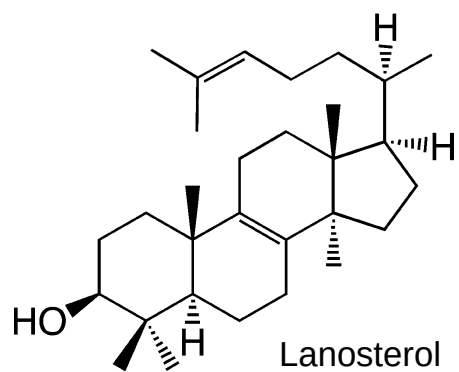
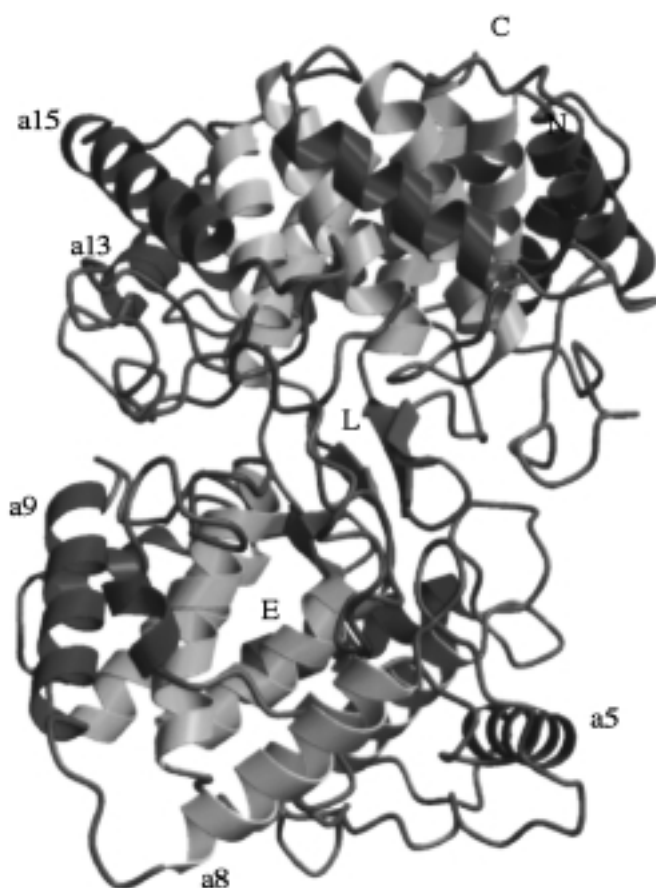
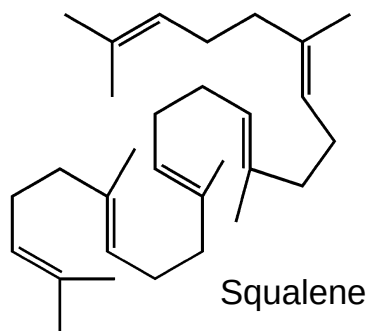
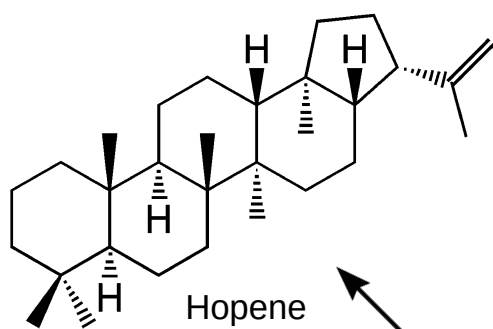


Cover Picture

K. Ulrich Wendt, Georg E. Schulz, Elias J. Corey, and David R. Liu

The cover picture shows the X-ray structure of a squalene–hopene cyclase (ribbon diagram) in the center. Squalene and 2,3-oxidosqualene cyclases catalyze the formation of up to five rings and nine stereocenters in transformations that are both complex and elegant. The triterpenes which are arranged around the structure outline the principal reaction pathways for the enzymatic conversion of oxidosqualene (upper semicircle) and squalene (lower semicircle). Further details about these fascinating reactions are discussed by Schulz, Liu et al. on p. 2812 ff.





Enzyme Mechanisms for Polycyclic Triterpene Formation

K. Ulrich Wendt, Georg E. Schulz,* Elias J. Corey, and David R. Liu*

The mechanisms by which triterpene cyclases transform olefins into complex and biologically important polycyclic products have fueled nearly half a century of intense research. Recent chemical and biological studies, together with previous findings, provide intriguing new insights into the enzymatic mechanism of triterpene formation and form a surprisingly detailed

picture of these elegant catalysts. It can be concluded that the role of the oxidosqualene cyclases involves protection of the intermediate carbocation against addition of water or deprotonation by base, thereby allowing the shift of the hydride and methyl groups along a thermodynamically and kinetically favorable cascade. Key questions in the areas of structural biology, site-

directed mutagenesis, and directed evolution are apparent, now that the first structure of a triterpene cyclase is known.

