**) and 4-methoxybenzyl butenyl ether (**12**) (*E*:*Z* $\approx$ 1:1) lead to the cycloadduct **16a** in a yield of 84% and with an asymmetric induction of greater than 20:1. Direct solvolysis (methanol/K₂CO₃) of **16a** without further purification followed by hydrogenation (10% Pd/C, 1 bar H₂) afforded the tetracycle **18a** as a single product in 67% yield (Scheme 3). All stereogenic centers in **18a** have the desired absolute configuration of hirsutine (**1**).^[17]



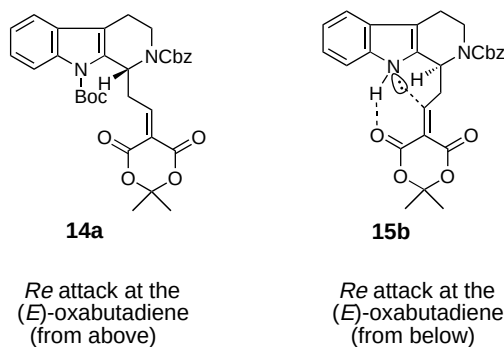
Scheme 3. Synthesis of **18** and **19** by a sequence of domino Knoevenagel–hetero-Diels–Alder, solvolysis, hydrogenation reactions with **3/4**, **11**, and **12/13**. **a**: 3*R* (β -H), **b**: 3*S* (α -H); PMB = *p*-methoxybenzyl; EDDA = ethylenediamine diacetate.

Similarly, the domino Knoevenagel–hetero-Diels–Alder reaction of **3b** with **11** and the (*Z*)-enol ether **13** gave the diastereomeric cycloadducts **17b** and *ent*-**17a** in a very good yield, although with a somewhat lower asymmetric 1,3-induction of 4.8:1 (Scheme 3). However, reaction of the product mixture with methanol/K₂CO₃ and subsequent hydrogenation afforded the desired product **19b** in only poor yields. The mixture of cycloadducts was therefore first converted into the *tert*-butoxycarbonyl derivatives **16b** and *ent*-**16a**, which were then treated with methanol/K₂CO₃ and hydrogenated. Chromatographic separation gave the enantiomerically pure diastereomers **18b** and *ent*-**18a** in 62 and 16% yield, respectively.

Cleavage of the *tert*-butoxycarbonyl group in **18a** and **18b** followed by condensation with methyl formate and treatment with diazomethane by known procedures gave the desired enantiomerically pure indole alkaloids hirsutine (**1**) and dihydrocorynantheine (**2**).

The synthetic sequence described contains firstly a Knoevenagel condensation of **3** and **4** with **11** with the formation of the 1,3-oxabutadienes **14** and **15**, respectively, which then undergo a facial-differentiating hetero-Diels–Alder reaction with the enol ethers **12** and **13**. In the reaction of **4a** with the

tert-butoxycarbonyl group on the indole nitrogen atom, the diastereomer **16a** with the 15*R* configuration is almost formed exclusively, whereas the 15*S* product **17a** is obtained preferentially from **3a** with an indole NH group (**3b** gives the 15*R* product **17b**). We assume that the 1-oxa-1,3-butadiene moiety in **14** and **15** is attached from the same face but exists in a different conformation in the transition state, so that *Re* attack occurs with **14a** with formation of the 15*R* configuration and *Si* attack with **15a** with the formation of the 15*S* configuration (**15b**: *Re* attack to 15*R*; Scheme 4). During



Scheme 4. Postulated conformation of 1-oxa-1,3-butadienes **14a** and **15b** in the transition state. Boc = *t*BuOCO.

solvolysis with methanol, the lactone is converted into a methyl ester with concomitant release of an aldehyde group. The Cbz group is cleaved during the following reaction under hydrogenation conditions, and the resulting secondary amine forms an enamine with the aldehyde which is finally hydrogenated selectively under stereoelectronic control to give the desired products **18a** and **19b**, respectively.

Thus, the biologically interesting alkaloid hirsutine (**1**) can be obtained with high selectivity and efficiency from the simple precursors **4a**, **11**, and **12** in a sequence consisting of a domino Knoevenagel–hetero-Diels–Alder reaction, solvolysis of the formed lactone with methanol/K₂CO₃, and subsequent hydrogenation. Dihydrocorynantheine (**2**) is formed analogously from **3b**, **11**, and **13**. Currently, the concept is being extended to the synthesis of analogues by the use of other enol ethers.

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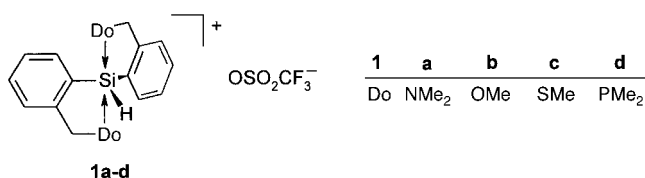
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- [14] **9**: Reaction of *rac*-**5** (14.5 g, 56.0 mmol) in CH₂Cl₂ (200 mL) and pyridine (13.5 mL) with (–)-camphanic acid chloride (12.1 g, 56.0 mmol) in CH₂Cl₂ (20 mL) at 0 °C; addition time 10 min, reaction time 8 h; yield 21.3 g (87 %). Separation: silica gel, ethyl acetate/petroleum ether (1/4); **9a**: *R*_f = 0.20; **9b**: *R*_f = 0.25.
- [15] Separation: silica gel, ethyl acetate/petroleum ether (1/2). **10a**: *R*_f = 0.19; m.p. 228 °C (ethyl acetate); [α]_D²⁰ = –58.0 (*c* = 1.0 in chloroform). **10b**: *R*_f = 0.40; m.p. 227 °C (ethyl acetate); [α]_D²⁰ = +80.5 (*c* = 1.0 in chloroform).
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- [17] **18a**: aldehyde **4a** (0.40 g, 0.89 mmol), **11** (0.15 g, 1.1 mmol), enol ether **12** (0.51 g, 2.7 mmol), and a few crystals of ethylenediamine diacetate (EDDA) in benzene (1.0 mL) were allowed to react in an ultrasonic bath at 50–60 °C for 12 h. Column filtration over silica gel (20 g) with petroleum ether/ethyl acetate (1/2) gave 0.55 g (84 %) of the cycloadduct as a yellow foam. The product (0.19 g, 0.28 mmol) was dissolved in methanol (5 mL) and treated with anhydrous potassium carbonate (20 mg) and palladium on carbon (10 %, 90 mg). The reaction mixture was stirred for 20 min at room temperature (TLC control) and then hydrogenated for 4 h (1 bar). After column filtration over silica gel with ethyl acetate, the solvent was evaporated under reduced pressure, and the crude product purified by chromatography on silica gel with petroleum ether/ethyl acetate (3/1); yield 80 mg (67 %) **18a** as a yellow oil. *R*_f = 0.29; [α]_D²⁰ = +93.0 (*c* = 0.3 in dichloromethane); ¹H-NMR (500 MHz, CDCl₃, 25 °C, TMS): δ = 7.92 (dd, ³*J* = 8.0, ⁴*J* = 1.5 Hz, 1H, 12-H), 7.39 (dd, ³*J* = 8.0, ⁴*J* = 1.5 Hz, 1H, 9-H), 7.24, 7.20 (2 × ddd, ³*J*₁ = ³*J*₂ = 8.0, ⁴*J* = 1.5 Hz, 2H, 10-H, 11-H), 4.03 (brs, 1H, 3-H), 3.71 (s, 3H, OCH₃), 3.00–2.52 (m, 8H, 5-H₂, 6-H₂, 21-H₂, 16-H₂), 2.10 (m, 1H, 15-H), 1.95 (ddd, ³*J*₁ = 13.0, ³*J*₂ = 3.5, ³*J*₃ = 3.0 Hz, 1H, 14-H_{eq}), 1.88 (ddd, ³*J*₁ = 13.0, ³*J*₂ = 9.0, ³*J*₃ = 5.0 Hz, 1H, 14-H_{ax}), 1.66 (s, 9H, *t*Bu-H), 1.58–1.49 (m, 3H, CH₂CH₃, 20-H), 0.91 (t, ³*J* = 7.5 Hz, 3H, CH₃).

Stabilization of Organosilicenium Ions by means of Intramolecular Coordination of O, S, or P Ligands

Uwe-H. Berlekamp, Peter Jutzi,* Andreas Mix, Beate Neumann, Hans-Georg Stammler, and Wolfgang W. Schoeller

Dedicated to Professor Edgar Niecke on the occasion of his 60th birthday

Organosilicenium ions, R_3Si^+ , are very strong electrophiles which can be stabilized by various donors.^[1, 2] An intramolecular coordination of N donors was described first by Corriu et al.^[3] and then by other authors;^[4] it leads to trigonal-bipyramidal siliconium ions. Surprisingly, other donors have not yet been employed in these systems. We now present compounds of the type **1b–d**, in which oxygen, sulfur, or phosphorus donors are used for the first time for stabilization purposes, compare them with each other and with the known N donor compound **1a**,^[4] and describe interesting differences with regard to structure and reactivity. Furthermore, we present quantum-chemical calculations on model systems.



The compounds **1b–d** (Table 1) are produced in excellent yield in the reaction of the corresponding chlorosilanes $(C_6H_4CH_2Do)_2Si(H)Cl$ ^[5] (Do = donor) with trimethylsilyl triflate ($Me_3SiOSO_2CF_3$) at $-80^\circ C$ in hexane. They are obtained as colorless solids (**1b, d**) or as a colorless oil (**1c**). The presence of 1:1 electrolytes in solution is confirmed by conductivity measurements. Statements concerning structure and bond type are made according to X-ray structure and multinuclear NMR data.

The molecular structure of an enantiomer of **1b** in the solid state is represented in Figure 1, together with important bond lengths and angles.^[6] The geometry at the silicon atom is almost perfect trigonal bipyramidal; the Si–O distances of 192 pm are comparatively short for donor–acceptor interactions.

According to the DNMR data, **1b–d** also have a trigonal-bipyramidal structure in solution. It is particularly interesting that dynamic processes in the sense of coordination equilibria (Scheme 1; “flip-flop” coordination^[7]) are observed for the

Table 1. Physical data for **1b–d**.

1b : ^1H NMR (500.1 MHz, CD_2Cl_2 , RT): δ = 4.19 (s, 6H, OCH_3), 5.18 (s, 1H, Si-H); 5.39, 5.63 (AB sys., $^2J(H,H)$ = 12.2 Hz, 4H, CH_2), 7.31 (d, $^3J(H,H)$ = 8.1 Hz, 2H, Ar-H), 7.41 (t, $^3J(H,H)$ = 7.5 Hz, 2H, Ar-H), 7.53 (t, $^3J(H,H)$ = 7.5 Hz, 2H, Ar-H), 7.56 (d, $^3J(H,H)$ = 7.5 Hz, 2H, Ar-H); $^{13}C\{^1H\}$ NMR (125.8 MHz, CD_3CN): δ = 62.7, (CH_2), 80.6 (CH_3), 124.1, 125.4, 129.7, 133.1, 135.1, 143.5 (arom. C); ^{29}Si NMR (99.4 MHz, $[D_6]DMSO$, RT): δ = -47.2 ($^1J(Si,H)$ = 272 Hz); $^{19}F\{^1H\}$ NMR (470.6 MHz, CD_3CN): δ = 142.78; IR (KBr): $\tilde{\nu}$ = 2170 cm^{-1} ; C,H analysis (%) calcd for $C_{17}H_{19}F_3O_3SiS$ (420.5): C 48.60, H 4.55; found: C 48.59, H 4.60
1c : ^1H NMR (500.1 MHz, CD_2Cl_2 , RT): δ = 1.36 (s, 6H, SMe), 3.89, 3.94 (br, AB sys., 4H, CH_2), 6.10 (s, 1H, Si-H), 6.97 (m, 4H, Ar-H), 7.05 (d, $^3J(H,H)$ = 7.32 Hz, 2H, Ar-H), 7.79 (d, $^3J(H,H)$ = 6.98 Hz, 2H, Ar-H); ^1H NMR (500.1 MHz, C_7D_8 , $90^\circ C$): δ = 1.47 (s, 6H, CH_3), 3.57 (s, 4H, CH_2), 6.19 (s, 1H, Si-H), 7.31–7.39 (m, 4H, Ar-H), 7.45 (t, $^3J(H,H)$ = 7.40 Hz, 2H, Ar-H), 7.77 (d, $^3J(H,H)$ = 7.40 Hz, 2H, Ar-H); $^{13}C\{^1H\}$ NMR (125.8 MHz, C_6D_6): δ = 14.9 (SMe), 38.8 (CH_2), 119.5 (q, $^1J(C,F)$ = 318.3 Hz, CF_3), 127.4, 129.6, 130.8, 130.9, 136.1, 143.3 (arom. C); ^{29}Si NMR (99.4 MHz, C_6D_6 , RT): δ = -47.9 ($^1J(Si,H)$ = 278.4 Hz); $^{19}F\{^1H\}$ NMR (470.6 MHz, C_6D_6): δ = 132.7; IR (KBr): $\tilde{\nu}$ = 2199 cm^{-1} ; C,H analysis (%) calcd for $C_{17}H_{19}F_3O_3S_3Si$ (452.6): C 45.11, H 4.23; found: C 44.99, H 5.76
1d : ^1H NMR (500.1 MHz, CD_2Cl_2 , $-80^\circ C$): δ = 1.54 (brs), 12H, CH_3 , 3.30, 3.41 (AB sys., $^2J(H,H)$ = 9.98 Hz, 4H, CH_2), 5.92 (t, $^2J(P,H)$ = 72.5 Hz, 1H, Si-H), 7.32 (m, 4H, Ar-H), 7.41 (m, 2H, Ar-H), 7.47 (m, 2H, Ar-H); ^1H NMR (500.1 MHz, CD_2Cl_2 , RT): δ = 1.54 (t, $^2J(P,H)$ = 3.5 Hz, 12H, PMe_2), 3.45 (t, $^2J(P,H)$ = 3.7 Hz, 4H, CH_2), 5.98 (t, $^2J(P,H)$ = 55.1 Hz, 1H, Si-H), 7.35 (t, $^3J(H,H)$ = 7.3 Hz, 2H, Ar-H), 7.42 (m, 4H, Ar-H), 7.52 (t, $^3J(H,H)$ = 7.5 Hz, 2H, Ar-H); $^{13}C\{^1H\}$ NMR (125.8 MHz, CD_2Cl_2 , $-80^\circ C$): δ = 5.8 (CH_3), 7.50 (CH_2), 30.1 (CH_2), 119.8 (q, $^1J(F,C)$ = 320 Hz, CF_3), 127.0, 129.3, 131.3, 134.8, 142.7 (arom. C); $^{13}C\{^1H\}$ NMR (125.8 MHz, C_6D_6 , RT): δ = 14.9 (SMe), 38.6 (CH_2), 127.4, 129.6, 130.8, 130.9, 136.1, 143.3 (arom. C); ^{29}Si NMR (99.4 MHz, CD_2Cl_2 , $-80^\circ C$): δ = -104.3 (t, $^1J(Si,P)$ = 37.8 Hz); ^{29}Si NMR (99.4 MHz, C_6D_6 , RT): δ = -67.8 ($^1J(Si,H)$ = 251, $^1J(P,Si)$ = 19.8 Hz); $^{29}Si\{^1H\}$ CP-MAS NMR: δ = -89.2; ^[10] $^{19}F\{^1H\}$ NMR (470.6 MHz, CD_2Cl_2): δ = 131.3; $^{31}P\{^1H\}$ NMR (202.5 MHz, CD_2Cl_2 , $-80^\circ C$): δ = -17.9; $^{31}P\{^1H\}$ NMR (202.5 MHz, CD_2Cl_2 , RT): δ = -22.1; IR (KBr): $\tilde{\nu}$ = 2159 cm^{-1} ; C,H analysis (%) calcd for $C_{19}H_{25}ClF_3O_3P_2SiS$ (480.50): C 46.16, H 5.38; found: C 44.00, H 5.22. A more accurate C,H analysis was impossible owing to the extreme sensitivity.

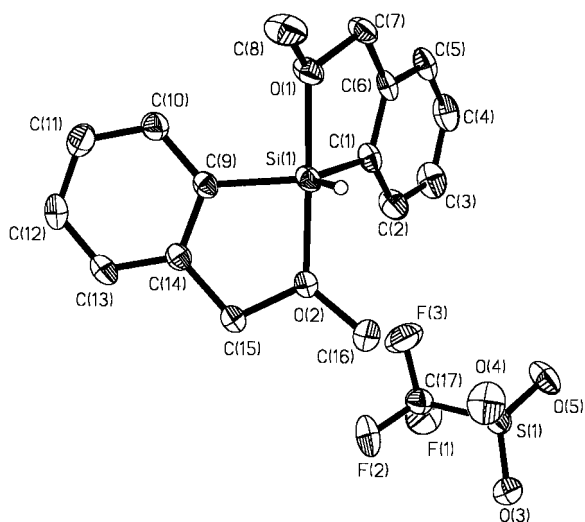
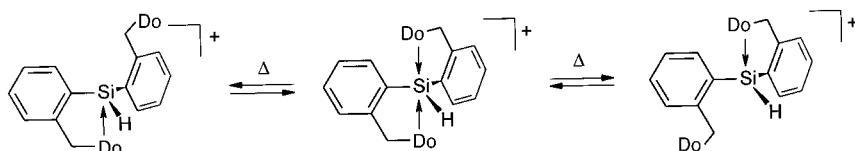


Figure 1. Molecular structure of **1b**; the ellipsoids correspond to a probability level of 50%. The hydrogen atoms were omitted for clarity. Selected bond lengths [pm] and angles $^\circ$: Si(1)–O(1) 191.8(3), Si(1)–O(2) 192.3(3), Si(1)–H 137(4); C(1)–Si(1)–C(9) 120.7(2), C(1)–Si(1)–H(1) 118(2), C(9)–Si(1)–H(1) 121(2), O(1)–Si(1)–O(2) 178.57(12), C(1)–Si(1)–O(1) 84.7(2), C(1)–Si(1)–O(2) 96.6(2), O(1)–Si(1)–H(1) 88(2), O(2)–Si(1)–H(1) 92(2), C(9)–Si(1)–O(1) 94.6(2), C(9)–Si(1)–O(2) 84.3(2).

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Scheme 1. Molecular dynamics in the cations of **1c**, **d**.

cations with “soft” ligands (**1c**, **d**), with activation energies of approximately 17 (**1c**) and 11 kcal mol^{−1} (**1d**). These dynamics can be proved by the cancellation of the diastereotopicity of the CH₂ units (**1c** and **1d**) and the PMe₂ unit (**1d**), as well as the temperature dependence of the $\delta(^{31}\text{P})$, $^2J(\text{P,H})$, and $^1J(\text{Si,P})$ values (**1d**).

For a comparison of **1a–d**, the SiH units are important NMR spectroscopic probes (Table 2). The $\delta(^{29}\text{Si})$ values are characteristic for trigonal-bipyramidal silicon centers (Table 1); the enormous upfield shift in **1d** is striking. The large

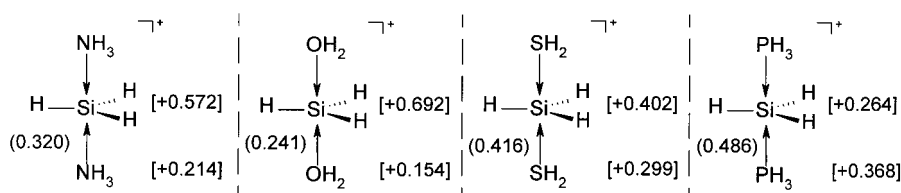
Table 2. Selected NMR data for **1a–d**.

	1a	1b	1c	1d
$\delta(\text{Si})$	−51.6	−47.2	−47.9	−104.3 ^[a]
$\delta(\text{H}(\text{Si}))$	4.60	5.18	6.10	5.92 ^[b]
$^1J(\text{Si,H})$ [Hz]	272	272	279	251

[a] $T = -80^\circ\text{C}$, $^1J(\text{Si,P}) = 37.8$ Hz (-80°C); [b] $^2J(\text{P,H}) = 72.5$ Hz (-80°C).

$^1J(\text{Si,H})$ coupling constants speak for an increased s proportion in the Si–H bond (towards sp² hybridization^[8]) in all cases. Significant differences are observed in the $\delta(\text{H}(\text{Si}))$ values: A downfield shift occurs in the systems with “soft” donors (**1c**, **d**), speaking for a decrease in hydride character. Confirmation of this, as well as for the dynamics in **1c**, **d**, is supplied by quantum chemical calculations.

Ab initio calculations (CCSD(t)/6-311 + g**//MP2/6-31g**) on the model system SiH₃⁺^[9] speak for clearly different bonding situations in complexes with “hard” (OH₂, NH₃) and with “soft” donors (SH₂, PH₃). A comparison of the bond energies [kcal mol^{−1}] for the tetrahedrally coordinated mono-addition products [Do → SiH₃]⁺ (Do = OH₂ (51.4), NH₃ (72.3), SH₂ (46.4), PH₃ (57.1)) with those for the trigonal-bipyramidal bis-addition products [Do → SiH₃ ← Do]⁺ (Do = OH₂ (74.3), NH₃ (100.2), SH₂ (59.0), PH₃ (67.8)) shows that the addition of a second donor molecule is less exothermic in the case of the “soft” donors. A comparatively easier dissociation—as in the process according to Scheme 1—thus becomes understandable. The differences in the Do → Si bonds of the bis-adducts [Do → SiH₃ ← Do]⁺ follow immediately from population analyses, which is documented in Scheme 2 with charge densities at atomic groups (values in



Scheme 2. Charge density at atomic groups (values in square brackets) and Wiberg bond orders (values in parentheses); average values over all H atoms are given for OH₂ and SH₂, respectively.

square brackets) and of Wiberg bond orders (values in parentheses) according to the NBO method (NBO = natural bond orbital).

Accordingly, a transfer of electron density to the SiH₃⁺ ion is much less distinctive for a “hard” donor than for a “soft” donor.

Therefore, Do → Si bonds with “soft” donors show a comparatively higher covalent character. GIAO calculations confirm the trend observed for the $\delta(\text{H}(\text{Si}))$ values (Table 2); population analyses yield comparatively less negative charge densities for the H atoms in **1c**, **d** (SiH₃⁺: −0.152, (NH₃)₂SiH₃⁺: −0.188, (OH₂)₂SiH₃⁺: −0.183, (PH₃)₂SiH₃⁺: −0.144, (SH₂)₂SiH₃⁺: −0.149).

The differences in structure between **1a–d** lead to marked differences in reactivity, as preliminary experiments have shown. Therefore, the susceptibility towards hydrolysis and the general reactivity towards nucleophiles increases drastically in the order **1b** < **1c** < **1d**. Surprisingly, **1b** reacts like an oxonium salt with pyridine, under transfer of a methyl group and formation of a neutral five-membered silyl ether; on the other hand, **1c**, **d** react under substitution of a donor molecule. The addition of acetonitrile leads to the exchange of a donor molecule in the case of **1c**, to the formation of a product with an as yet unknown structure in the case of **1d**, and to no reaction in the case of **1b**.

The stabilization of a silicenium ion with O-, S-, or P-donors described here has interesting consequences in terms of structure and bond theory, and also offers the possibility of control of reactivity. This concept should be transferable to other element cations of the type R_xElⁿ⁺.

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Synthesis and Structural Characterization of Graphite-Like [(Me₃Sn)₃O]Cl**

Bodo Räke, Peter Müller, Herbert W. Roesky,* and Isabel Usón

Dedicated to Professor Hubert Schmidbaur on the occasion of his 65th birthday

In our investigations on weakly coordinating cations and anions, we were interested in the properties of the sterically demanding oxonium cation [(Me₃Sn)₃O]⁺. We have already isolated tris(trimethylsilyl)methylaluminum compounds which exhibit weak cation–anion interactions. In the anions of both [Ag(toluene)₃]⁺[(SiMe₃)₃C]₂Al₂F₅₂Li[–] and [AlF₂(thf)₄]⁺[(SiMe₃)₃C]₂Al₂F₅[–] the fluorine atoms bridge the aluminum atoms. Weak ionic interactions are the result of the efficient shielding of the anionic centers by the tris(trimethylsilyl)methyl ligands.^[1]

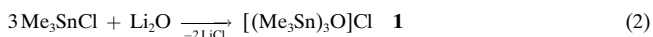
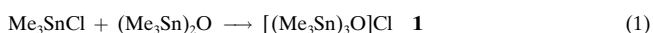
Dehnicke et al. have already described trimethylstannyl-substituted ammonium salts that exhibit an interesting ionic

arrangement. In [NH₂(SnMe₃)₂][SnMe₃Cl₂], the chlorine atoms bridge two tin atoms and one hydrogen atom, which leads to a symmetrical three-dimensional lattice.^[2, 3]

Interactions of alkyltin compounds with Lewis bases are not rare. The tin atoms in SnF₄ and Me₂SnF₂ as well as in the [SnCl₆]^{2–} dianion are surrounded by their ligands in an octahedral arrangement.^[4, 5]

There is an almost trigonal-bipyramidal configuration of tin and oxygen in Me₃SnN(SO₂Me)₂·Me₃SnOH, whereby the oxonium ion [(Me₃Sn)₂OH]⁺ is formed.^[6] The synthesis and properties of [(Me₃Sn)₃O]I have already been discussed by Harada, however, without a structural investigation of this compound.^[7]

H₃O⁺ and the cation of the Meerwein salt are the best known oxonium ions. Our work is directed to the synthesis and the structural characterization of the oxonium salt [(Me₃Sn)₃O]Cl (**1**). This compound was synthesized by refluxing (Me₃Sn)₂O^[8] and Me₃SnCl in THF for 12 h. After concentration of the solution and storage at –23 °C, **1** precipitated as colorless crystals [Eq. (1)]. Compound **1** was investigated by single-crystal X-ray structural analysis, NMR and IR spectroscopy as well as mass spectrometry. Compound **1** is also accessible by the treatment of Me₃SnCl with Li₂O in refluxing THF [Eq. (2)].



The solvent was removed under vacuum after 15 h of heating. Excess starting material was removed during the workup of **1**: Me₃SnCl was removed with the solvent under vacuum, and Li₂O was removed by filtration. The formation of **1** was confirmed by ¹H NMR spectroscopy. Unfortunately, we were not able to obtain single crystals; traces of LiCl probably prevent crystallization. In the ¹H NMR spectrum there is a singlet at δ = 0.21. Coupling with the ¹¹⁷Sn and ¹¹⁹Sn nuclei gives a satellite pair with a coupling constant of 56 Hz, which is, however, not split.^[8] There is only one signal in both the ¹³C NMR spectrum (δ = –2.40) and in the ¹¹⁹Sn NMR spectrum (δ = 120.02). The satellite pair in the ¹³C NMR spectrum is hidden by background noise. The elemental analysis only deviates slightly from the calculated values. The mass spectrum only exhibits fragments of the starting materials, namely, (Me₃Sn)₂O and Me₃SnCl, which are produced on heating **1** in the mass spectrometer. The molecular peak of (Me₃Sn)₂O minus a methyl group (*m/z* 331) and the molecular peak of Me₃SnCl (*m/z* 200) are observed in the mass spectrum.

The single-crystal X-ray structural analysis of **1** reveals that it crystallizes in the hexagonal space group *P*6₃/*m* with a sixth of the molecule in the asymmetric unit; the remaining five sixths are produced by a threefold axis and the mirror plane. The structure consists of a positively charged oxygen center that is surrounded by three SnMe₃ groups in a trigonal-planar manner as well as a chlorine atom (Figure 1). In the crystal, each chloride ion is coordinated to three Sn atoms to produce an infinite two-dimensional hexagonal structure. Each of these hexagons is formed by a 12-membered Sn₆Cl₃O₃ ring

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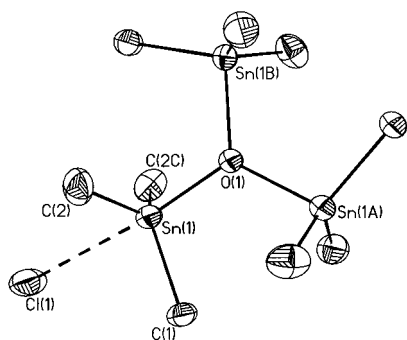


Figure 1. Crystal structure of **1** (the H atoms have been omitted for clarity. The vibration ellipsoids represent 50% probability levels). Selected bond lengths [Å] and angles [°]: Sn(1)–O(1) 2.0999(4), Sn(1)–C(1) 2.124(5), Sn(1)–C(2) 2.125(4), Sn(1)–Cl(1) 3.0856(6); O(1)–Sn(1)–C(1) 103.7(2), O(1)–Sn(1)–C(2) 96.4(1), C(1)–Sn(1)–C(2) 113.2(1), C(2)–Sn(1)–C(2C) 126.8(2), Sn(1)–O(1)–Sn(1A) 120.0(0), O(1)–Sn(1)–Cl(1) 174.37(1).

(Figure 2). Two of these hexagonal layers lie over one another so that the chlorine atoms are positioned over the chlorine atoms of the next layer and the oxygen atoms of one layer lie over the center of the hexagon of the next as well as the

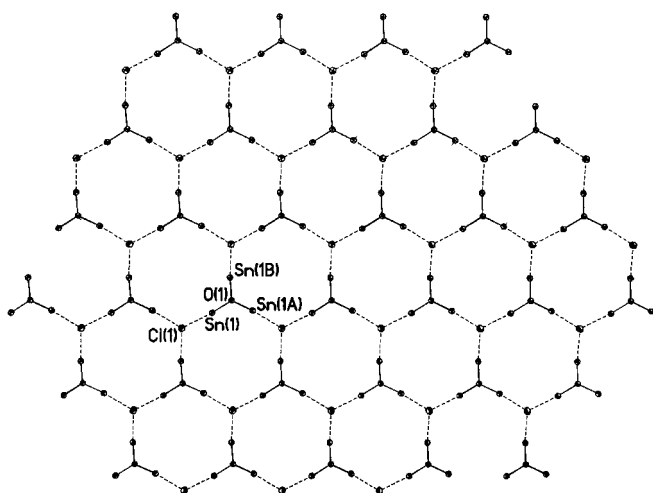


Figure 2. Honeycomb structure of the $\text{Sn}_6\text{Cl}_3\text{O}_3$ rings in **1**. The C and H atoms have been omitted for clarity.

previous layer (Figure 3). In this way a hexagonal AB layer structure is formed (distance between two neighboring layers 6.32 Å), which is very similar to the α modification of graphite (graphite: space group $P6_3/mc$, hexagonal AB layer structure).^[9] The carbon atoms do not participate in the formation of the lattice layers. Each tin atom is in a distorted trigonal-bipyramidal environment, and the three equatorial methyl groups are bent away from the axial oxygen atom, which is placed opposite to the chloride ion in the pyramid (the Sn atom lies 0.319(4) Å outside the plane through the three C atoms), so that the original tetrahedral coordination of the Me_3SnO unit is still recognizable.

Thus, we have demonstrated that in $[(\text{Me}_3\text{Sn})_3\text{O}]\text{Cl}$ there are strong cation–anion interactions in the solid state. Our results provide many possibilities for the investigation of corresponding systems by exchange of the anion. The prediction of possible structures is, however, risky.

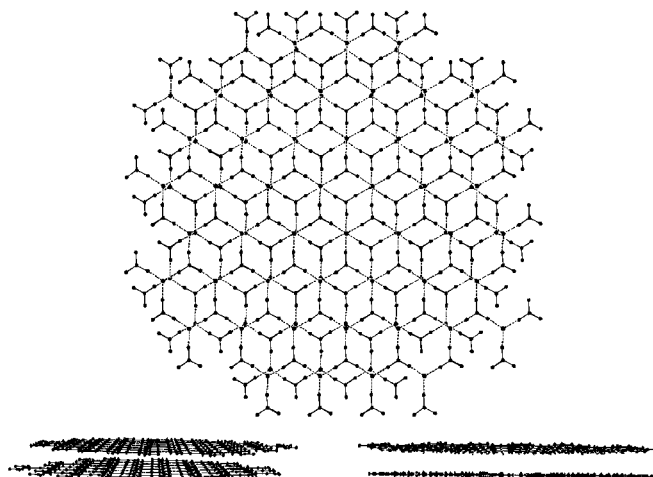


Figure 3. The AB layer structure in **1**. The C and H atoms have been omitted for clarity.

Experimental Section

1. Me_3SnCl (0.12 g, 1 mmol) was refluxed with $(\text{Me}_3\text{Sn})_2\text{O}$ (0.34 g, 1 mmol) in THF (50 mL) for 12 h. The solution was concentrated (5 mL) and left to stand at -23°C to give compound **1** as colorless crystals. Yield: 0.2 g (37%).

2. Me_3SnCl (1.39 g, 7 mmol) was refluxed with Li_2O (0.06 g, 2 mmol) in THF (80 mL) for 15 h under reflux. The solid residue was filtered off, and volatile components were removed under vacuum. Yield **1**: 0.3 g (28%).

M.p. 94°C ; ^1H NMR (250 MHz, C_6D_6 , TMS): $\delta = 0.21$ (s, 27 H, $\text{Sn}(\text{CH}_3)_3$, $J_{\text{SnCH}} = 56$ Hz); ^{13}C NMR (62 MHz, C_6D_6 , TMS): $\delta = -2.40$ (s, 9 C); ^{119}Sn NMR (149 MHz, C_6D_6 , SnMe_4): $\delta = 120.02$ (s, 3 Sn); IR (KBr, Nujol): $\tilde{\nu} = 3360, 3187, 2359, 1305, 1261, 1194, 1184, 1170, 1155, 1031, 975, 919, 772, 736, 723, 546, 516, 455$ cm^{-1} ; MS (EI): m/z (%): 331 (67, $M^+ - \text{Me}_3\text{SnCl} - \text{Me}$), 200 (3, $M^+ - (\text{Me}_3\text{Sn})_2\text{O}$), 165 (100, $M^+ - (\text{Me}_3\text{Sn})_2\text{O} - \text{Cl}$); elemental analysis calcd for $\text{C}_9\text{H}_{27}\text{ClO}_3\text{Sn}_3$: C 19.91, H 5.01, Cl 6.53; found: C 19.46, H 5.27, Cl 6.80.

Structural analysis of $\text{C}_9\text{H}_{27}\text{ClO}_3\text{Sn}_3$: $M_r = 542.83$, crystal dimensions: $0.70 \times 0.70 \times 0.60$ mm, hexagonal, space group $P6_3/m$, $a = b = 8.971(1)$, $c = 12.646(3)$ Å, $V = 881.4(3)$ Å³, $\rho_{\text{calcd}} = 2.045$ g cm⁻³, $Z = 2$, absorption coefficient $\mu = 4.348$ mm⁻¹, maximum resolution $2\theta = 57.4^\circ$; of 15939 reflections measured, 794 were independent ($R_{\text{int}} = 0.0719$). Data/restraints/parameter ratio: 794/11/29, final R values: $R1 = 0.0263$, $wR2 = 0.0648$ for reflections with $I > 2\sigma(I)$ and $R1 = 0.0274$, $wR2 = 0.0654$ for all reflections, goodness of fit $S = 1.229$; ($R1 = \sum ||F_o| - |F_c|| / \sum |F_o|$, $wR2 = [\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2]^{1/2}$, $S = [\sum w(F_o^2 - F_c^2)^2 / \sum (n - p)]^{1/2}$); weighting scheme: $w^{-1} = \Sigma^2(F_o)^2 + (0.0226P)^2 + 1.9493P$; $P = [F_o^2 + 2F_c^2]/3$; max./min. residual electron density 0.734/−0.474 e Å⁻³.