Keywords: bioorganic chemistry • cyclases • enzymatic catalysis • steroids • structural biology

1. Introduction

For nearly half a century the remarkable enzymatic cyclizations of squalene (**1**) and 2,3-oxidosqualene (**2**; Figure 1) to form polycyclic triterpenes have provided both fascination and challenge in the molecular sciences.^[1–10] These polyolefins are stereoselectively cyclized and skeletally rearranged in a single enzyme-catalyzed reaction by a wide range of microorganisms and higher eukaryotes to yield tetracyclic and pentacyclic triterpenoids including hopene (**3**), diplopterol (**4**), tetrahymanol (**5**), lanosterol (**6**), cycloartenol (**7**), and β -amyrin (**8**; Figure 1). These triterpenes in turn serve as the precursors of all hopanoids and steroids, including cholesterol, glucocorticoids, estrogens, androgens, and progesterones. The remarkably powerful and efficient transformation catalyzed by the triterpene cyclases stimulated the interest of pioneering organic chemists nearly a half century ago.^[1–9, 11] Their early chemical studies on these enzymes identified the

intermediacy of oxidosqualene in steroid biosynthesis and clarified the stereochemical course of the cyclization as well as the 1,2-hydride and methyl group migrations which follow the cyclization.^[3–9]

Early studies on triterpene cyclases focused on the use of substrate analogues to define the range of structures which the cyclases can transform and the nature of any resulting products.^[9] While these chemical studies have provided useful mechanistic information, more recent research employing molecular and structural biology in conjunction with bioorganic chemistry has yielded a wealth of new data. Taken together, these findings offer surprisingly detailed insights into the complex series of molecular events involved in squalene and oxidosqualene cyclization. Below we review these studies as applied to unraveling the complexities of triterpene cyclization.

2. The Reaction Pathway: Chemical Studies

The formation of tetracyclic or pentacyclic triterpenes from squalene (**1**) or oxidosqualene (**2**) has never been achieved efficiently in purely nonenzymatic experiments, despite numerous attempts to duplicate this powerful one-step enzyme-catalyzed transformation, outlined briefly in Figure 2. The formation of a six-membered ring by intramolecular addition of a carbocation to a carbon–carbon double bond is highly exothermic ($\Delta E = E_{\sigma\text{-CC}} - E_{\pi\text{-CC}} \approx 20 \text{ kcal mol}^{-1}$) and requires little activation (zero to a few kcal mol^{-1} , depending on solvation).^[12] Accordingly, cationic cyclizations of conformationally mobile substrates are difficult to control

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because numerous reaction modes of low activation energy compete effectively and lead to a variety of products.

2.1. Oxidosqualene – Lanosterol Cyclase

2.1.1. Substrate Conformation

It is therefore abundantly clear that tight enzymatic control of substrate conformation is a prerequisite for the highly selective formation of products such as lanosterol or hopene. As is evident from Figure 2, the enzyme-imposed substrate conformation is notably different for the formation of lanosterol (**6**) and hopene (**3**). While squalene–hopene cyclase allows an all chair conformation of squalene, oxidosqualene–lanosterol cyclase enforces a chair–boat–chair conformation of its substrate, as demonstrated by the configuration of trapped intermediates described below.

Experiments with the 20-thia analogue **9** of oxidosqualene (Figure 3) and the lanosterol synthase of *Saccharomyces cerevisiae* demonstrated that this compound was neither a substrate nor an efficient inhibitor of the enzyme,^[13] in dramatic contrast to 20-oxa-oxidosqualene (**10**)^[13, 14] which undergoes cyclization to two tetracyclic products (see below). It has been argued that the larger size of sulfur relative to CH₂ or O may result in a somewhat different conformation of **9**, poorer binding to the cyclase, and an enzyme–substrate complex in which the enzymatic acid is not correctly positioned to activate the oxirane function. The reported lack of action of rat liver lanosterol synthase on 11-fluoro-oxidosqualene (**11**)^[15] may be caused by a similar effect, since the steric or electronic differences in this analogue may disfavor the chair–boat–chair folding arrangement, possibly due to steric repulsion between the fluorine at C11 and the methyl group at C6, as is evident in Figure 2. Implicit in this argument is the assumption that the conformer of oxidosqualene which binds to and complements the enzyme

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Karl Ulrich Wendt was born in 1968 in Essen, Germany. He received his Diploma (1994) and Ph.D. (1998) from the University of Freiburg, Germany, where he worked under the direction of G. E. Schulz on the expression, purification, and X-ray structural analysis of squalene–hopene cyclase. In 1998 he joined the groups of P. G. Schultz and R. C. Stevens at the University of California, Berkeley, working on the structural consequences of antibody maturation. He has done further postdoctoral work in the protein crystallography group in the Department of Protein Engineering at Genentech, Inc. in South San Francisco, USA.