The crystal was removed from the Schlenk flask under an inert gas, mounted on a glass fiber with perfluoro-polyether, and shock-frozen.^[10] The data was collected at $T = 133$ K on a Stoe–Siemens–Huber four-circle diffractometer equipped with a Siemens CCD area detector, with graphite monochromatic $\text{MoK}\alpha$ radiation ($\lambda = 0.71073$ Å) performing φ and ω scans. The structure was solved by direct methods (SHELXS-97^[11]) and refined against F^2 (SHELXL-97^[12]) in a full-matrix least-squares procedure. All the non-hydrogen atoms were anisotropically refined, and the hydrogen atoms were added to the model in calculated geometric positions and refined with a riding model. Crystallographic data (excluding structure factors) for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-114479. 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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Keywords: oxonium ions • oxygen • layered compounds • tin

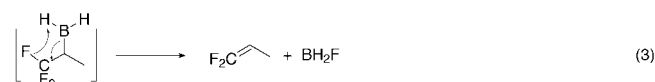
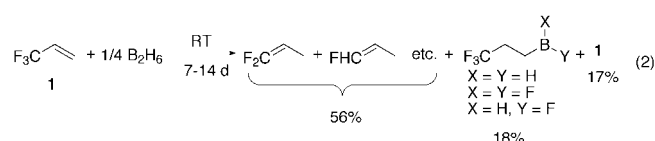
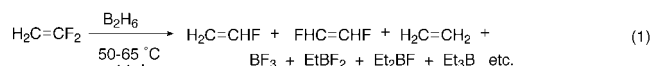
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Markovnikov Hydroboration of Perfluoroalkylethylenes**

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Michael P. Jennings,
and P. Veeraraghavan Ramachandran*

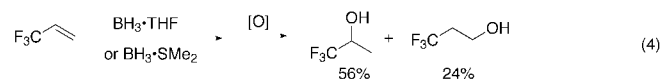
Fluoroorganic compounds are increasingly being used in analytical, biological, medicinal, organic, and polymer chemistry as well as materials science.^[1] The introduction of fluorine in place of hydrogen often improves the biological properties of organic compounds.^[1] The application of organoboranes has become a routine affair in many synthetic laboratories.^[2] Yet, it is surprising that relatively little organoborane chemistry has been utilized to prepare fluoroorganic compounds.^[3] For example, the ether-catalyzed hydroboration of olefins has been known for over 40 years.^[4] However, there are no reports in the literature describing the liquid-phase addition of borane to fluorine-containing unsaturated molecules. Several gas-phase reactions of diborane with fluoroolefins have been described. The first such study by Barotcha and Stone described the reaction of diborane with tetra-, tri-, 1,1-di-, and monofluoroethylene at high and room temperatures resulting in the formation of a complex mixture of products.^[5] For example, the reaction of 1,1-difluoroethylene provided a series of products resulting from the exchange of

fluorine and hydrogen between the olefin and borane [Eq. (1)]. Stone and co-workers later reported that the reaction of 3,3,3-trifluoro-1-propene (**1**) with 0.25 equivalents of diborane at room temperature for 1–2 weeks resulted in several products. 1,1-Difluoropropene and its reduced species constituted 56% of the products, and (3,3,3-trifluoro-1-propyl)borane derivatives accounted for another 18% [Eq. (2)]. They reasoned that the former product originated from the addition of borane to the internal carbon atom, followed by a β -elimination [Eq. (3)].^[6]



Several hydroboration agents of differing steric and electronic requirements have since become commercially available.^[2] However, none of these have been tested with fluoroolefins. The fascinating results of our systematic study of the hydroboration of perfluoroalkylethylenes ($\text{R}_f\text{CH}=\text{CH}_2$) with these reagents are presented herein.

When **1** was treated with one equivalent of $\text{BH}_3 \cdot \text{THF}$ at room temperature, the ^{11}B NMR spectrum of the mixture after five minutes revealed three peaks at $\delta = 0.12$ (q), 5.5 (t), and 9.5 (d). These correspond to the unchanged $\text{BH}_3 \cdot \text{THF}$, a monoalkylborane, and a dialkylborane, respectively. The concentration of the dialkylborane increased with time until no further change was observed after four hours. The ^{11}B NMR spectrum now revealed the presence of BH_3 , RBH_2 , and R_2BH in a ratio of 2.5:4.5:1. Oxidation of the reaction mixture with alkaline H_2O_2 provided a gas chromatographic (GC) yield of 82% for the product alcohols 1,1,1-trifluoro-2-propanol and 3,3,3-trifluoro-1-propanol in a ratio of 7:3 [Eq. (4)]. The reaction of **1** with one equivalent of $\text{BH}_3 \cdot \text{SMe}_2$ at room temperature followed by oxidation with alkaline H_2O_2 revealed results similar to those realized in hydroboration with $\text{BH}_3 \cdot \text{THF}$ [Eq. (4)].

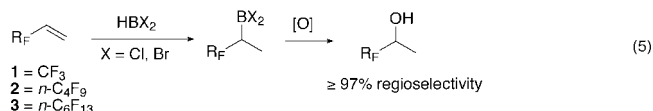


The hydroboration of **1** with $\text{ClBH}_2 \cdot \text{SMe}_2$ in Et_2O was slow and complete in 24 h. Oxidation of this mixture resulted in a 3:2 ratio of secondary and primary alcohols. The increase in the amount of the primary alcohol may be the result of an enhanced formation of the dihydroborated intermediate. The second hydroboration might be occurring at the terminal carbon atom due to the large bulk of the R_fBHCl reagent formed in the initial hydroboration.

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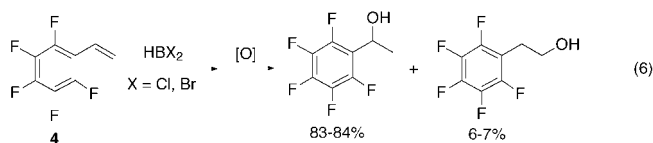
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We envisaged that hydroboration with reagents containing only one hydrogen atom on the boron atom, such as 9-borabicyclo[3.3.1]nonane (9-BBN), catecholborane (CB), or dichloroborane, might provide the secondary alcohol selectively. However, 9-BBN was very slow to react with this olefin, even after 24 h. Catecholborane did not show any indication of reaction, even after days. To our gratification, the reaction of Cl_2BH —prepared by liberation from $\text{Cl}_2\text{BH} \cdot \text{SMe}_2$ upon addition of BCl_3 ^[7] or by Matteson's procedure ($\text{BCl}_3 + \text{R}_3\text{SiH}$)^[8]—was instantaneous, and oxidation of the organoborane intermediate produced the secondary (Markovnikov) alcohol in 98 % isomeric purity (GC) along with 2 % of the primary alcohol [Eq. (5)].



We then conducted the hydroboration of 3,3,4,4,5,5,6,6,6-nonafluoro-1-hexene (**2**) and 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-1-octene (**3**) with Cl_2BH and $\text{Cl}_2\text{BH} \cdot \text{SMe}_2$. The results of hydroboration were similar, except that the product ratio from the hydroboration with $\text{Cl}_2\text{BH} \cdot \text{SMe}_2$ showed improved regioselectivity as compared to **1**, providing the secondary and primary alcohols in a ratio of 84:16. Cl_2BH achieved essentially quantitative regioselectivity in providing the secondary alcohol [Eq. (5)]. The product alcohols were isolated in 72–86 % yields from these olefins.

Subsequently, we studied the hydroboration of a representative perfluoroarylethylene, 2',3',4',5',6'-pentafluorostyrene (**4**). It has been reported that hydroboration of styrene with $\text{BH}_3 \cdot \text{THF}$ provides a 4:1 mixture of primary and secondary alcohols.^[2] The reaction of **4** with $\text{BH}_3 \cdot \text{THF}$ at room temperature was complete within five hours, and the ^{11}B NMR spectrum revealed a 4:1 ratio of mono- and dihydroborated products. Oxidation of this mixture provided the secondary and primary alcohols in a 4:1 ratio, which could be isolated in 66 % yield. On the contrary, Cl_2BH reacted instantaneously to provide, after oxidation, a 92:8 mixture of the secondary and primary alcohols in 90 % [Eq. (6)].



The hydroboration of **1–4** with Br_2BH provided results comparable to those achieved with Cl_2BH . The data are summarized in Table 1.

In conclusion, we have studied the hydroboration of a series of perfluoroalkylethylenes and 2',3',4',5',6'-pentafluorostyrene with several hydroboration agents. Cl_2BH and Br_2BH hydroborate these olefins with an unprecedented Markovnikov regioselectivity of 92 % or higher. The utilization of these fluoroalkylboranes for the synthesis of representative fluoro-organic derivatives is in progress.

Table 1. Hydroboration of perfluoroalkyl(aryl)ethylenes $\text{R}_\text{F}\text{CH}=\text{CH}_2$ with representative hydroborating agents at room temperature

Olefin	R_F	Reagent	Solvent	Reaction time [h] ^[a]	Product alcohol yield [%] ^[b]	sec:pri ^[c]
1	CF_3	$\text{BH}_3 \cdot \text{SMe}_2$	Et_2O	3	79 ^[d]	70:30
1	CF_3	$\text{BH}_3 \cdot \text{THF}$	THF	4	82 ^[d]	71:29
1	CF_3	$\text{BH}_2\text{Cl} \cdot \text{SMe}_2$	Et_2O	24	80 ^[d]	59:41
1	CF_3	$\text{BHCl}_2 \cdot \text{SMe}_2$	hexane	inst.	81 ^[d]	98:2
1	CF_3	BHCl_2	hexane	inst.	81 ^[d]	98:2
1	CF_3	BHBr_2	hexane	inst.	85 ^[d]	97:3
2	$n\text{-C}_4\text{F}_9$	$\text{BH}_3 \cdot \text{SMe}_2$	Et_2O	3	71	84:16
2	$n\text{-C}_4\text{F}_9$	$\text{BH}_3 \cdot \text{THF}$	THF	4	73	85:15
2	$n\text{-C}_4\text{F}_9$	$\text{BH}_2\text{Cl} \cdot \text{SMe}_2$	Et_2O	24	68	84:16
2	$n\text{-C}_4\text{F}_9$	$\text{BHCl}_2 \cdot \text{SMe}_2$	hexane	inst.	75	99:1
2	$n\text{-C}_4\text{F}_9$	BHCl_2	hexane	inst.	85	≥ 99 ^[e]
2	$n\text{-C}_4\text{F}_9$	BHBr_2	hexane	inst.	86	99:1
2	$n\text{-C}_4\text{F}_9$	9-BBN	THF	very slow reaction		
2	$n\text{-C}_4\text{F}_9$	CB	THF	no perceivable reaction		
3	$n\text{-C}_6\text{F}_{13}$	$\text{BH}_3 \cdot \text{SMe}_2$	Et_2O	3	72	80:20
3	$n\text{-C}_6\text{F}_{13}$	$\text{BH}_3 \cdot \text{THF}$	THF	4	74	83:17
3	$n\text{-C}_6\text{F}_{13}$	$\text{BH}_2\text{Cl} \cdot \text{SMe}_2$	Et_2O	24	74	84:16
3	$n\text{-C}_6\text{F}_{13}$	$\text{BHCl}_2 \cdot \text{SMe}_2$	hexane	inst.	77	≥ 99 ^[e]
3	$n\text{-C}_6\text{F}_{13}$	BHCl_2	hexane	inst.	77	≥ 99:1 ^[e]
3	$n\text{-C}_6\text{F}_{13}$	BHBr_2	hexane	inst.	80	99:1
4	C_6F_5	$\text{BH}_3 \cdot \text{THF}$	THF	5	66	79:21
4	C_6F_5	$\text{BHCl}_2 \cdot \text{SMe}_2$	hexane	inst.	90	92:8
4	C_6F_5	BHBr_2	hexane	inst.	90	94:6

[a] inst. = instantaneous. [b] Yields of isolated product unless otherwise stated. [c] Determined by GC analysis. [d] Yields determined by GC analysis. [e] No primary alcohol was detected.

Experimental Section

All operations were carried out under an inert atmosphere.^[9] A typical experimental procedure for the hydroboration of **3** with Cl_2BH is as follows. Trimethylsilane (1.48 g, 20 mmol) was added to a solution of BCl_3 (20 mL, 1.0 M in hexanes, 20 mmol) and **3** (6.92 g, 20.0 mmol), precooled to -78°C . The hydroboration was completed essentially instantaneously as shown by the ^{11}B NMR spectrum ($\delta = 60.4$). The solution was brought to room temperature, treated with water (5 mL), and neutralized with saturated Na_2CO_3 . The volatile components were removed under vacuum, and the residue was dissolved in diethyl ether and oxidized with NaOH (20.0 mmol) and H_2O_2 (20.0 mmol). The crude product was analyzed by GC using a Carbowax 20 column. No primary alcohol was detected. Distillation ($76^\circ\text{C}/25$ Torr) provided 5.61 g (77 %) of 3,3,4,4,5,5,6,6,6-nonafluoro-2-hexanol.

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Ta₄BTe₈: Tantalum Telluride Cluster Chains with Encapsulated Boron Atoms**

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Clusters of the types $M_6X_{12}^{n+}$ and $M'_8X_8^{m+}$ have been known for many years for Group 5 and 6 transition elements.^[1, 2] For the more common M_6X_{12} type, various binary and ternary niobium and tantalum compounds have been made that contain $M_6X_{12}^{2+/3+}$ clusters along with different halide anions.^[3, 4] The electronic rules for the stability of these cluster compounds are mainly dictated by the (idealized) cubic symmetry and the connectivity within the cluster cores. The $M_6X_{12}^{n+}$ cluster phases are generally stable for electron counts between 14 and 16;^[5] typical electron counts for M_6X_8 clusters range between 19 and 24 with an upper limit of 24.^[6] Given the generality that something close to these "magic" electron counts is required for cluster stability, it is clear that the M_6X_8 type is preferred for Group 6 chalcogenides, as observed in the Chevrel phases,^[7] whereas the M_6X_{12} type is adopted by Group 5 halides such as Ta₆Cl₁₅.^[8]

Analogous clusters of the Group 3 and 4 elements will be seriously electron deficient, but this shortage may be compensated for by encapsulating heteroatoms in the center of the cluster where the heteroatoms donate their electrons to the (otherwise) empty cluster orbitals.^[9] Alternatively, to electronically saturate electron-deficient cluster species, counterreduction of the clusters may be achieved by the inclusion of cations into the cluster network.^[10] The knowledge of the electronic and structural principles has lead to the discovery of an enormous variety of centered early transition metal halide clusters during the past decade.^[11]

So far, a corresponding chalcogenide interstitial cluster chemistry does not exist. McCarley^[12] as well as Simon and Köhler^[13] developed a systematic chemistry of the "reduced" niobates and molybdates such as NaMo₄O₆.^[14] The structures of these materials are based on isolated and fused M_6O_{12} units

whose inherent electron deficiency is compensated for by charge-balancing cations. In contrast, the structures of early transition metal sulfide, selenide, or telluride clusters are almost exclusively based on Mo₆Q₈ cluster units. Alternative cluster topologies are infinite chains of interpenetrating icosahedral^[15] or face-sharing square-antiprismatic units;^[16] Ta₂S₂C, which contains layers of edge-sharing C-centered Ta₆ octahedra, is a notable exception.^[17]

Here we report on the synthesis, structure, and properties of an unusual centered M_6X_{12} chalcogenide cluster phase. From structural chemistry it is well known that size effects and radius ratios control the structure of materials to a major extent. Similarly, the size of the heteroelement determines the structure of compounds in the system M-A-Te (M = Nb, Ta; A = Group 13 element). In attempts to replace gallium (covalent radius $r_{\text{cov}} = 1.26 \text{ \AA}$) in the ternary phase Ta₁₃Ga₃Te₂₄^[18] by the isoelectronic, but much smaller, group homologue boron ($r_{\text{cov}} = 0.88 \text{ \AA}$) leads to the formation of the novel cluster compound Ta₄BTe₈ with an interstitial boron atom.^[19]

The structure of Ta₄BTe₈ is shown in Figure 1.^[20] Ta₄BTe₈ crystallizes in the space group *Pbam* with two formula units per unit cell, and all atoms are situated on mirror planes perpendicular to *c* at *z* = 0 and $\frac{1}{2}$. The structure consists of

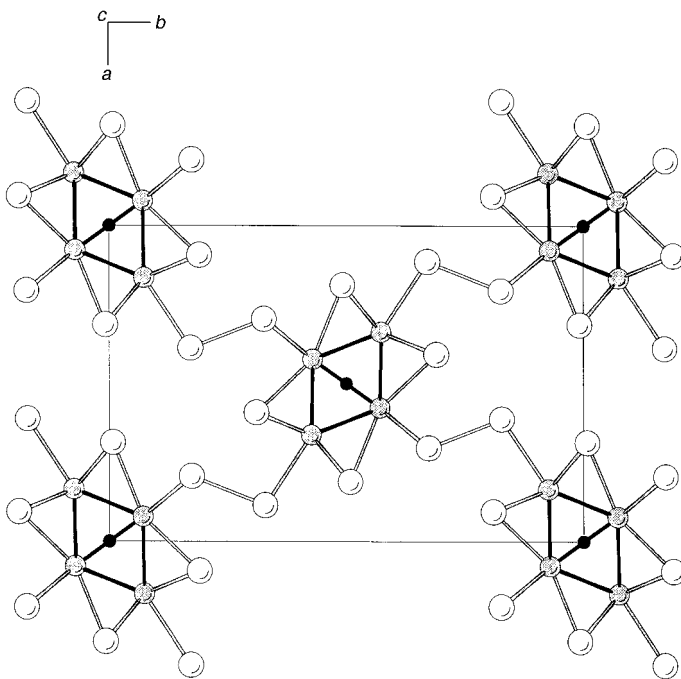


Figure 1. View of the Ta₄BTe₈ structure along *c* (Te: white open spheres, Ta: gray spheres, B: black spheres).

Ta₆BTe₁₂ clusters, whose twofold axis is along the viewing direction and which contain a B atom in the center. The individual clusters are condensed by sharing of common edges. As a result, linear chains of centered edge-sharing Ta₆-octahedra are obtained which are coordinated by Te atoms above all free edges and exhibit the connectivity $\infty^1[\text{Ta}_{1/2}\text{Ta}_{2/2}(\text{B})\text{Te}_{1/4/2}\text{Te}_{2/4/2}\text{Te}_{3/2}\text{Te}_{4/4/2}]$, according to the nomenclature of Schäfer and von Schnering.^[2] Figure 2 shows in

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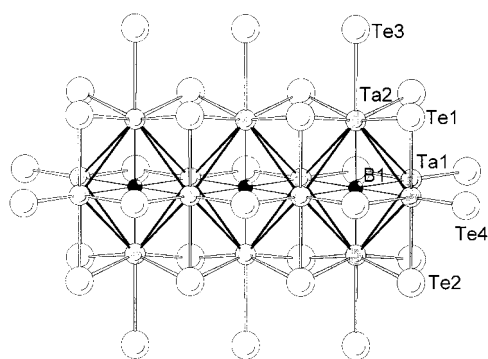


Figure 2. Portion of one infinite cluster chain in the structure of Ta_4BTe_8 with the atomic labeling scheme. Selected distances [Å]: Ta1–B1 2.309(2), Ta2–B1 2.198(3), Ta1–Ta1 2.938(7), Ta1–Ta1 3.564(1), Ta1–Ta2 3.174(4), Ta1–Ta2 3.203(4), Ta2–Ta2 3.564(1), Te1–Ta1 2.803(6), Ta1–Te2 2.735(6), Ta1–Te4 2.848(5), Ta2–Te1 2.783(5), Ta2–Te2 2.752(5), Ta2–Te3 2.939(6), Te3–Te4 2.915(3).

a side view of a portion of one infinite cluster chain of the composition $(\text{Ta}_2)(\text{Ta}_{4/2})\text{B}(\text{Te}_2)_{4/2}(\text{Te})_4 = \text{Ta}_4\text{BTe}_8$. The four crystallographically distinct Te atoms in Figure 2 have three different functions: Four Te1 and Te2 atoms bridge the top and bottom edges of the cluster, two Te4 atoms bridge the equatorial plane, and the Te3 atoms, which are not shared by adjacent clusters of the chain, provide links to parallel but rotated chains ($d_{\text{Te-Te}} = 2.786(8)$ Å) according to $\text{Ta}_4\text{B}(\text{Te}^i)_4(\text{Te}^{i-a})_2(\text{Te}^a)_2$. Thus, the structure is three-dimensional, but highly anisotropic, and the crystals extremely fibrous.

A structural description of Ta_4BTe_8 is based on the hierarchy of interactions. The Ta atoms are surrounded in a pseudooctahedral fashion by Te and B atoms. Ta2, which is situated at the apical position of the Ta_6 octahedra, is surrounded by five Te atoms, and Ta1 at the equatorial plane of the octahedra is surrounded by four Te atoms. The octahedral coordination of Ta2 is completed by one, the coordination environment of Ta1 by two Ta–B contacts ($d_{\text{Ta1-B}} = 2.309(2)$ Å ($2 \times$), $d_{\text{Ta2-B}} = 2.198(3)$ Å). The equivalence of the two symmetry-independent Te atoms is apparent from the Ta–Te bond lengths, but may also be seen from the computed Ta–Te overlap populations (see below). The average Ta1–Te distance is 2.809 Å and Ta(2)–Te distance 2.802 Å, in accordance with the structures of related compounds.^[21]

The presence of the interstitial boron atom is reflected in the metal–metal distances. Interstitial atoms expand the cluster; thus the average Ta–Ta distance is significantly longer than typical Ta–Ta distances of 2.9–3.0 Å in tantalum halide $[\text{M}_6\text{X}_{12}]^{n+}$ clusters.^[2] The idealized octahedra from which these chains are constructed are characteristically distorted. The Ta–Ta distances at the shared edge ($d_{\text{Ta1-Ta1}} = 2.938(7)$ Å) between two adjacent Ta_6 octahedra are comparable to the Ta–Ta distances in Ta metal ($d_{\text{Ta-Ta}} = 2.92$ Å). The van der Waals repulsion between the Te atoms along the cluster chains ($d_{\text{Te-Te}} = 3.564(3)$ Å) leads to a strong elongation of the Ta_6 octahedra along the chain direction *c*, whereas the average Ta–Ta distance between the apical and equatorial positions of the octahedron is 3.189 Å. Furthermore, the presence of two distinct Ta–B separations may be related to the distortion of the Ta_6 clusters. Ta–B distances correspond to the average

M–B distances in B-centered Zr_6 clusters (e.g. $d_{\text{Zr-B}} = 2.304$ Å in $\text{K}_2\text{Zr}_6\text{BCl}_{15}$),^[22] but they are considerably shorter than typical Ta–B distances in binary borides (e.g. 2.45 Å in Ta_2B).^[23] Thus, many structural features of the $[\text{Ta}_6\text{BTe}_{12}]$ species match those of related clusters among the rare earth halides. Remarkably, chalcogenide cluster compounds containing interstitial atoms are extremely scarce; $\text{Ta}_2\text{S}_2\text{C}$,^[17] $\text{M}_{4+x}\text{ATe}_4$ (M = Nb, Ta; A = Ga, Si, Cr, Fe, Co),^[16] and $\text{Nb}_4\text{Te}_3\text{OI}_4$ ^[24] are the only known examples so far.

Chains of related construction have been found in $\text{Sc}_4\text{Cl}_6\text{Z}$ (Z = B, N)^[25] and NaMo_4O_6 .^[14] The principal difference is that in the structure of $\text{Sc}_4\text{Cl}_6\text{Z}$ and NaMo_4O_6 the chains are interconnected by isolated chlorine and oxygen atoms, respectively (instead of Te_2^{2-} groups in the case of Ta_4BTe_8), and sodium atoms are located in the cavities between four chains in NaMo_4O_6 .

It is difficult to describe the electronic structure of Ta_4BTe_8 in simple valence terms. Formal electron counting leads to the formulation $[(\text{Ta}^{2.25+})_4(\text{B}^{3+})(\text{Te}^{2-})_4(\text{Te}_2^{2-})_2]$. According to a theoretical analysis for NaMo_4O_6 , the optimum number of M–M bonding electrons is 13^[14b] (compared to 11 for Ta_4BTe_8). Bond order summations using Pauling's formula^[26] do not lead to conclusive results. We have performed band-structure calculations using the extended Hückel method^[27] to obtain a better understanding of the electronic structure. The density of states (Figure 3) is situated at a small local

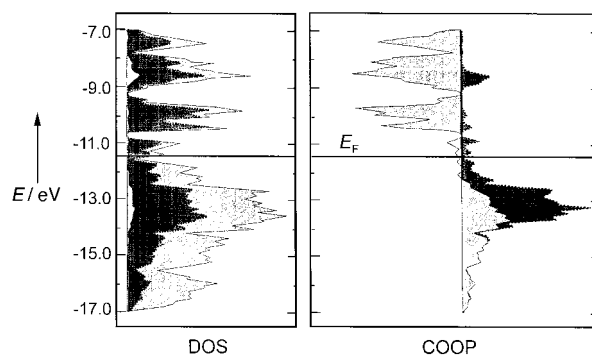


Figure 3. Left: Calculated density of states diagram for Ta_4BTe_8 (light gray: total density, dark gray: Ta contribution, white: B contribution). Right: Orbital population analysis of Ta_4BTe_8 (COOP curves; light gray: Ta–Ta, dark dark: Ta–B). The Fermi level is marked by a horizontal bar.

maximum. The projections of the Ta and B contributions in Figure 3 show that the energy states below –15 eV are mainly localized on the Te atoms, whereas the states in the energy window between –15 and –11.5 eV have Ta 5d and Te 5p character with some B 2p admixture. An indication for the highly covalent interactions is the strong mixing of Ta- and Te-centered states in this energy interval. The orbital population analysis^[6] (Figure 3) revealed that for Ta_4BTe_8 all strongly Ta–B and Ta–Ta bonding states are occupied, whereas the Ta–Te and Te–Te antibonding states are beginning to be filled. These results are compatible with the picture that, as a consequence of short Te–Te interactions along the chain direction, a fraction of the “anion” states is raised above the Fermi level; therefore, the electron deficiency of Ta_4BTe_8 is less than expected from a comparison with the related oxide

NaMo₄O₆. Conductivity studies reveal, in agreement with the computational results, metallic properties for the title compound.

Four-probe single-crystal conductivity measurements along the needle axis *c* show that Ta₄BTe₈ exhibits metallic behavior over the temperature range 4–250 K, with a room-temperature conductivity of 2 mΩ⁻¹cm⁻¹ (Figure 4). No indications of a metal–insulator transition were observed. The electrical conductivity is indicative of a material with a partially filled band at the Fermi level.

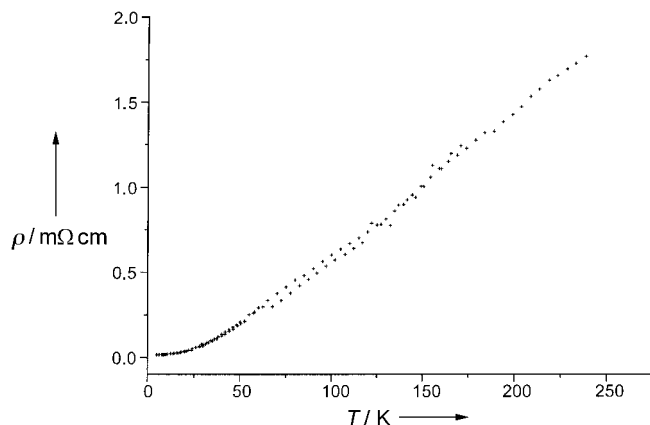


Figure 4. Plot of conductivity versus temperature for a single crystal of Ta₄BTe₈ (measured along the needle axis *c*).

Ta₄BTe₈ provides a conceptual link between the condensed, empty cluster systems encountered among the oxoniobates and oxomolybdates and the condensed rare earth and zirconium halide clusters with interstitial atoms. In the former class of compounds, matrix effects^[21] (i.e., the size of the metal oxide clusters) prevents interstitial atoms from being encapsulated in the cluster; the electron deficiency of the cluster is compensated for by counteranions in intercluster cavities. On the other hand, the cluster halides of Group 3 and 4 transition metals can “optimize” the cluster electron count by a broad variety of interstitial atoms and additional counteranions. Matrix effects do not prevent Group 5 tellurides—formally isoelectronic to the oxoniobates—from balancing their electron deficit by the formation of centered clusters. The smooth encapsulation of boron in Ta₄BTe₈ may be due to thermodynamic (competing boron telluride phases are not stable), structural (cluster proportions optimal for the bonding of boron interstitials), or electronic factors (electron count).

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- [20] Data were collected on a Syntex P2₁ diffractometer using monochromated MoK_α radiation (λ = 0.71073 Å) and variable scan speeds of 1.9–19.3° min⁻¹. Crystal size 0.05 × 0.03 × 0.18 mm³; θ–2θ scan, 2θ_{max} = 54°, 1719 reflections, empirical absorption correction (ψ scan), structure solved and refined using the SHELXTL program system, 850 independent reflections, 419 with *F* > 6σ(*F*_o), *R*(*F*_o)/*R*_w(*F*_o) = 0.081/0.067. The B atom was located as the most prominent peak from a difference Fourier synthesis after the Ta₄B₈ cluster portion of the structure had been refined. Subsequent refinements, where the *U* factor of the B atom was fixed at 0.01 and the occupancy factor was allowed to refine, indicated a full occupancy of the interstitial atom position. Further details on the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49)7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository number CSD-410843.
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Template-Mediated Synthesis of a Polymeric Receptor Specific to Amino Acid Sequences**

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Francis Mulholland, and Evgeny N. Vulfson*

The idea of template-mediated assembly in biological systems was first introduced by Linus Pauling to explain the working of the immune system.^[1] We now know this elegant hypothesis to be incorrect but the principle of using a target molecule to create its own recognition site was fruitfully exploited by chemists in the preparation of artificial polymeric receptors by molecular imprinting.^[2] The resulting materials have been shown to display specificities similar to polyclonal antibodies^[3,4] and bind ligands with a single dissociation constant.^[4,5] However, it is the recognition of oligonucleotide, -saccharide, or -peptide sequences that is often seen as the real test of the ability of “plastic antibodies” to compete with their natural counterparts. Herein we will address the latter problem by describing the preparation of imprinted polymers specific for amino acid sequences.

Several considerations were taken into account when designing a suitable oligopeptide template^[6] for the purpose of this study. Firstly, it was preferable to have a relatively short target molecule such that it could be synthesized in the required quantities by conventional solution-state chemistry. A relatively small target peptide was also preferred as a consequence of the necessity for chemical modification, recovery, and characterization of the template. Secondly, a highly functionalized oligopeptide would enable us to probe the contribution of individual interactions/functional groups by substitutions in the amino acid sequence. At the same time, a net charge of zero would be advantageous to eliminate possible artefacts that arise from nonspecific “ion-exchange type” interactions between the oligopeptide and receptor. Finally, the incorporation of an aromatic amino acid residue in the sequence should facilitate the analysis. Thus, a sequence containing lysine (Lys) and aspartic acid (Asp) in the terminal

positions with a bulky tryptophan (Trp) residue in the middle was selected as satisfying all of the above requirements.

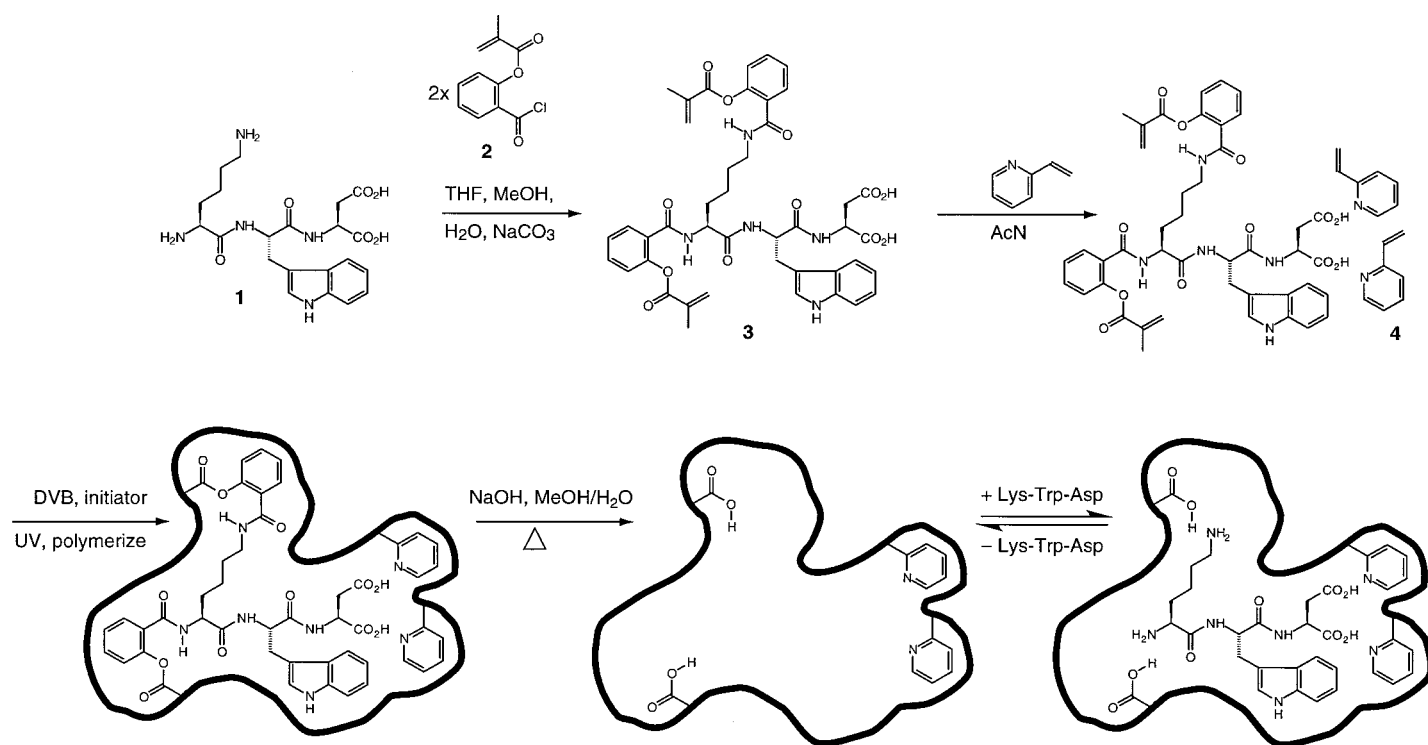
The peptide Lys-Trp-Asp (**1**) was preferred to Asp-Trp-Lys for purely synthetic reasons, and the target **1** was prepared in seven steps in 13 % overall yield. The synthesis was accomplished by using conventional activated-ester coupling from suitably protected amino acid precursors. A portion of the tripeptide product was then further modified to prepare the template. Carboxylic acids were the natural choice of functionality to interact with amino groups of the lysine residue in the polymer's binding sites. Hence, the tripeptide **1** was treated with 2-methacryloyloxybenzoyl chloride^[7] (**2**) to obtain the template **3**. The key feature of this approach is the presence of a relatively labile ester bond between the methacrylic acid residue and the hydroxybenzamide moiety, the cleavage of which would leave the carboxy group in the precise spatial arrangement to interact with the target tripeptide as well as a small “void” in the polymer to facilitate the template removal and re-binding of **1** (Scheme 1).

This simple approach also enabled us to position carboxy groups exclusively in the recognition sites, thus minimizing nonspecific interactions between ligands and the polymeric matrix itself.^[8] The carboxylic acid groups of the template **3** were targeted by noncovalent complexation with 2-vinylpyridine^[9] (**4**), as depicted in Scheme 1. In order to achieve a reasonable degree of complexation **4** was used in a fourfold molar excess over the template **3**, and the polymerization reaction was carried out in acetonitrile. The choice of solvent was influenced by template solubility and the necessity to stabilize hydrogen-bonding interactions that were essential for the successful imprinting of the tripeptide template. Not only was this important to promote the formation of the complex between the template **3** and monomers **4**, but also to restrict the conformation of the *o*-hydroxybenzamide spacer groups by intramolecular hydrogen bonds. The latter should help to position the methacrylic acid residue precisely in the polymer binding site.^[10] Divinylbenzene was selected as the cross-linker to ensure that no degradation of the polymer matrix would occur under the hydrolytic conditions used for template removal. The polymers were prepared photochemically at 4 °C (see the experimental section). Nonimprinted polymer was synthesized under exactly the same conditions, the template being replaced by two equivalents of methacrylic acid, to ensure that the final chemical composition of the polymers were identical.

Once prepared, the polymers were tested with solutions of **1** in aqueous acetonitrile to evaluate their binding properties. Specific binding was seen at all ratios of aqueous/organic solvent (inset in Figure 1) but the binding of **1** to the polymer dropped significantly from 47 % in acetonitrile/water 4:1 to 28 % in pure water, where the tripeptide was noticeably more soluble. Binding studies were therefore made at 80 % acetonitrile where high specific binding and a reasonable range of ligand concentration could be used. Isotherms were then determined for two polymer concentrations (15 and 90 mg mL⁻¹; Figure 1). It is evident from these data that the imprinted polymer shows considerable binding of the peptide, even at concentrations as low as 0.02 mM, while the non-imprinted and the unhydrolysed imprinted polymer (not

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Scheme 1. Covalent modification of Lys-Trp-Asp with 2-methacryloyloxybenzoyl chloride and preparation of the imprinted polymer.