Elias J. Corey, born in 1928 in Methuen, 30 miles north of Boston, studied chemistry from 1945–1950 at the Massachusetts Institute of Technology, where he gained his doctorate for work on synthetic penicillins under the supervision of J. C. Sheehan. In January 1951 he joined the University of Illinois at Urbana-Champaign as an Instructor in Chemistry and was promoted in 1956 to full Professor. Since 1959 he has been at Harvard University. For as long as he can remember he has enjoyed study, adventure, and discovery.

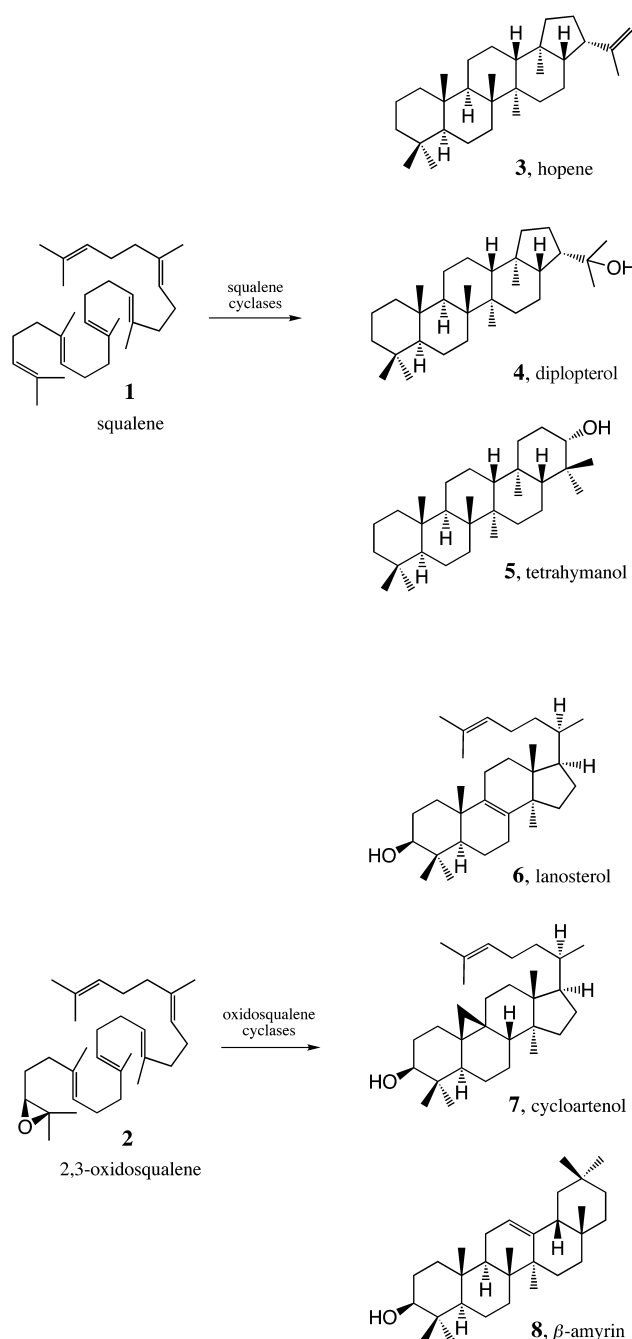


Figure 1. Substrates and products of several triterpene cyclase enzymes.

pocket has a molecular geometry which allows cyclization to proceed with a minimum of conformational change in the substrate once the reaction is initiated. This notion is attractive from a chemical point of view because it restricts the modes of reaction which are available to any intermediate carbocations and leads to the most efficient channeling of the cyclization to one product.

Consistent with the above possibilities, it has been reported that neither 14-fluorooxidosqualene (**12**) nor 14,15-dihydrooxidosqualene (**13**) afford any cyclic products when incubated with rat liver oxidosqualene cyclase (Figure 3).^[16] The 14-fluoro substituent may interact repulsively with either the methyl group at C10 or a nearest neighbor from the

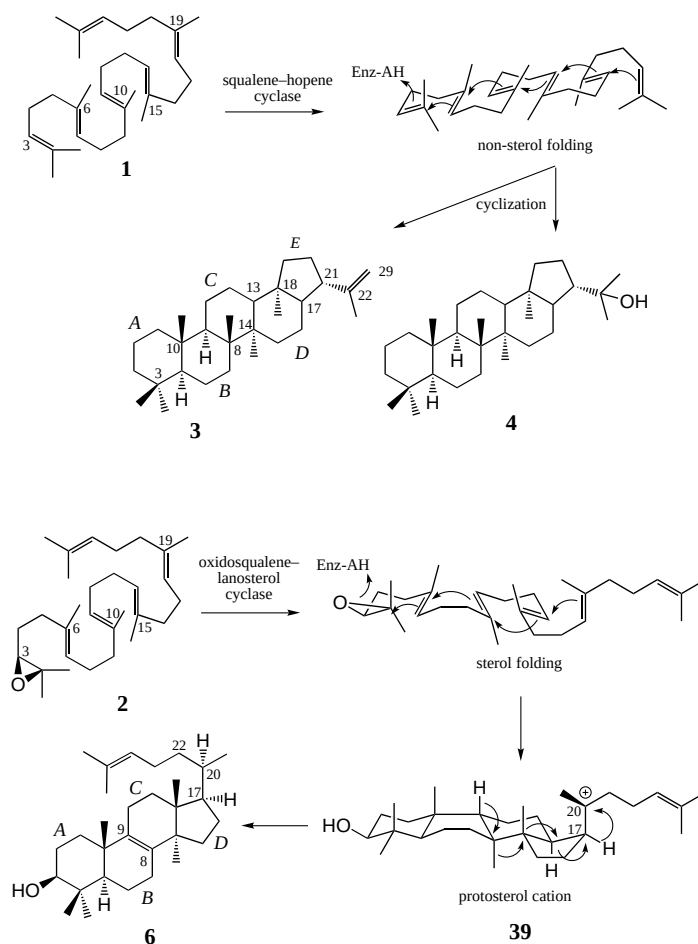


Figure 2. Putative substrate conformation and overall mechanism for reactions catalyzed by oxidosqualene-lanosterol cyclase and squalene-hopene cyclase.

enzyme binding pocket which would lead to incorrect folding of substrate and loss of epoxide activation.

2.1.2. Epoxide Opening and A-Ring Formation

The chemical cyclization of oxidosqualene must be initiated by electrophilic epoxide activation. Oxidosqualene is completely stable in neutral media and is even quite stable in glacial acetic acid at room temperature for up to one day. A more powerful Brønsted acid such as trichloroacetic acid is required for sufficiently strong epoxide activation to promote cyclization on a shorter timescale.^[10] As a single aspartic acid residue (D456) in the oxidosqualene cyclase (lanosterol synthase) of *S. cerevisiae* was found to be critical for cyclization^[17, 18] and Lewis acidic metals are absent in the purified active enzyme,^[17] a kinetic and mechanistic study of proton catalysis of cyclization was undertaken with simpler models of oxidosqualene **14–16** (Figure 4).^[10]

The rates of proton-catalyzed carbocyclization of substrates **14** and **15** were considerably greater than the rate of proton-catalyzed carbocation formation from the noncyclizable substrate **16**, consistent with concerted C–O cleavage and cyclohexane C–C bond formation for **14** and **15**.^[10] As expected, anchimeric assistance to C–O cleavage by the

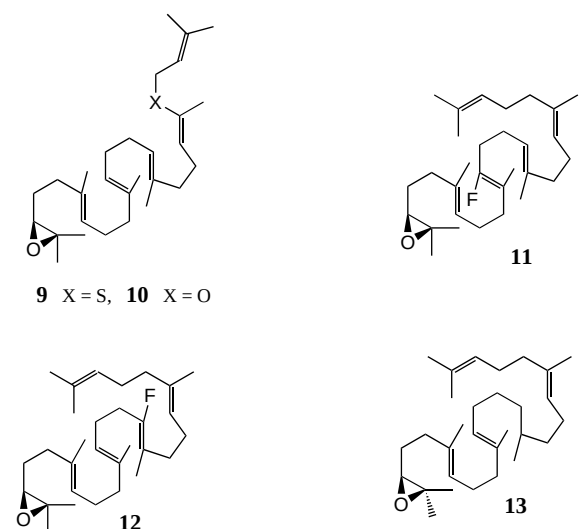


Figure 3. Substrate analogues of oxidosqualene-lanosterol cyclases which provide insight into substrate conformation.^[13–16]

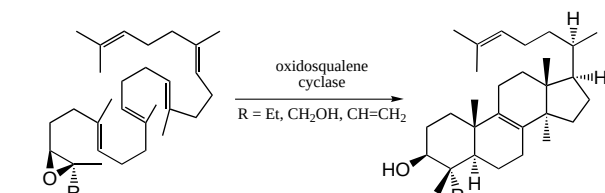
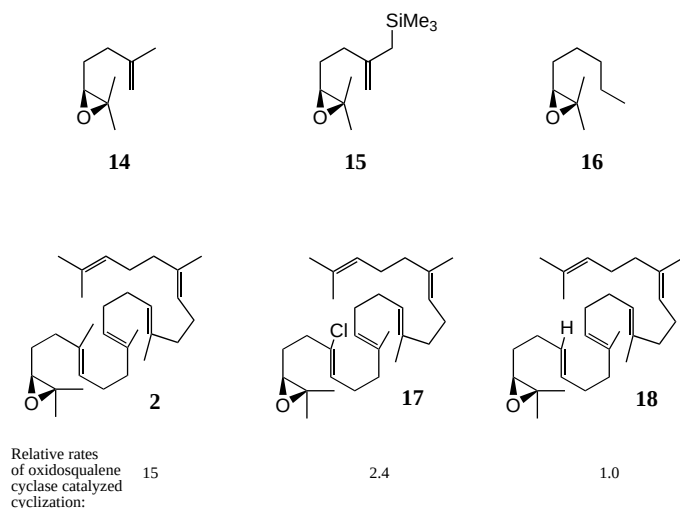