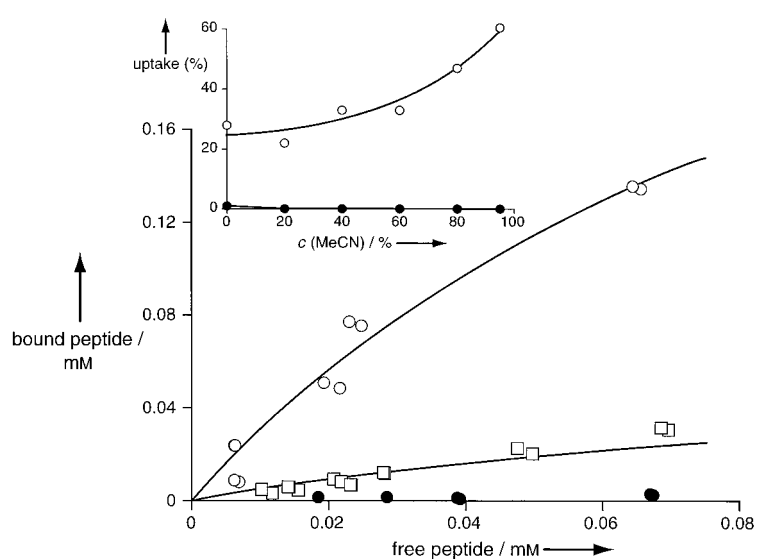


Figure 1. Polymer binding characteristics in acetonitrile/water mixtures. Main figure: Isotherms for the binding of Lys-Trp-Asp to imprinted (15 and 90 mg mL⁻¹, □ and ○, respectively) and nonimprinted (15 mg mL⁻¹, ●) polymers in acetonitrile/water 4/1. Fitted curves are calculated using the following parameters: $K_d = 113 \mu\text{M}$ and capacity = $4.1 \mu\text{mol g}^{-1}$. Inset: Dependence of binding on solvent composition in acetonitrile/water mixtures for Lys-Trp-Asp (0.1 mM) incubated with imprinted (○) and nonimprinted (●) polymer at 90 mg mL⁻¹. (Binding experiments were carried out as described in the experimental section.)

shown) have negligible affinity for **1**. In fact nonimprinted polymer showed a slightly “negative uptake” at 90 mg mL⁻¹, which was attributed to polymer swelling in the incubation mixture.^[5] Binding parameters, calculated by curve-fitting the isotherm data, were determined as $K_d = 113 \pm 47 \mu\text{M}$ at a capacity of $4.1 \pm 1.2 \mu\text{mol g}^{-1}$.

We then proceeded to probe the polymer selectivity with structural analogues of the tripeptide **1**, which were prepared by conventional solid-state peptide synthesis, purified by HPLC, and characterized by high resolution mass spectrometry. Initially, the three tripeptides **5–7** were designed with the N-terminus lysine exchanged for arginine, leucine and glutamine (Table 1). The binding of these tripeptides relative to **1** would provide information on the importance (and relative contribution) of the two carboxy groups that were introduced in the recognition site by the covalent method. Leucine and glutamine were chosen because leucine has a non-functionalized side chain of similar overall size to lysine and the γ -amide group of glutamine is capable of hydrogen bonding with carboxylic acids. Binding

Table 1. Sequence-specific binding of peptides to the Lys-Trp-Asp-imprinted polymer.

Peptide	Sequence	Percentage bound ^[a]	
		imprinted polymer	nonimprinted polymer
1	Lys-Trp-Asp	43 %	11 %
5	Arg-Trp-Asp	24 %	9 %
6	Leu-Trp-Asp	< 2 %	6 %
7	Gln-Trp-Asp	< 2 %	< 2 %
8	Lys-Phe-Asp	4 %	< 2 %
9	Lys-Trp-Glu	5 %	3 %
10	Lys-Trp ^[b]	35 %	17 %
11	Lys-Phe ^[c]	9 %	5 %
12	Lys-Val ^[c]	15 %	4 %

[a] Peptides (1 mM in acetonitrile/water 4:1) were incubated with polymer (30 mg mL⁻¹) as described in the experimental section. [b] Obtained from Bachem. [c] Obtained from Sigma.

studies were carried out at tripeptide concentrations of 1 mM as all the ligands were soluble in 80% acetonitrile at this concentration.

It is evident from the results presented in Table 1 that the replacement of lysine with arginine led to a significant decrease in binding from 43 to 24%, although the polymer still recognized the latter tripeptide reasonably well. This is not too dissimilar to the specificity of enzymes and antibodies, which often do not discriminate well between sequences containing these two amino acids. However, when the polymer was challenged with leucine- and glutamine-containing peptides, **6** and **7**, respectively, with the aim of eliminating the interaction between one of the carboxy groups and the side chain amino group of lysine, the binding was negligible. This result confirmed that the tight binding of **1** to the imprinted polymer was a result, at least in part, of the cooperative interaction between the lysine amino groups and the two carboxylic acids in the recognition sites. Ligands capable of participating in only one of these interactions exhibited a significantly lower affinity to the polymer.

We then replaced the tryptophan residue with phenylalanine ($\rightarrow$ **8**), and, once again, the binding was dramatically reduced (Table 1). The amplitude of the effect was somewhat surprising however, as the central amino acid residue was not expected to be strongly involved in any of the functional group interactions and hence a high degree of selectivity at the second position was not predicted. Perhaps the smaller phenylalanine residue of **8** cannot adopt a suitable conformation to allow it to occupy the "space" created by the apparently much bulkier side chain of **1**. As the indole group of tryptophan is connected through the pyrrole ring, it is possible that the void created in the polymer simply cannot accommodate a phenyl group. Although such a "tight fit" seems unlikely, it would explain the observed selectivity. In this case an interaction between the indole N proton and vinylpyridine could be responsible. Such interactions have been reported between 4-vinylpyridine and indole templates in the preparation of imprinted polymer catalysts for the benzisoxazole isomerization reaction.^[11]

Substitution of the C-terminus aspartic acid with the structurally very similar glutamic acid ($\rightarrow$ **9**) also resulted in dramatically reduced binding. Two explanations can be advanced to account for the difference in binding between the template **1** and the glutamic acid containing tripeptide **9**. Firstly, the poor binding of **9** could simply arise from the loss of the hydrogen bond between the side chain carboxyl group and the corresponding polymer-bound pyridine residue.^[12] Alternatively some other more subtle effects may be at play. For example, the binding of the slightly larger glutamate residue in the aspartate binding pocket could prevent the lysine or tryptophan residues (or both) occupying their parts of the imprint site because the peptide as a whole cannot adopt a conformation that satisfies all of the binding interactions. In order to distinguish between these possibilities the polymer was tested with the dipeptide Lys-Trp (**10**). We reasoned that if the loss of affinity occurred primarily because of the disruption of hydrogen bonding **10** should be as poor a ligand for the polymer as **9**. However, if the overall size and/or conformation of the glutamic acid residue was responsible for

the reduced binding of **9** then the dipeptide should bind noticeably better than **9**, but not as well as **1**. It was found that uptake of Lys-Trp (**10**) was only slightly weaker than that of **1**. This observation confirmed that complexation by vinylpyridine groups contributed a significant but somewhat lesser part of the overall energy of binding and highlights the advantage of the covalent "sacrificial spacer" method.

Finally, we determined the uptake of two more dipeptides, Lys-Phe (**11**) and Lys-Val (**12**), which were chosen to probe the fit of the polymer binding site in the "hydrophobic pocket". In accordance with our previous result the replacement of tryptophan with phenylalanine led to a significant decrease in binding, however the uptake increased slightly in the case of **12**, presumably as the side chain of valine is less sterically demanding than that of phenylalanine and therefore **12** fits somewhat loosely in the site.

In conclusion, we have shown the feasibility of preparing polymeric materials that can discriminate between very similar amino acid sequences with a specificity approaching that of antibodies and other biological receptors. Polymers of this type are expected to find applications in affinity separations for the purification of biologically active oligopeptides from natural sources or as sensing elements in biomedical devices.^[13]

Experimental Section

Template synthesis: Lys-Trp-Asp-2CF₃COOH · H₂O (520 mg, 0.75 mmol) was dissolved in aqueous methanol (15 mL, 30%) containing Na₂CO₃ (200 mg) and cooled to 0 °C. The aqueous mixture was added to a solution of 2-methacryloyloxybenzoyl chloride (505 mg, 2.25 mmol) in THF (15 mL), also at 0 °C. The reaction mixture was stirred at room temperature overnight. The organic solvent was removed by rotary evaporation, and HCl (0.1 N) was added to precipitate the product. The solid was collected and dissolved in ethyl acetate and the solution combined with an ethyl acetate extract of the filtrate. The combined organic extracts were washed with water (2 ×), HCl (0.1 N, 3 ×), and water (3 ×), dried over Na₂SO₄, and evaporated. The crude product was purified by preparative HPLC to give the pure product in 49% yield.

Polymer synthesis: Template (116.4 mg), 2-vinylpyridine (74.3 mg), divinylbenzene (technical grade, 80%, 809.3 mg) and azobiscyclohexanecarbonitrile (29.8 mg) were dissolved in acetonitrile (2 mL) in a test tube fitted with a stopcock. The polymerization mixture was degassed by a series of three freeze–thaw cycles and polymerized photochemically for 48 h at 4 °C with 366 nm radiation. The polymer was ground to an average particle size of 30 µm using a mechanical mortar and the template removed by treatment with refluxing NaOH (7.5 N) in methanol (template removal 58%, by amino acid analysis). The control polymer was prepared in exactly the same manner, except that the template was replaced by methacrylic acid (27.4 mg).

Binding experiments: The polymer (10 mg) was shaken overnight at room temperature with peptide solution (1 mM acetonitrile/water (4/1), 0.333 mL). The solutions were filtered through a 13 mm diameter, 0.2 µm PTFE membrane syringe filter (HPLC Technology, Macclesfield, UK) fitted to a disposable syringe before analysis by HPLC (LiChrospher C18 column (Merck), isocratic elution with 20% methanol and UV detection).

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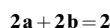
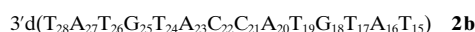
Keywords: imprinting • molecular recognition • peptides • polymers • receptors

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A New Platinum Anticancer Drug Forms a Highly Stereoselective Adduct with Duplex DNA**

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Much attention is currently focused on the design of new generations of platinum anticancer complexes that circumvent cisplatin resistance. Such resistance often involves the recognition of platinated DNA adducts by proteins and enzymes in the excision–repair systems in cells.^[1] It is therefore vital to understand how ligand design influences the nature of platinated DNA lesions. A complex with high activity against cisplatin-resistant cell lines is the 2-picoline (2-Pic, 2-methylpyridine) complex *cis*-[PtCl₂(NH₃)(2-Pic)] (**1**, ZD0473), a new anticancer Pt^{II} drug currently in phase I clinical trials.^[2, 3] The steric effect of 2-picoline plays an important role in determining the rates of hydrolysis and substitution reactions with the nucleotide guanosine-5'-monophosphate (5'-GMP).^[4, 5] As a result of slow rotation about the Pt–N(2-Pic) bond (0.62 s^{–1}, 296 K), together with the non-C₂-symmetrical structure of complex **1**, four isomers of the bis(GMP) adduct are possible, and these are formed in equal amounts.^[5] We show here, in dramatic contrast, that reactions of **1** with the 14mer DNA duplex **2** give predominantly a single stereoisomer, whereas relatively little stereoselectivity is observed for reactions with the deoxydinucleotide d(GpG) or the 14-mer single strand **2a**. The structural basis for this unusually high stereoselectivity has been revealed by NMR studies.



Reactions of complex **1** with both d(GpG) and 14-mer single strand **2a** at a 1:1 molar ratio (1 mM, pH 6.0, 9 mM phosphate, 100 mM NaClO₄, H₂O/D₂O 90/10, 298 K) were followed by 1D ¹H and 2D [¹H, ¹⁵N] HSQC NMR spectro-

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scopy. In each case the GG chelate gave rise to four cross peaks of similar intensities, which correspond to similar populations of the four possible stereoisomers: 2-picoline orientated towards 5'-G or 3'-G, and slow rotation about the Pt–N(2-Pic) bond.^[6] When the temperature was increased, the four $^1\text{H}/^{15}\text{N}$ cross peaks for the d(G*pG*) adducts began to merge into two, as observed for the bis(GMP) adducts.^[5]

The reaction of complex **1** with duplex **2** at a 1:1 molar ratio was studied under the same conditions, and followed in the same way. After one week one major cross peak was observed (Figure 1, $\delta = 4.51/-67.46$), which accounted for 90 % of the

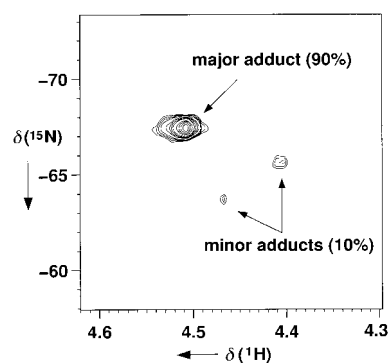


Figure 1. 2D [$^1\text{H},^{15}\text{N}$] HSQC NMR spectrum recorded after one week of reaction of *cis*-[PtCl₂($^{15}\text{NH}_3$)(2-Pic)] (**1**, 1 mol equiv) with the 14-mer DNA duplex **2** (298 K, 100 mM NaClO₄, pH 6.0, 9 mM phosphate buffer, H₂O/D₂O 90/10). The integral percentages of the major peak and the two minor peaks are indicated.

total quantity of platinated adducts. Peaks for the H6 ($\delta = 8.88$) and 2-methyl ($\delta = 3.15$) protons of the 2-picoline ligand of unreacted **1** were replaced by two new signals at $\delta = 9.20$ and 3.19, respectively. A feature of the new 2-picoline signals was their broadness relative to free **1**, as a result of the 2-picoline complex taking on the T_2 relaxation characteristics of a macromolecule by irreversible binding to DNA. New NMR resonances were also observed outside the usual windows expected for both aromatic and methyl protons of DNA. These signals enabled further assignments to be made. We explored the use of NMR methods to determine the structure around the platination site of the major adduct even though the NMR spectra were complicated by the presence of peaks for the minor adducts and free duplex **2**. A ^1H 2D NOESY NMR spectrum (mixing time 200 ms) was acquired for the same sample and compared to that of the cisplatin GG bischelated adduct of the same DNA duplex.^[7] The key ^1H NMR assignments derived from these data are listed in Table 1. They are based on the following observations: a clear strong NOE between G7 H8 and G8 H8 with chemical shifts similar to those observed for the cisplatin–DNA adduct of DNA duplex (Figure 2 A),^[7, 8] an NOE between 2-picoline H6 and G7 H8, a cross peak between a resonance in the usual H2'/H2'' region and a resonance at $\delta = 0.42$ assignable to T6 H2' (which is significantly shifted to low frequency relative to that for DNA platinated by cisplatin, $\delta = 1.37$ for duplex **2**,^[7] and $\delta = 1.40$ for a reported 12-mer duplex^[8]), and a strong cross peak between 2-picoline CH₃ and T6 CH₃, which also showed NOEs to T6 H1' and T6 H2'. Confirmation of the 2-picoline

Table 1. Assignments of key ^1H resonances for the G*G* chelate of duplex **2** with complex **1**.

Assignment (2-picoline)	δ	Assignment (DNA)	δ
H6	9.20	T6 CH ₃	1.16
H5	7.67	T6 H1'	5.82
H4	8.18	T6 H2'	0.43
H3	7.59	T6 H2''	1.91
2-CH ₃	3.19	T6 H3'	4.69
		T6 H4'	4.00
		G7 H8	8.55
		G8 H8	8.18

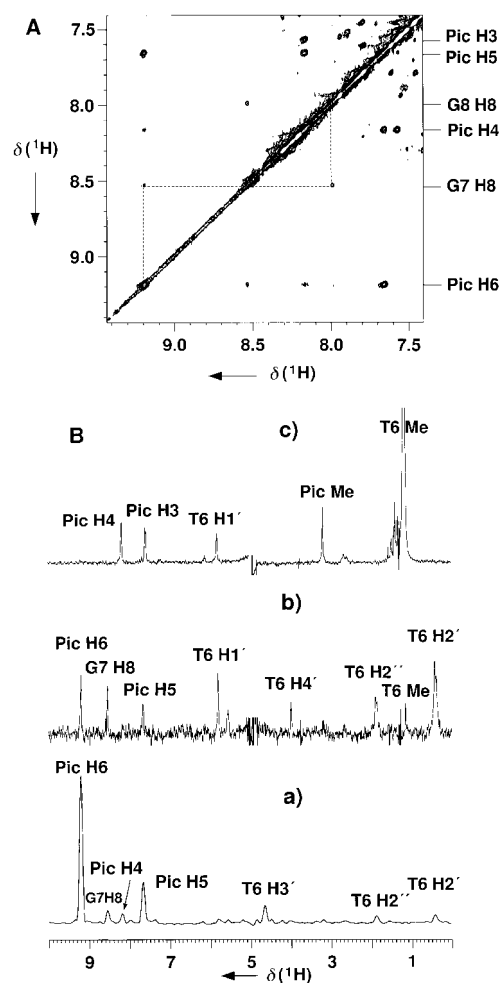


Figure 2. A) Expansion of the aromatic region of the 200 ms 2D NOESY NMR spectrum for the same sample as Figure 1 in 99.9 % D₂O. Assignments are shown on the right-hand side (Pic refers to the 2-methylpyridine ligand), and the dashed line shows the connectivity from Pic-H6 to G7 H8 and G7 H8 to G8 H8; B) Slices through the 2D NOESY spectrum at the chemical shifts of a) 2-picoline H6, b) T6 H2', and c) T6 CH₃, showing NOE connectivities to other protons. Detailed peak assignments and NOE information are listed in Tables 1 and 2, respectively.

ring proton assignments was obtained from a ^1H 2D DQF-COSY NMR spectrum. The assignments of resonances for T6 were further confirmed by the NOE observed between T6 H2' and G7 H8. Figure 2 B shows slices taken through the NOESY data at the chemical shifts of T6 CH₃, T6 H2', and 2-picoline H6. Key assignments of NOEs around the platination site are listed in Table 2.

Table 2. Key NOE signals observed between 2-picoline and DNA protons after platination of DNA duplex **2** with the platinum anticancer drug **1**. The corresponding distances in the four models are listed for comparison. Distances over 5 Å are beyond the limit for NOEs, and are labeled (–).