Figure 4. Evidence for concerted epoxide opening and A-ring closure catalyzed by oxidosqualene – lanosterol cyclases.^[17]

proximate double bond was greater for the more nucleophilic olefin **15** than for the less π -nucleophilic **14**.^[10] Since the conformers of **14** and **15** which correspond to the optimal geometry for cation– π -olefin cyclization constitute only a minor fraction of the rotamers of **14** and **15** which are present in solution, there would presumably be even greater anchimeric acceleration for the cyclization of the optimally prefolded conformers. Thus, the combination of optimal placement of the activating proton source relative to the epoxide oxygen and the optimal folding of the substrate could together produce rapid catalytic cyclization, which would explain how an enzyme-provided carboxylic acid could

initiate cyclization of oxidosqualene. Even greater accelerations would be expected on chemical and mechanistic grounds if the enzyme provides one or two hydrogen-bond donors to the key catalytic carboxylic acid.

Early qualitative studies of Lewis acid initiated cyclizations of oxidosqualene analogues suggested that during SnCl_4 -catalyzed reaction in benzene, epoxide cleavage and cyclohexane ring formation are concerted.^[19] Recent kinetic studies of the *S. cerevisiae* lanosterol synthase demonstrate that this enzyme catalyzes the cyclization of epoxides **2**, **17**, and **18** with the relative rates shown in Figure 4. The correlation between the observed rates of cyclization and π -nucleophilicity of the C6,C7 double bond of substrates **2**, **17**, and **18** provides additional evidence for concerted C–O cleavage and A-ring formation for the enzymatic cyclization.^[17] The configuration of products resulting from the enzymatic cyclization of oxidosqualene analogues bearing modified α -C2 methyl groups (Figure 4)^[20–23] demonstrates that A-ring closure occurs faster than rotation about the C2–C3 bond. This observation is also consistent with, although not exclusively indicative of, a concerted mechanism. Finally, recent computational results are also in agreement with these experimental findings suggesting concerted epoxide opening and A-ring formation.^[12, 24]

2.1.3. B- and C-ring cyclization

Formation of the B ring as a boat conformer probably occurs very rapidly once positive charge builds on C5 of the A ring. It is not known, however, if there is any temporal overlap between the formation of the A and B rings. The closure of the third ring has also long been enigmatic because direct formation of a six-membered C ring would represent an anti-Markovnikov mode of addition to a double bond. Closure of a six-membered ring would, however, be stereo-electronically somewhat more favorable than Markovnikov cyclization to form a five-membered C-ring. A key piece of information relevant to this dilemma was obtained from the products arising from the cyclization of 20-oxa-oxidosqualene (**10**) by the lanosterol synthase of *S. cerevisiae*. In addition to the expected 6-6-6-5 fused ring product **19** (40%), incubation of **10** also affords the 6-6-5 fused ring product **20** (3.4%).^[13] The formation of **20** is most easily explained by internal trapping of the carbocation **21** through intramolecular reaction with the electron-rich C18,C19 double bond (Figure 5).

Additional substrate analogue incubations provide further evidence for a five-membered C-ring closure followed by a ring expansion (Figure 6). Oxidosqualene cyclase processes 15-ethyloxidosqualene (**22**) into a 71:29 mixture of ethyl lanosterol and a 6-6-5 tricyclic product,^[25] converts 18,19-dihydrooxidosqualene (**23**) into a 6-6-5 tricyclic product,^[26] transforms the 20-carbon truncated analogue **24** of oxidosqualene into three 6-6-5 tricycles,^[27] and cyclizes 10,15-didemethyloxidosqualene (**25**) into predominantly a 6-6-5-4 system in the product **26** (Figure 6).^[28] The structural diversity of these substrate analogues makes it unlikely that they all cyclize following a pathway that is not relevant to that of the natural substrate. It should be noted that the substrate

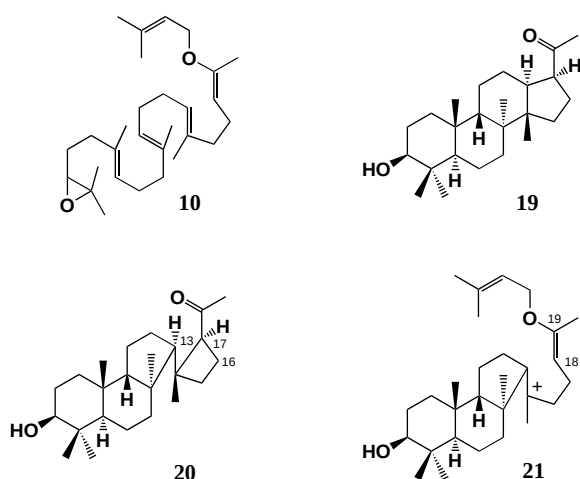


Figure 5. Substrate analogues and products of oxidosqualene cyclase which are suggestive of a five-membered C-ring intermediate.^[13]

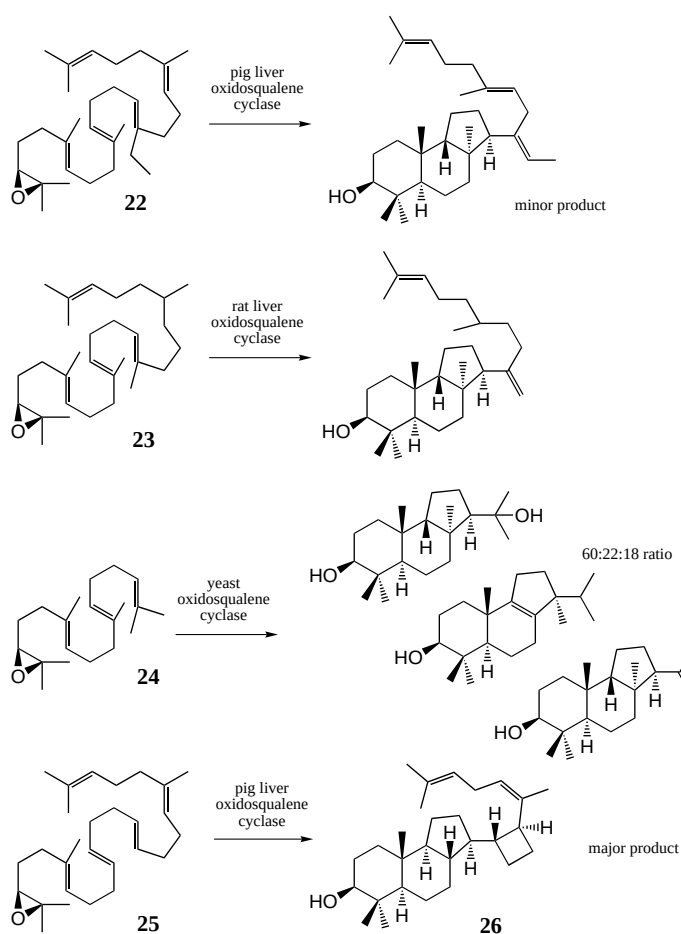


Figure 6. 6-6-5 cyclization products formed by oxidosqualene cyclases.^[25–28]

conformation which is required to explain the stereochemistry of the conversion $25 \rightarrow 26$ is the nonsteroidal B-chair type, implying that the cyclase not only recognizes the difference between methyl and hydride substituents at C10, but also that this structural feature is critical to the proper double bond face selectivity in the closure of the B ring. This finding also argues that the fit between the substrate and the enzyme

pocket is remarkably precise in some areas. Collectively these results suggest that substrate perturbations around C10, C15, and C19 of oxidosqualene reduce the enzyme's ability to effect ring expansion relative to further cyclization of the C15 carbocation. Computational evidence provides further support for the 6-6-5 to 6-6-6 rearrangement pathway.^[12]

2.1.4. Protosterol Cation Formation and Rearrangement to Lanosterol

Early analyses of the stereochemistry of sterol biosynthesis assumed that the enzymatic conversion of oxidosqualene to lanosterol proceeds via a protosterol cation in which the cationic side chain at C17 is α -oriented (steroid notation) as shown in compound **27** (Figure 7).^[3] A problem with the

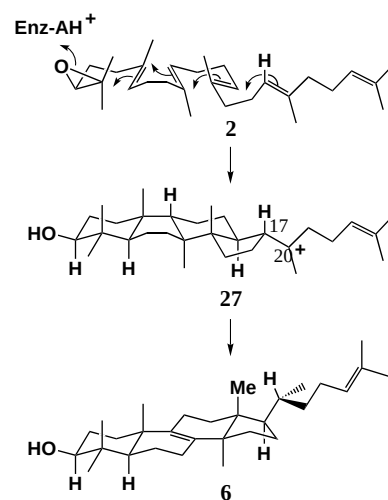


Figure 7. The formerly postulated, incorrect C17 stereochemistry of protosterol requires a large side-chain rotation prior to rearrangement to account for the observed stereochemistry at C20.^[31]