Observed NOE 1 ... 2	Distances in models [Å]			
	3a	3b	3c	3d
H6...G7 H8	4.45	3.47	5.70(–)	5.15(–)
H6...T6 H3'	4.88	4.53	7.21(–)	7.06(–)
H6...T6 H2''	4.98	4.50	7.08(–)	7.02(–)
H6...T6 H2'	3.33	3.52	5.57(–)	5.88(–)
H5...T6 H2'	3.78	3.85	7.86(–)	8.09(–)
CH ₃ ...T6 CH ₃	8.22(–)	4.03	9.07(–)	5.31(–)
H3...T6 CH ₃	7.26(–)	2.73	9.66(–)	7.35(–)
H4...T6 CH ₃	5.42(–)	4.70	9.02(–)	9.15(–)

Four molecular models of the GG adduct of duplex **2** were built by docking [Pt(NH₃)(2-Pic)]²⁺ onto the G*G* site of the 14-mer DNA duplex **2**. The structure of the latter was based on that of the cisplatin adduct.^[7] The models differed as follows: 2-picoline *trans* to G8 (3'-G) with the 2-methyl group orientated either towards the phosphate backbone (Methyl-Out, **3a**) or towards the centre of the major groove (Methyl-In, **3b**); 2-picoline *trans* to 5'-G also with either Methyl-Out (**3c**) or Methyl-In (**3d**) orientations (See Scheme 1). Distance restraints were applied to each case, based on the data shown in Table 2, and the models minimized in the region of the bischelate site in order to satisfy the data. Of the four models only that with 2-picoline *trans* to 3'-G with the Methyl-In orientation (**3b**) satisfied all of the observed NOE constraints, as shown in Figure 3.

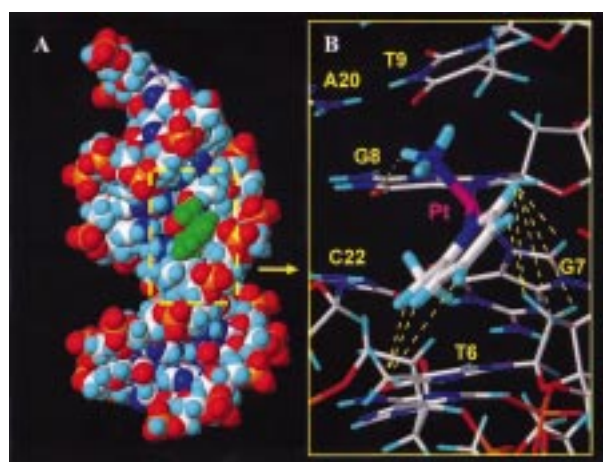


Figure 3. A) Space-filling model of *cis*-[Pt(NH₃)(2-Pic)]-d(ATA-CATG*G*TACATA)·d(TATGTACCATGTAT), with *cis*-[Pt(NH₃)(2-Pic)]²⁺ shown in green. The perfect fit and alignment of the 2-picoline ligand along the major groove can be seen. B) Close-up view of the Pt binding site showing NOE connectivities (yellow dashed lines, listed in Table 2) between 2-picoline and DNA. Hydrogen bonding between Pt-NH₃ and O6 of G8 is shown as a yellow dotted line. Color code: C: white, H: cyan, N: blue, O: red, P: orange, Pt: purple.

The first notable feature of model **3b** is that the picoline ring fits perfectly into the major groove of the DNA duplex and lies along the phosphate backbone, as shown in Figure 3A. Models **3c** and **3d**, in which the 2-picoline ligand is *trans* to 5'-G with Methyl-Out or Methyl-In orientations, have the 2-picoline ring in an almost perpendicular orientation with

respect to the planes of the nearby nucleotide bases. This steric clash causes models **3c** and **3d** to be far less stable than those with 2-picoline *trans* to 3'-G. The other notable feature is the orientation of the 2-picoline ring which lies almost perpendicular to the Pt square plane (angle 100°), as in the crystal structure of **1** (103°).^[4] Model **3b** satisfies several other features of the NMR data. As shown in Table 1 the T6 H2' resonance at $\delta = 0.44$ is significantly shifted to low frequency from its normal position for a G*G* DNA duplex/cisplatin adduct (usually near $\delta = 1.39$). It can be seen in Figure 3B that T6 H2' for molecule **3b** lies directly beneath, and within 3 Å of, the center of the 2-picoline ring. This is consistent with strong aromatic ring current shielding effects.^[9, 10] The ¹H chemical shift of the NH₃ signal of the platinated duplex is shifted to high frequency by $\delta = 0.4$ relative to that for **1** itself. This is consistent with the presence of hydrogen bonding between the NH₃ of [Pt(NH₃)(2-Pic)]²⁺ and O6 of DNA residue G8 in model **3b**. Additional stabilization of complex **3b** may arise from van der Waals contact between the 2-methyl group of 2-picoline and the T6 methyl group of DNA (C...C: 3.78 Å).^[11] This contact is not observed in the Methyl-Out model **3a** (distance between 2-CH₃ and T9 CH₃: 5.13 Å) and appears to be important in distinguishing between the Methyl-In and Methyl-Out models.

In previous reports of the stereoselective formation of platinated GG adducts,^[12, 13] the selectivity has been low and with the opposite orientation (NH₃ *cis* to 5'-G) compared to that observed here. In the case of *cis*-[PtCl₂(NH₃)-(C₆H₁₁NH₂)], a metabolite of the orally active drug JM216, the most abundant platinated G*G* stereoisomer (isomer ratio 2:1) has the cyclohexylamine ligand directed towards the 3'-G of calf thymus DNA.^[14] This preference was attributed partly to Pt–NH₃ H-bonding with the 5'-phosphate. The same ratio of orientational isomers (2:1) has been observed for an 11-mer DNA duplex platinated at G*G* with *cis*-[Pt(NH₃)(4-aminoTEMPO)]²⁺ (TEMPO = 2,2,6,6-tetramethylpiperidine-N-oxide (radical)).^[15]

Preliminary kinetic studies of the reaction of complex **1** with duplex **2** show that the monoaqua species with 2-picoline *trans* to water reacts faster with DNA, and therefore monofunctional adducts with GN7 *trans* to 2-picoline are formed in preference to those with NH₃ *trans* to GN7 (Scheme 1). This is probably a result of unfavorable steric interactions when 2-picoline is *cis* to GN7. This contrasts with the formation of monofunctional adducts of *cis*- and *trans*-[PtCl(NH₃)(4-MePy)]⁺ where the methyl substituent is further away from the coordination plane,^[16] and emphasizes further the important role played by the 2-methyl group of **1** that is close to the Pt ion. Monofunctional 3'-G-platinated adducts of DNA duplexes usually form faster and ring-close faster than 5'-G monofunctional adducts.^[17] If this is also the case for adducts of **1**, then this, together with the preference for substitution *trans* to 2-picoline, would explain why the bifunctional adduct with 2-picoline *cis* to 5'-G is the preferred product.

In conclusion, a number of factors appear to drive the high stereoselectivity in reactions of the anticancer drug **1** with DNA duplex **2**. These include: unfavorable steric interactions between the 2-picoline ring and the nearby nucleotide bases in models **3c** and **3d**, favorable hydrogen bonding between the



NH₃ ligand and O6 of G8 in model **3b**, and van der Waals interactions between the 2-picoline CH₃ group and the CH₃ group of nearby T6, which is absent in model **3a**. Both thermodynamic and kinetic factors appear to favor the formation of complex **3b**. The high stability of **3b** may be important in determining the lack of cross-resistance between **1** and cisplatin. Such a lesion may be recognized differently from cisplatin–DNA adducts by the excision-repair systems in cells.^[18, 19]

2-Picoline was purchased from Aldrich. The sodium salts of HPLC-purified 14-mer oligonucleotides were prepared by Oswel (Southampton, UK). *cis*-[PtCl₂(¹⁵NH₃)(2-Pic)] (**1**) was prepared from *cis*-[PtCl₂(¹⁵NH₃)₂] by a similar procedure to that described for natural abundance mixed-ligand ammine/amine Pt^{II} complexes.^[20]

NMR spectra were acquired, as previously described,^[4, 5] on both Bruker DMX500 and Varian ^{Unity}INOVA600 NMR spectrometers operating at ¹H resonance frequencies of 500.13 and 599.842 MHz, respectively. 2D ¹H NOESY data sets were acquired for both the H₂O/D₂O 90/10 sample and the 99.9 % D₂O sample with a 200 ms mixing time at 278 K. 2D DQF-COSY data were acquired only on the 99.9 % D₂O sample.

Molecular modeling was carried out using Sybyl version 6.3 (Tripos Inc.). DNA duplex structures were based on energy-minimized models of a cisplatin–DNA duplex calculated from NMR data.^[7] The X-ray crystal

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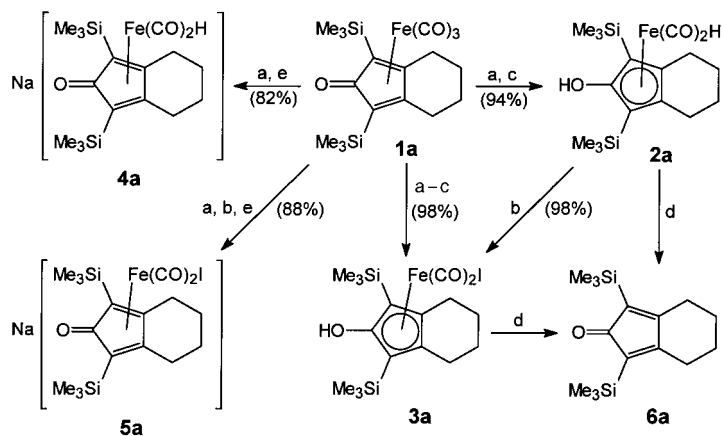
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Demetalation of Tricarbonyl(cyclopentadienone)iron Complexes Initiated by a Ligand Exchange Reaction with NaOH—X-Ray Analysis of a Complex with Nearly Square-Planar Coordinated Sodium**

Hans-Joachim Knölker,* Elke Baum, Helmut Goesmann, and Rüdiger Klauss

The [2+2+1] cycloaddition of two alkynes and carbon monoxide in the presence of pentacarbonyliron represents a useful method for the construction of five-membered ring systems.^[1, 2] Applications of the resulting tricarbonyl(η^4 -cyclopentadienone)iron complexes to organic synthesis are feasible by demetalation to the free cyclopentadienones. This transformation was achieved by oxidation with trimethylamine *N*-oxide.^[1, 3] Recently we reported a novel method for the demetalation of tricarbonyl(diene)iron complexes by a photochemically induced exchange of the carbonyl ligands by acetonitrile.^[4] Herein we describe an alternative procedure for the ligand exchange at tricarbonyl(η^4 -cyclopentadienone)iron complexes and the subsequent demetalation in the air.

Tricarbonyl(η^4 -cyclopentadienone)iron complexes undergo a transformation similar to the Hieber reaction.^[5] Thus, reaction of complex **1a** with aqueous NaOH in THF leads to an equilibrium of the corresponding hydrido complexes **2a** and **4a** in a ratio of about 13:1 (Scheme 1). Tricarbonyl(cyclohexa-1,3-diene)iron complexes are inert under these conditions. Addition of H_3PO_4 affords **2a** in 94% yield, while reaction with NaH shifts the equilibrium towards the salt **4a**



Scheme 1. a) 1M NaOH/THF (1/2); b) $C_5H_{11}I$; c) H_3PO_4 ; d) air, daylight, Et_2O/THF , $Na_2S_2O_3$, Celite, 3 h; e) NaH, Et_2O/THF .

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(82% yield). Reaction of the hydrido complex **2a** with 1-iodopentane provides the iodo complex **3a** in 98% yield. A related transformation is reported for the hydrido complex $[CpFe(CO)_2H]$.^[6] The addition of 1-iodopentane after the reaction of **1a** with NaOH affords an equilibrium of the iodo complexes **3a** and **5a** that is shifted again by addition of H_3PO_4 or NaH, respectively. Preparation of the iodo complex **3a** without isolation of the intermediate hydrido complex **2a** increases the yield (98% based on complex **1a**).

The ^{13}C NMR and the IR data of the hydrido complex **2a** and the iodo complex **3a** suggest an η^5 -coordinated hydroxycyclopentadienyl ligand for both compounds.^[7] A characteristic structural feature of the hydrido complex **2a** is the unsymmetrical arrangement of the coligands, which is apparent from two CO signals in the ^{13}C NMR spectrum. This assignment was confirmed by an X-ray structure determination of complex **2a** (Figure 1),^[8] which shows an η^5 -coordinated hydroxycyclopentadienyl ligand and a C1–O1 bond length of 1.366 Å.^[9] A loss of C_s symmetry was also found for the hydrido complex **4a** from the ^{13}C NMR spectrum, which exhibits the two signals for the carbonyl ligands and a peak at $\delta = 170.13$ for C1.^[7]

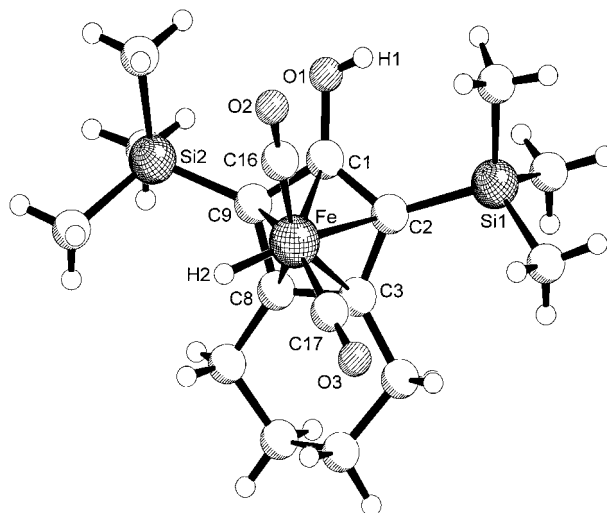


Figure 1. Molecular structure of **2a** in the crystal. Selected bond lengths [Å]: Fe–C1 2.1353(13), Fe–C2 2.1485(14), Fe–C3 2.0992(14), Fe–C8 2.0981(14), Fe–C9 2.1087(14), Fe–H2 1.38(2), Fe–C16 1.752(2), Fe–C17 1.741(2), C16–O2 1.143(2), C17–O3 1.153(2), C1–C2 1.437(2), C2–C3 1.452(2), C3–C8 1.430(2), C8–C9 1.445(2), C1–C9 1.445(2), C1–O1 1.366(2), O1–H1 0.75(2), C2–Si1 1.8876(14), C9–Si2 1.887(2).

The iodo complex **5a** was crystallized from pentane/THF (6/1). The X-ray analysis of **5a** shows a dimeric structure for the solid state (Figure 2a).^[8] The oxygen atoms O1 and O1* are both coordinated to the sodium ions Na1 and Na1*. Each sodium ion is further coordinated with an iodine and one THF molecule. However, the sodium ions exhibit an extraordinary nearly square-planar arrangement of the ligands instead of the usual tetrahedral coordination. The bulky cyclopentadienone ligand with the trimethylsilyl (TMS) substituents and the $Fe(CO)_2I$ fragment forces the sodium ion into the unusual square-planar coordination geometry which is only slightly distorted. Each sodium ion and the three oxygen ligands are in one plane and the iodo ligand is only slightly out of this

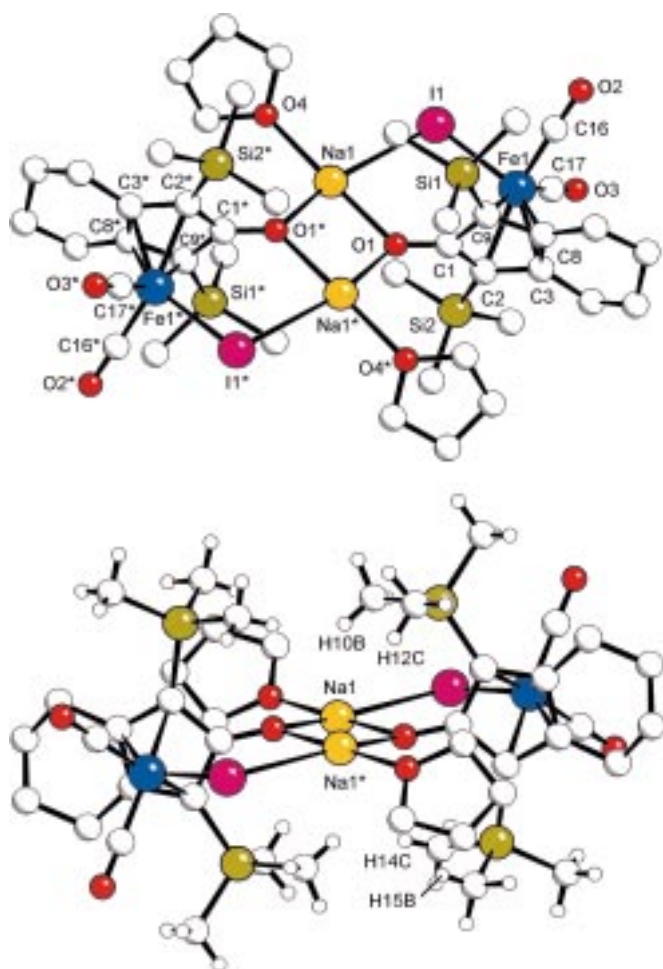


Figure 2. Dimeric structure of **5a** in the crystal. a) View perpendicular to the plane formed by the ligands coordinated at the sodium center; hydrogen atoms have been omitted for clarity. Selected bond lengths [Å] and angles [°]: Fe1–C2 2.144(4), Fe1–C3 2.075(4), Fe1–C8 2.063(4), Fe1–C9 2.136(4), Fe1–I1 2.6409(8), Fe1–C16 1.766(6), Fe1–C17 1.763(5), C16–O2 1.151(6), C17–O3 1.148(6), C1–C2 1.468(6), C2–C3 1.453(6), C3–C8 1.419(6), C8–C9 1.442(6), C1–C9 1.466(6), C1–O1 1.268(5), Na1–O1 2.240(3), Na1–O1* 2.280(3), Na1–O4 2.298(4), Na1–I1 3.165(2); O1–Na1–O1* 83.13(12), O1–Na1–O4 179.02(14), O1*–Na1–O4 97.65(13), O1–Na1–I1 82.25(9), O1*–Na1–I1 162.86(10), O4–Na1–I1 96.88(10), Na1–O1–Na1* 96.87(12). b) Side view; only the hydrogen atoms of the TMS groups are shown. Selected atom distances [Å] and angles [°]: Na1–H10B 2.625, Na1*–H12C 3.014, Na1–H14C 3.249, Na1*–H15B 3.805; C10–H10B–Na1 118.32, C12–H12C–Na1* 148.62, C14–H14C–Na1 128.48, C15–H15B–Na1* 142.83.

plane.^[10] The corresponding bond angles O1–Na1–O4 and O1*–Na1–I1 are 179.02(14) and 162.86(10)°, respectively. Presumably, the shielding of the sodium ion by the TMS groups from both sides above and below the almost square-planar arranged ligands prevents the coordination of further ligands to the axial positions of the sodium ions, which would lead to octahedral coordination (Figure 2b). The cyclopentadienone ring consists of two planes, one is formed by the coordinated butadiene, and the second, defined by C2, C1, and C9, is bent away from the iron atom. The dihedral angle between these two planes is 13.9°, which is typical for cyclopentadienone-iron complexes (12–20°).^[1a, 2a, 11] The IR spectrum of **5a** in KBr exhibits a carbonyl stretching frequency for the cyclopentadienone of 1492 cm^{−1}. The

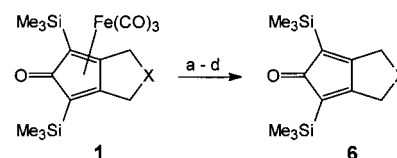
¹³C NMR spectrum of **5a** in CD₃OD shows a chemical shift of $\delta = 176.05$ for C1, which indicates a monomeric structure in solution.^[7]

An X-ray crystal structure determination also confirmed that complex **4a** has a dimeric structure in the solid state. In this case a square-planar arrangement of the ligands at the sodium ions is realized by coordination of a carbonyl ligand. In solution complex **4a** is monomeric, as shown by the absence of the carbonyl bands in the IR spectrum at 1859, 1836, and 1819 cm^{−1}, which were present in the IR spectrum in KBr.^[7]

We next investigated the demetalation behavior of the complexes **2a** and **3a**. The crystals of complex **2a** can be handled in air for a short period of time and can be stored in an inert gas atmosphere at −30 °C. Stirring a solution of **2a** in diethyl ether for 5 h in the air leads to demetalation and provides the free ligand **6a** in 80% yield based on **1a** (Scheme 1). A related demetalation of a tricarbonyl(η^4 -cyclopentadienone)iron complex with concomitant oxidation of the ligand was achieved on treatment of an aqueous alkaline solution with molecular oxygen.^[11d] However, these conditions are too harsh in the present case since bases cause an isomerization and subsequent desilylation.^[1b]

The iodo complex **3a** remains nearly unchanged after stirring a solution of **3a** in the air for 42 h in the dark. However, stirring a solution of **3a** in the air with exposure to daylight leads to a highly selective demetalation within 3 h and provides the free cyclopentadienone **6a** in 95% yield based on complex **1a**. In the optimized procedure some Celite is added to remove iron particles and photochemically generated iodine is reduced with sodium thiosulfate (see experimental procedure). A photolytically induced exchange of the carbon monoxide ligands was found for the related complex [CpFe(CO)₂I].^[12] The removal of the carbon monoxide ligand in the air is irreversible and leads to demetalation.