intermediacy of **27** is that it would be expected to lead, after hydride migration from C17 to C20, not to the natural sterol $20R$ configuration, but to the unnatural $20S$ arrangement. Starting from the geometry of **27** produced immediately after D ring closure, a rotation about the C17–C20 bond of 120° is required prior to hydride migration in order to produce the natural $20R$ configuration of lanosterol (**6**), whereas only a 60° rotation is required to produce the unnatural $20S$ configuration (Figure 7). Perhaps because of this difficulty, it was proposed that the initial tetracyclic intermediate may not be cation **27** but a covalent adduct formed by reaction of **27** with a nucleophilic group of the cyclase.^[29, 30] Isolation of a trapped protosterol (methyl ketone **19**) from the enzyme-catalyzed cyclization of 20-oxa-oxidosqualene (**10**), however, demonstrated the β (pseudoaxial side chain) rather than α (pseudoequatorial side chain) stereochemistry at C17 of protosterol (Figure 8).^[31] This finding removed the need to invoke either large (120°) rotation around the C17–C20 bond prior to rearrangement, or covalent attachment of a transient enzyme nucleophile. Interestingly, protosterol analogue **28** is only a weak inhibitor of yeast oxidosqualene cyclase (with an IC_{50} which is 55-fold higher than that of 2,3-iminosqualene (**29**)),

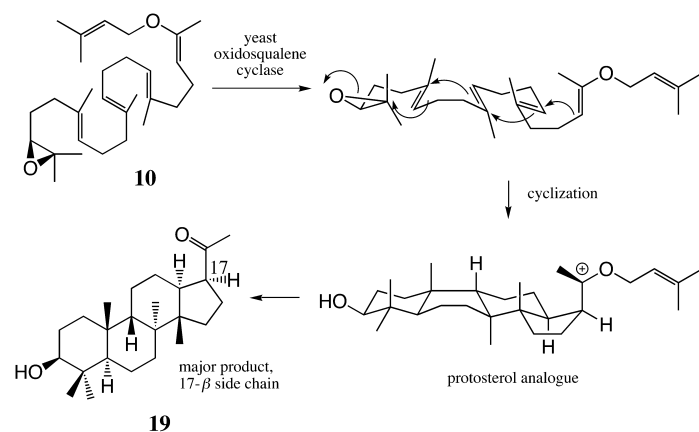


Figure 8. Trapping of an enzyme-cyclized protosterol analogue.^[31]

suggesting that the protosterol cation is not bound very snugly by the enzyme (Figure 9).^[32] The 17 β orientation of the side chain in the protosterol cation was also demonstrated by the action of the yeast cyclase on 20,21-dehydrooxidosqualene (**30**). Enzymatic cyclization of **30** yielded protosterol derivative **31** (Figure 10), which was identical with a totally synthetic sample.^[14]

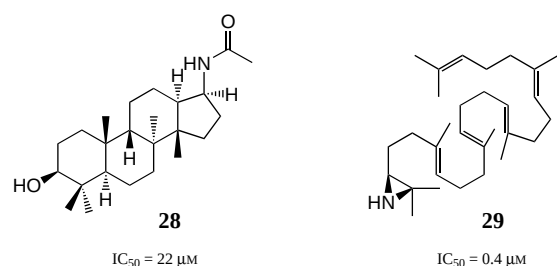


Figure 9. A comparison of oxidosqualene cyclase inhibitors suggests that the protosterol cation is weakly bound by the enzyme.^[32]

The isolation of this trapped protosterol analogue enabled the first examination of the rearrangement process uncoupled from the cyclization events. Nonenzymatic reaction of the benzoate of **32** (that is, **33**) with BF_3 in dichloromethane at $-90^\circ C$ for 3 min produced 24,25-dihydroparkeol benzoate (**34**) via the protosterol cation **35** (Figure 10).^[31] Similarly, the BF_3 -induced rearrangement of the C20 diastereomer of **33** produced mainly the C20 diastereomer of **34**.^[14] The BF_3 -catalyzed rearrangements of the 17 α epimer of **33**, and its C20 epimer, in contrast, are nonselective at C20, each giving a 1:1 mixture of C20 epimers of dihydroparkeol benzoate (**36**).^[33] Consistent with these nonenzymatic findings, the enzymatic incubation of substrate analogues bearing significant structural differences from oxidosqualene still leads to some hydride and methyl group rearrangement. The cyclase-catalyzed cyclization of a precyclized (D ring) analogue of oxidosqualene (**38**) yields rearranged lanosterol-like products (Figure 11).^[34, 35] while the enzymatic cyclization of a truncated substrate analogue (**24**) which yields unnatural 6-6-5 tricycles is also accompanied by methyl group migration (Figure 6).^[27] Importantly, even the nonenzymatic Lewis acid catalyzed cyclization of oxidosqualene affords at least one