The application of the novel one-pot demetalation procedure to a series of bicyclic tricarbonyl(η^4 -cyclopentadienone)iron complexes **1**^[1a,b] provided the corresponding free ligands **6a–d** in excellent yields (Scheme 2, Table 1). Thus, the results for demetalation of the complexes **1** obtained by our previous photolytically induced ligand exchange were improved once again.^[4] Only the demetalation of complex **1e** to **6e** afforded a poor yield, which is a consequence of concomitant ester cleavage.



Scheme 2. a) 1M NaOH/THF (1/2); b) C₅H₁₁I; c) H₃PO₄; d) air, daylight, Et₂O/THF, Na₂S₂O₃, Celite, 3 h.

Table 1. Demetalation of the tricarbonyl(η^4 -cyclopentadienone)iron complexes **1** via the intermediate iodo complexes **3**.

1	X	Yield (6) [%]
a	(CH ₂) ₂	95
b	CH ₂	93
c	O	89
d	S	87
e	C(COOMe) ₂	12

In conclusion, we have developed a novel procedure for the demetalation of tricarbonyl(η^4 -cyclopentadienone)iron complexes, which proceeds by a ligand exchange initiated by sodium hydroxide. The X-ray analysis of an intermediate dimeric complex revealed an extraordinary nearly square-planar coordinated sodium ion.

Experimental Section

General procedure for the demetalation of the bicyclic tricarbonyl(η^4 -cyclopentadienone)iron complexes **1** by NaOH-initiated ligand exchange: A solution of complex **1** (0.478 mmol) in THF (8 mL) and aqueous 1M NaOH (4 mL) was stirred for 2.5 h under an argon atmosphere. Then 1-iodopentane was added (0.15 mL, 228 mg, 1.15 mmol) and the yellow solution turned brown. After stirring the mixture for an additional 15 min under argon, H_3PO_4 (0.15 mL) was added, the organic layer was separated, and the aqueous layer was extracted with diethyl ether. The combined organic layers were dried over Na_2SO_4 and filtered through a short path of silica gel. After addition of $\text{Na}_2\text{S}_2\text{O}_3$ (200 mg) and Celite (200 mg) the filtrate was stirred slowly in the air for 3 h in the presence of daylight. Filtration through a short path of Celite, evaporation of the solvent, and flash chromatography (pentane/diethyl ether) of the residue on silica gel provided the free ligand **6**.

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- [7] Spectroscopic data: **2a**: IR (KBr): $\tilde{\nu}$ = 3581, 1991, 1932 cm^{-1} ; ^1H NMR (400 MHz, C_6D_6): δ = –11.62 (s, 1H), 0.30 (s, 18H), 1.23 (m, 2H), 1.45 (m, 2H), 2.01 (m, 2H), 2.36 (m, 2H), 3.75 (s, 1H); ^{13}C NMR and DEPT (100 MHz, C_6D_6): δ = 1.48 (6CH₃), 23.65 (2CH₂), 26.09 (2CH₂), 70.73 (2C), 103.61 (2C), 146.18 (C), 217.57 (CO), 217.64 (CO); elemental analysis calcd for $\text{C}_{17}\text{H}_{28}\text{FeO}_3\text{Si}_2$: C 52.03, H 7.19; found: C 51.97, H 7.21. **3a**: IR (KBr): $\tilde{\nu}$ = 3584, 2011, 1968 cm^{-1} ; ^1H NMR (400 MHz, C_6D_6): δ = 0.35 (s, 18H), 1.03 (m, 2H), 1.13 (m, 2H), 1.87 (m, 2H), 2.14 (m, 2H), 4.34 (s, 1H); ^{13}C NMR and DEPT (125 MHz, C_6D_6): δ = 1.34 (6CH₃), 22.81 (2CH₂), 24.92 (2CH₂), 75.65 (2C), 102.15 (2C), 147.74 (C), 216.57 (2CO); elemental analysis calcd for $\text{C}_{17}\text{H}_{27}\text{FeIO}_3\text{Si}_2$: C 39.39, H 5.25; found: C 39.54, H 5.10. **4a**: IR (KBr): $\tilde{\nu}$ = 2000, 1967, 1943, 1859, 1836, 1819 cm^{-1} ; IR (CH_3OH): $\tilde{\nu}$ = 1997, 1970, 1937, 1904 cm^{-1} ; ^1H NMR (500 MHz, CD_3OD): δ = –13.05 (s, 1H), 0.25 (s, 18H), 1.56 (m, 2H), 1.72 (m, 2H), 2.30 (m, 2H), 2.52 (m, 2H); ^{13}C NMR and DEPT (125 MHz, CD_3OD): δ = 1.56 (6CH₃), 24.60 (2CH₂), 27.49 (2CH₂), 68.47 (2C), 102.53 (2C), 170.13 (C=O), 221.06 (CO), 221.18 (CO). **5a**: IR (KBr): $\tilde{\nu}$ = 1993, 1957, 1911, 1492 cm^{-1} ; IR (CH_3OH): $\tilde{\nu}$ = 1992, 1937 cm^{-1} ; ^{13}C NMR and DEPT (125 MHz, CD_3OD): δ = 1.11 (6CH₃), 23.93 (2CH₂), 26.67 (2CH₂), 65.41 (2C), 106.29 (2C), 176.05 (C=O), 219.17 (2CO); elemental analysis calcd for $\text{C}_{21}\text{H}_{34}\text{FeINaO}_4\text{Si}_2$: C 41.19, H 5.60; found: C 41.14, H 5.52.
- [8] X-ray crystal structure analyses: **2a**: yellow needles, $\text{C}_{17}\text{H}_{28}\text{FeO}_3\text{Si}_2$; M_r = 392.42 g mol^{-1} , triclinic, space group $P\bar{1}$, λ = 0.71073 Å, a = 6.7797(4), b = 11.2561(8), c = 13.9334(12) Å, α = 104.343(5), β =

92.265(5), γ = 97.624(4)°, V = 1018.14(13) Å³, Z = 2, μ = 0.868 mm^{-1} , ρ_{calcd} = 1.280 g cm^{-3} , T = 200(2) K, θ range: 2.70–27.50°; 4598 independent reflections; full-matrix least squares on F^2 ; R_1 = 0.0272, wR_2 = 0.0710 [$I > 2\sigma(I)$]; maximal residual electron density 0.620 e Å^{-3} . All hydrogen atoms were determined by Fourier difference calculation and refined isotropically. **5a**: brown cubes, $\text{C}_{17}\text{H}_{26}\text{FeINaO}_3\text{Si}_2 \cdot \text{C}_4\text{H}_8\text{O}$; M_r = 612.40 g mol^{-1} , monoclinic, space group $P2_1/n$, λ = 0.71073 Å, a = 11.254(2), b = 15.508(3), c = 15.254(3) Å, β = 93.52(3)°, V = 2657.2(9) Å³, Z = 4, ρ_{calcd} = 1.531 g cm^{-3} , μ = 1.859 mm^{-1} , T = 200(2) K, θ range: 1.87–25.50°; 4950 independent reflections; full-matrix least squares on F^2 ; R_1 = 0.0390, wR_2 = 0.0712 [$I > 2\sigma(I)$]; maximal residual electron density 0.565 e Å^{-3} . Programs: G. M. Sheldrick, SHELXS-86 (Göttingen, 1986), SHELXL-93 (Göttingen, 1993); E. Keller, SCHAKAL-97 (Freiburg im Breisgau, 1997). 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-114303 (**5a**) and CCDC-114304 (**2a**). 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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A Molybdenum – Iron – Sulfur Cluster Containing Structural Elements Relevant to the P-Cluster of Nitrogenase**

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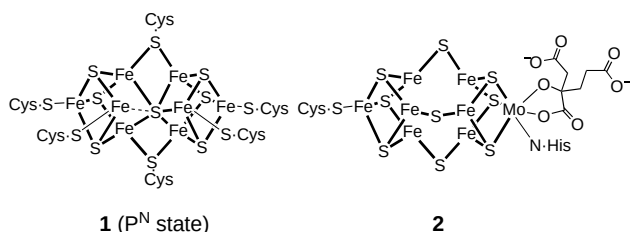
The commonly occurring iron – sulfur cluster cores—rhombohedral Fe_2S_2 , cuboidal Fe_3S_4 , and cubane-type Fe_4S_4 —have been synthesized in research directed toward an understand-

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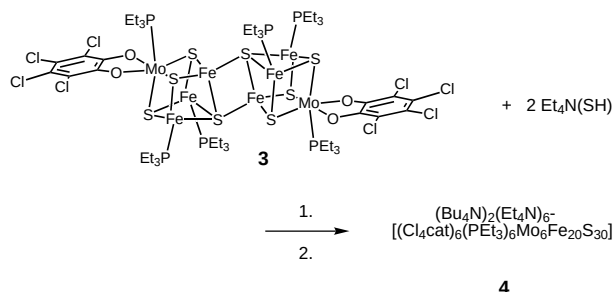
ing of the formation, structure, and function of these clusters in biology.^[1] Our current endeavors are directed toward the synthesis of more complicated metal site structures, described as bridged cluster assemblies^[2] and including the two cluster types in the MoFe protein of nitrogenase.^[3] The P-cluster **1** contains an Fe₈S₇ core formed by two cuboidal Fe₄S₃ fragments bridged by a central sulfur atom, with additional bridging by two cysteinyl sulfur atoms.^[4] The FeMo cofactor



(FeMoco) cluster **2** has an MoFe₇S₉ core with cuboidal Fe₄S₃ and MoFe₃S₄ clusters bridged by three μ₂-S atoms. The P-cluster appears to mediate electron transfer from the Fe protein to FeMoco, which is the catalytic site.^[5]

Recently, we reported a compositional analogue of FeMoco in the form of the sulfido-bridged double cubane core [Fe₄S₄-S-MoFe₃S₄].^[6] The symmetrical double cubane [MoFe₃S₄-S-MoFe₃S₄] has also been prepared. In these species, the individual clusters are bridged by μ₂-sulfide units and are in their oxidized states ([Fe₄S₄]²⁺, [MoFe₃S₄]³⁺). The iron populations of as-isolated FeMoco (*S* = 3/2) and P-cluster (P^N state) are substantially reduced. For the cofactor, the ⁵⁷Fe isomer shift of δ = 0.47 mm s⁻¹ at 4.2 K^[7] is not inconsistent with trigonal Fe^{II}S₃ coordination,^[8] and a recent ⁵⁷Fe ENDOR analysis leads to the valence description Mo^{IV} + Fe^{III} + 6 Fe^{II}.^[9] The isomer shifts of the P-cluster (δ ≈ 0.64 mm s⁻¹ at 4.2 K) are consistent with an all-Fe^{II} P^N state.^[10] Consequently, we have initiated a second synthetic approach to FeMoco and P-cluster utilizing as precursors cubane-type clusters in more reduced states.

The rhomb-bridged double cubane [(Cl₄cat)₂Mo₂Fe₆S₈-(PEt)₆]^[11] (**3**) is one of several species whose reduced cores ([Fe₄S₄]¹⁺, [MoFe₃S₄]²⁺) are stabilized in isolable compounds by phosphane ligation.^[12] A suspension of **3** in acetonitrile was treated with two equivalents of (Et₄N)(SH) to give a dark solution (Scheme 1). Evaporation of solvent to remove PEt₃



Scheme 1. Preparation of (Bu₄N)₂(Et₄N)₆[**4**]. 1. Stirring in CH₃CN at room temperature for 12 h followed by evaporation of all volatile components (CH₃CN, PEt₃, H₂S) in vacuo. 2. Extraction of the solid residue with NMF, addition of solid (Bu₄N)(PF₆), and crystallization from NMF/diethyl ether.

and H₂S followed by extraction of the solid residue with *N*-methylformamide (NMF) afforded a black solution, whose major component could be crystallized after addition of (Bu₄N)(PF₆). Vapor diffusion of diethyl ether into the solution for three days caused separation of black crystals of (Bu₄N)₂(Et₄N)₆[(Cl₄cat)₆(Et₃P)₆Mo₆Fe₂₀S₃₀] · 9 NMF in 33 % yield. This compound contains cluster **4** and was identified crystallographically; the NMF molecules are uncoordinated. This is the highest nuclearity Fe–S or heterometallic Fe–S cluster currently known. Certain of its fragments are of interest in relation to the P-cluster of nitrogenase.

Two perspectives of the [Mo₆Fe₂₀S₃₀]⁸⁻ core of **4** are presented in Figure 1, from which it is evident that the structure is centrosymmetric with idealized C_{2h} symmetry. The

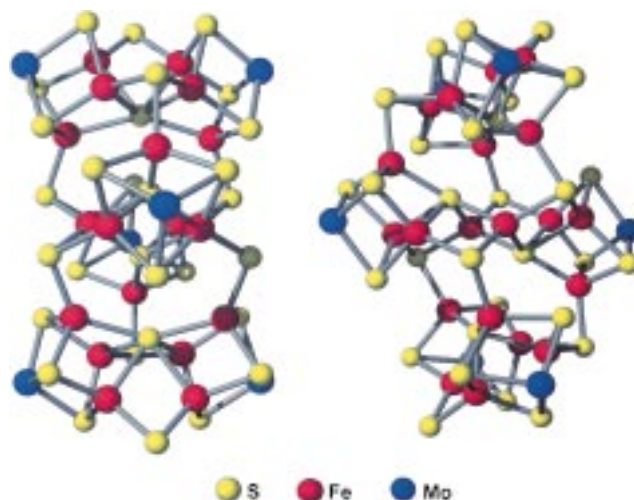


Figure 1. Front (left) and side views (right) of the Mo₆Fe₂₀S₃₀ core of cluster **4**, which has crystallographic inversion symmetry and idealized C_{2h} symmetry. The upper and lower Mo₂Fe₆S₉ fragments (Figure 2) are connected to the central Mo₂Fe₈S₁₂ fragment (Figure 3) by six μ₃-Fe-S bridges. Terminal ligands on the molybdenum atoms are omitted for clarity. Selected mean bond lengths [Å] for the distorted octahedral Mo(Cl₄cat)-(PEt₃)S₃ coordination unit: Mo–O 2.126(8), Mo–P 2.63(3), Mo–S 2.37(3).

initial double cubane structure of precursor **3** has undergone fracture and rearrangement, resulting in removal of terminal ligands at the iron sites; in this sense, **4** resembles the cyclic cluster [Na₂Fe₁₈S₃₀]⁸⁻.^[13] The sulfur atoms divide into the bridging modes 2 × μ₂ + 24 × μ₃ + 2 × μ₅ + 2 × μ₆. The structure can be described in terms of three fragments: The upper and lower fragments Mo₂Fe₆S₉ are symmetry-related and have the structure shown in Figure 2. The central fragment Mo₂Fe₈S₁₄ (see Figure 3) is connected to the upper and lower fragments by six μ₃-S–Fe bridges (2.230(4)–2.273(5) Å) involving S(8,14,15) and their symmetry-related counterparts. Within both types of fragments, the Fe–S distances increase in the order Fe–μ₂-S < Fe–μ₃-S < Fe–μ₅-S < Fe–μ₆-S.

The Mo₂Fe₆S₉ fragment (Figure 2) has idealized C_s symmetry and contains two MoFe₃S₄ cubane units, as does **3**. However, one sulfur atom is common to both, resulting in an S(9)Fe₆(1–6) grouping and a μ₆ bridging function for S(9). Atoms Fe(2,5) and Fe(1,4) are additionally bridged by μ₂-S(7) and μ₃-S(8). Consequently, S(9) is shared by eight nonplanar rhomb-like Fe₂S₂ units, an opposite pair of which

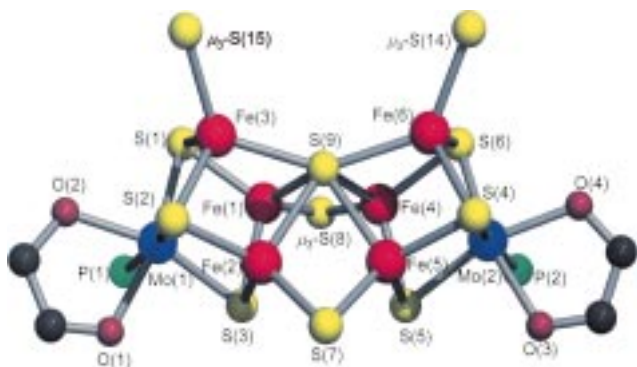


Figure 2. Structure of the upper and lower $\text{Mo}_2\text{Fe}_6\text{S}_9$ fragment of cluster **4** with idealized C_s symmetry. Atom $\mu_3\text{-S}(8)$ bridges to $\text{Fe}(7a)$ of the central fragment and atoms $\mu_3\text{-S}(14,15)$, which bridge to $\text{Fe}(3,6)$, are part of that fragment (Figure 3); atom $\text{S}(7)$ is bound to two Fe atoms in a μ_2 manner. At $\text{Mo}(1,2)$, the catecholate chelate rings and coordinated P atoms of PET_3 ligands are indicated. Selected (mean) bond lengths [$\text{\AA}$] and angles [$^\circ$]: Mo-Fe 2.74(1), intracubane Fe-Fe 2.74(2), $\text{Fe}(1)\text{-Fe}(4)$ 2.635(3), $\text{Fe}(2)\text{-Fe}(5)$ 2.681(3), $\text{Fe-S}(9)$ 2.40(2), $\text{Fe}(1,2,4,5)\text{-S}(9)$ 2.39(1), $\text{Fe-S}(1\text{-}6)$ 2.24(2), $\text{Fe}(2,5)\text{-S}(7)$ 2.190(8), $\text{Fe}(3)\text{-S}(9)\text{-Fe}(6)$ 145.3(2), $\text{Fe}(2)\text{-S}(7)\text{-Fe}(5)$ 75.5(1), $\text{Fe}(1)\text{-S}(8)\text{-Fe}(4)$ 71.9(1), $\text{Fe-S}(9)\text{-Fe}$ 69.0(6). Atoms (n) and (na) are related by symmetry.

($\text{Fe}_2(1,2)\text{S}_2(3,9)$ and $\text{Fe}_2(4,5)\text{S}_2(5,9)$) are faces of the MoFe_3S_4 cubanes. The $\text{S}(7,8)$ bridging interactions force a large $\text{Fe}(3)\text{-S}(9)\text{-Fe}(6)$ angle of $145.3(2)^\circ$. Intracubane bond angles at $\text{S}(9)$ occur in the range of $68.9\text{--}70.4^\circ$. The intracubane mean Mo-Fe (2.74(1) $\text{\AA}$) and Fe-Fe distances (2.74(2) $\text{\AA}$) are both substantially longer than the corresponding mean values in **3** (2.677(5) and 2.639(2) $\text{\AA}$,^[11] respectively). However, intracubane Fe-S distances are unchanged (2.24(2) $\text{\AA}$). The $\mu_6\text{-S}$ bridging mode is preceded—in $[\text{Ni}_9\text{S}(\text{tBu})_8]^{1-}$ ^[14] the core topology resembles that of **2**—but is otherwise unknown in Fe-S clusters.

The most striking feature of the $\text{Mo}_2\text{Fe}_6\text{S}_9$ fragment is its structural resemblance to the $\text{Fe}_8\text{S}_7(\text{SR})_2$ portion of the P-cluster **1**, whose composition can be derived by replacing $\text{Mo}(\text{Cl}_4\text{cat})(\text{PET}_3)$ with $\text{Fe}^{\text{II}}(\text{SR})$ and $\text{S}(7,8,14,15)$ with RS^- . The topologies of the fragment and **1** are clearly similar but with some structural differences. In the P^{N} state, the central sulfur atom exhibits unsymmetrical $5 + 1$ coordination with one long Fe-S bond of 2.9 $\text{\AA}$,^[4] and is best considered as $\mu_5\text{-S}$. In the fragment, the six $\text{Fe-S}(9)$ distances are in the range of 2.381(4)–2.429(5) $\text{\AA}$ with a mean of 2.40(2) $\text{\AA}$; the sulfur atom is clearly $\mu_6\text{-S}$. Otherwise, the adjacent Fe-Fe separations in the P^{N} state obtained from X-ray (2.4–2.8 $\text{\AA}$)^[4] and EXAFS analyses^[15] (mean values 2.57, 2.75 $\text{\AA}$) cover the range observed for the fragment (2.635(3)–2.771(3) $\text{\AA}$).

Certain distortions not found elsewhere in the $\text{Mo}_2\text{Fe}_6\text{S}_9$ fragment occur at $\text{Fe}(1,4/2,5)$, which are bound to $\mu_2\text{-S}(7)/\mu_3\text{-S}(8)$. The FeS_4 units are distorted toward trigonal-pyramidal coordination with $\text{S}(9)$ as the axial ligand. At these sites, differences between axial and mean equatorial bond lengths are 0.16–0.17 $\text{\AA}$, axial bond angles average to $105(1)^\circ$, and the range of equatorial bond angles is $103\text{--}124^\circ$. Distortions of this type occur in FePS_3 sites of Mo/V-Fe-S clusters.^[16] Limiting elongated distortion generates trigonal-planar coordination, similar to that observed for six iron sites in **2**. Note that the structures of **1** and **2** are formally interconvertible.

Movement of the $\mu_5\text{-S}$ atom out of the center of the Fe_6 trigonal prism with attendant cleavage of four Fe-S bonds and transformation of tetrahedral FeS_4 into trigonal FeS_3 sites recovers the topology of the cofactor core. Signs of this transformation are integrated into the structure of the $\text{Mo}_2\text{Fe}_6\text{S}_9$ fragment, whose structure could be interpreted as transitional between P-cluster and FeMoco topology.

The structure of the central $\text{Mo}_2\text{Fe}_8\text{S}_{12}$ fragment of cluster **4** is presented in Figure 3. Metric parameters of the molybdenum coordination unit and analogous $\text{Fe-}\mu\text{-S}$ interactions are

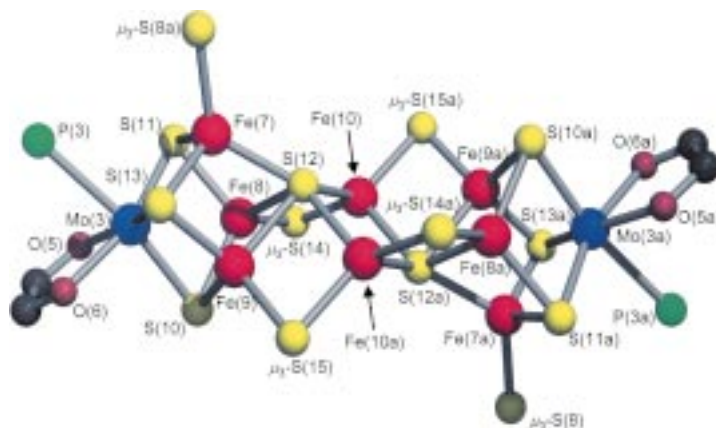


Figure 3. Structure of the central $\text{Mo}_2\text{Fe}_8\text{S}_{12}$ fragment of cluster **4** with a crystallographic inversion center and idealized C_{2h} symmetry. Atoms $\mu_3\text{-S}(8,8a)$ are parts of two $\text{Mo}_2\text{Fe}_6\text{S}_9$ fragments, and atoms $\mu_3\text{-S}(14/14a)$ and $\mu_3\text{-S}(15/15a)$ bridge to $\text{Fe}(6/6a)$ and $\text{Fe}(3/3a)$, respectively, of those fragments. Selected (mean) bond lengths [$\text{\AA}$] and angles [$^\circ$]: $\text{Fe}(7)\text{-S}(12)$ 2.292(4), $\text{Fe}(8\text{-}10)\text{-S}(12)$ 2.346(2), $\text{Fe}(10)\text{-S}(15a)$ 2.275(4), $\text{Fe}(10)\text{-Fe}(10a)$ 3.462(3), $\text{Fe}(7)\text{-S}(12)\text{-Fe}(10)$ 132.5(2), $\text{S}(12)\text{-Fe}(10)\text{-S}(12a)$ 85.0(2), $\text{Fe}(10)\text{-S}(12)\text{-Fe}(10a)$ 95.0(2), $\text{S}(14)\text{-Fe}(10)\text{-S}(12a)$ 126.9(2), $\text{S}(15a)\text{-Fe}(10)\text{-S}(12)$ 124.5(2).

practically identical to those shown in Figure 2. Two MoFe_3S_4 cubanes are linked through symmetry-related $\text{S}(12)\text{-Fe}(10/10a)\text{-S}(12a)$ bridges that generate an interior Fe_3S_2 rhomb in which $\text{Fe}(10,10a)$ are additionally bridged to the cubanes over two $\mu_3\text{-S}$ atoms. Tetrahedral coordination at these atoms is severely distorted by two S-Fe-S angles near 125° . In the $\text{Mo}_2\text{Fe}_8\text{S}_{12}$ fragment, the cubane units do not share a common atom, but $\text{S}(12)$ performs a μ_5 function by bridging within the cubane and the central rhomb. A relationship does exist between the central and terminal fragments in that $\mu_5\text{-S}(12)$ may be related to $\mu_6\text{-S}(9)$ and $\mu_3\text{-S}(14,15)$ to $\mu_2\text{-S}(7)/\mu_3\text{-S}(8)$. Possibly one fragment is an intermediate in the formation of the other. Note that $\text{Mo}_2\text{Fe}_8\text{S}_{12}$ can be formally recovered from $\text{Mo}_2\text{Fe}_6\text{S}_9$ by insertion of $\text{Fe}(10,10a)$ and $\text{S}(12a,14a,15a)$ and attendant structural rearrangement.

The zero-field Mössbauer spectrum of polycrystalline complex **4** at 150 K in Figure 4A consists of a doublet ($\delta = 0.47 \text{ mm s}^{-1}$, quadrupole splitting $\Delta E_Q = 0.8 \text{ mm s}^{-1}$) and a (non-Lorentzian) linewidth of 0.45 mm s^{-1} . The broad lines suggest that ΔE_Q values of the six inequivalent iron sites differ somewhat and occur in the range of $0.75\text{--}1.1 \text{ mm s}^{-1}$. The lines in the spectrum at 4.2 K are even broader and have broad wings reminiscent of paramagnetic relaxation. To determine whether **4** is diamagnetic or paramagnetic, the cluster has been studied in applied fields up to 8.0 T. The

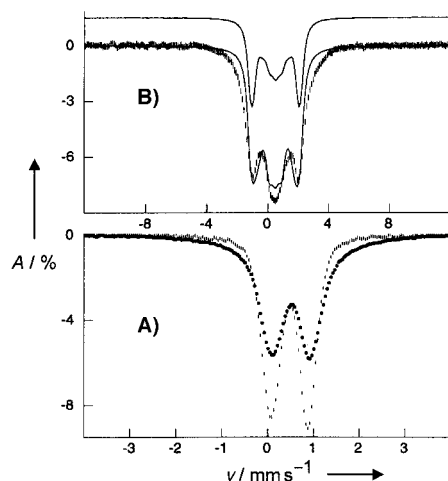


Figure 4. Mössbauer spectra of polycrystalline complex **4**. A) Spectra in zero field at 150 K (hash marks) and 4.2 K (solid circles). B) Spectrum at 4.2 K in a parallel applied field of 8.0 T. The solid line above the data is a simulation assuming that all iron sites reside in diamagnetic environments. The solid line drawn through the data is a simulation assuming one site with $\Delta E_Q = 0.8 \text{ mm s}^{-1}$, $\delta = 0.52 \text{ mm s}^{-1}$, and $\eta = 1$ (see text). The simulation assumes that the compound is paramagnetic with the undetermined ground-state spin S and involves zero-field splitting parameters and an A tensor chosen such that $H_{\text{int}}(x) = -4 \text{ T}$ and $H_{\text{int}}(y) \approx H_{\text{int}}(z) \approx 0$. The simulated spectrum is meant to illustrate how the shape of the high-field spectrum may arise.

spectrum in Figure 4B has a rather odd shape. While the splitting conforms to that of a diamagnet, there is additional intensity in the middle of the spectrum which cannot be explained by assuming, for instance, inequivalent iron sites. The increased intensity in the center of the spectrum at 8.0 T, however, can be understood as follows: If the asymmetry parameter of the electric field gradient tensor, $\eta = (V_{xx} - V_{yy})/V_{zz}$, has the value 1.0, the component of the field gradient along x is zero. If there is a negative internal magnetic field of $H_{\text{int}} = -4 \text{ T}$ along x , the effective field $H_{\text{eff}} = H_{\text{int}} + H_{\text{applied}}$ will be $H_{\text{eff}} = +4 \text{ T}$. Because there is no quadrupole splitting along x , the x direction contributes a single line in the center of the spectrum that is broadened by the action of H_{eff} . The solid line through the data (Figure 4B) was obtained by assuming one iron site with $\eta = 1$ and $H_{\text{int}}(x) = -4.0 \text{ T}$. The spectra of **4** exhibit magnetic features at 4.2 K, but it is not clear whether the system of 20 iron sites should be considered as an isolated paramagnet with unknown ground state spin or whether incipient long range magnetism gives rise to the internal magnetic field observed at 4.2 K.

We have demonstrated that reaction of **3** with HS^- induces substantial structural rearrangement to afford **4**, whose fragments can be related to different stages of the reaction. Taking the isomer shifts of Fe^{III} –rubredoxin (0.25 mm s^{-1}) and Fe^{II} –rubredoxin (0.70 mm s^{-1}) as criteria, the iron sites of **4** ($\delta = 0.52 \text{ mm s}^{-1}$ at 4.2 K) have an oxidation state of $+2.4$.^[17] The $\text{Mo}_2\text{Fe}_6\text{S}_9$ fragment provides the closest synthetic approach thus far to the topology of the P-cluster **1**. The Mössbauer spectrum of the P^{N} state shows sites with $\Delta E_Q \approx 3.0 \text{ mm s}^{-1}$ (two sites), 1.3 mm s^{-1} (one site), and 0.9 mm s^{-1} (five sites). Cluster **4** exhibits the sites with small ΔE_Q values characteristic of the P-cluster, but not the sites corresponding to the

large ΔE_Q values. Lastly, we associate the formation of higher nuclearity Fe–S and Mo–Fe–S clusters (Fe_nS_n , $n = 8, 16$;^[12b] **3**,^[11] **4**) with their dominant Fe^{II} character.

Experimental Section

All operations were carried out under a pure dinitrogen atmosphere; solvent was removed in vacuo at room temperature. NMF (Aldrich) was stored over NaHCO_3 and distilled in vacuo. A solution of $(\text{Et}_4\text{N})(\text{SH})$ (99 mg, 0.61 mmol; Aldrich, recrystallized from acetonitrile/diethyl ether) in acetonitrile (20 mL) was added with stirring onto solid $[(\text{Cl}_4\text{cat})_2\text{Mo}_2\text{Fe}_6\text{S}_9(\text{PET}_3)_6]$ (600 mg, 0.30 mmol).^[11] The brown suspension was stirred overnight, causing most of the solid to dissolve. The mixture was taken to dryness to remove H_2S and PET_3 . The solid residue was dissolved in acetonitrile (10 mL), the solution was stirred for 2 h, and the solvent was evaporated. The black residue was extracted with acetonitrile (20 mL). The extract was filtered through Celite, and $(\text{Bu}_4\text{N})(\text{PF}_6)$ (80 mg, 0.21 mmol) was added to the filtrate, which was then reduced to dryness. The black residue was extracted with a minimal volume (4 mL) of NMF/diethyl ether (1/1 v/v). Vapor diffusion of diethyl ether into the extract over 3 d resulted in the separation of a highly crystalline solid, which was collected, washed with NMF/diethyl ether (1/1 v/v) and diethyl ether, and dried in vacuo. The product was obtained as 200 mg (33 % based on Fe) of an air-sensitive black crystalline solid, $(\text{Bu}_4\text{N})_2(\text{Et}_4\text{N})_6[(\text{Cl}_4\text{cat})_6(\text{PET}_3)_6\text{Mo}_6\text{Fe}_{20}\text{S}_{30}] \cdot 9 \text{ NMF}$. UV/Vis (acetonitrile): λ_{max} (ϵ_{M}) = 224 (350 000), 316 (146 000), 450 nm ($111\,000 \text{ M}^{-1} \text{ cm}^{-1}$); cyclic voltammetry (acetonitrile, 20 mV s^{-1} , V vs. SCE): -0.45 , -0.78 (oxidation), -1.01 , -1.35 , -1.56 , -1.69 (reduction). Isomer shifts of ^{57}Fe are relative to that of iron metal at 298 K.

Diffraction-quality crystals were grown by diffusion of diethyl ether into the NMF extract obtained as described above. Crystal data for $(\text{Bu}_4\text{N})_2(\text{Et}_4\text{N})_6[\text{4}] \cdot 9 \text{ NMF}$: $\text{C}_{170}\text{H}_{327}\text{Cl}_{24}\text{Fe}_{20}\text{Mo}_6\text{N}_{17}\text{O}_{21}\text{P}_6\text{S}_{30}$, $M_r = 6636.55 \text{ g mol}^{-1}$, monoclinic, space group $P2_1/c$, $a = 19.3022(3)$, $b = 18.3633(1)$, $c = 36.9683(4) \text{ \AA}$, $\beta = 91.501(1)^\circ$, $V = 13099.0(3) \text{ \AA}^3$, $Z = 2$. Data were collected on a Siemens SMART diffractometer ($\text{MoK}\alpha$, 153 K). Of the 59 074 reflections measured, 22 676 were employed in structure solution and refinement. An empirical absorption correction was applied (SADABS). The structure was solved by direct methods and difference Fourier maps, and was refined by a full-matrix least-squares treatment on F^2 . All non-hydrogen atoms were described anisotropically except for those of disordered solvate molecules and cations. Hydrogen atoms at calculated positions with thermal parameters $1.2 \times$ those of bonded carbon atoms were included in the final refinement. Because of disorder, some cation and solvate molecule atoms were refined on split positions. At convergence, $R_1 = 0.0868$, $wR_2 = 0.1956$, and $\text{GOF} = 1.099$. 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-116378. 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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Deposition of Data from X-Ray Structure Analyses

In order to make life easier for authors and referees the Cambridge Crystallographic Data Centre (CCDC) and the Fachinformationszentrum Karlsruhe (FIZ) have unified their procedures for the deposition of data from single-crystal X-ray structure analyses.

Prior to submitting a manuscript please deposit the data for your compound(s) **electronically** at the appropriate data base, that is, at the CCDC for organic and organometallic compounds and at the FIZ for inorganic compounds. Both data bases will be pleased to provide help (see our *Notice to Authors* in the first issue of this year). In general, you will receive a depository number from the data base within two working days after electronic deposition; please include this number with the appropriate standard text (see our Notice to Authors) in your manuscript. This will enable the referees to retrieve the structure data quickly and efficiently if they need this information to reach their decision.

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