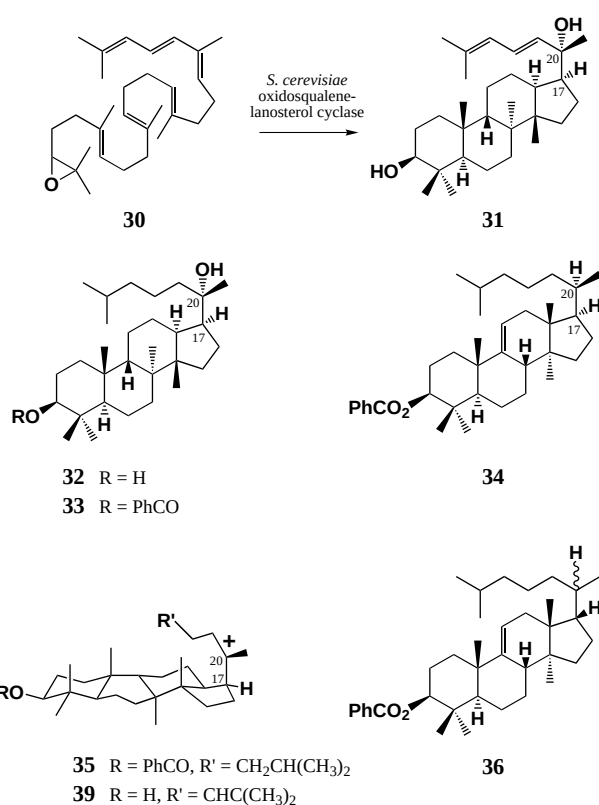


Figure 10. Products which are suggestive of the stereochemical pathway of nonenzymatic rearrangement.^[33]

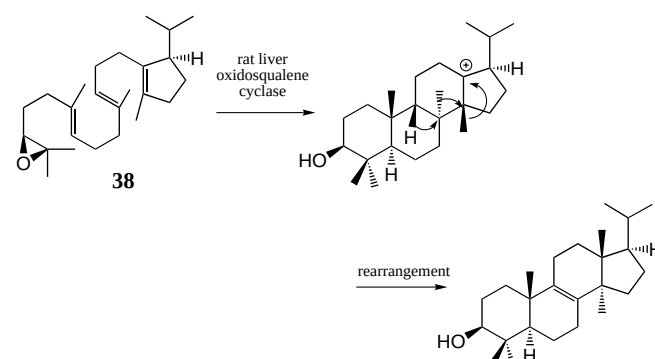


Figure 11. Enzymatic cyclization and rearrangement of a precyclized oxidosqualene analogue.^[34]

product arising from methyl group migration, further suggesting the ease with which these rearrangement processes naturally occur (Figure 12).^[36]

These results provide several crucial insights into the molecular details of the cyclization and rearrangement steps of lanosterol biosynthesis. First, the protosterol cation **39** (Figures 2 and 10) undergoes C17–C20 hydride migration to form the 20 R product at a rate which is rapid relative to rotation about the C17–C20 bond. Second, this bond rotation is greatly retarded in the 17 β protosterol side-chain arrangement, probably because of repulsion with the nearby 14 β -methyl group of **39**. Third, the pathway involving a 17 β side chain for the protosterol cation, as in **39**, allows for much more rigorous control by the enzyme of the C20 configuration in lanosterol than would the hypothetical pathway with the 17 α

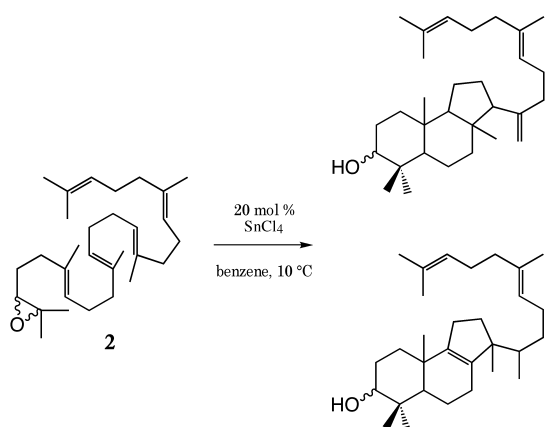


Figure 12. Nonenzymatic cyclization of oxidosqualene.^[36]

orientation of the side chain, corresponding to **27** of Figure 7. Finally, it is clear that the rearrangements of hydride and methyl substituents which convert the protosterol cation into lanosterol can proceed spontaneously without intervention by lanosterol synthase, except for the final proton removal step. This deprotonation clearly must be under rigorous control by the enzyme. The enzyme's primary role during the rearrangement of protosterol, therefore, may simply be to protect the intermediate cations from quenching by water (by exclusion of water from the active site) and from elimination (by exclusion of bases from the active site) prior to deprotonation by a well-positioned enzymatic base.

2.2. Squalene–Hopene Cyclase

2.2.1. Initiation and Early Cyclization Events

The bacterial squalene cyclases catalyze a stereochemically and mechanistically simpler process than the eukaryotic oxidosqualene cyclases. These squalene cyclases do not employ the same substrate conformation as lanosterol synthase but instead allow the substrate to adopt an all chair-like geometry (Figure 2). Furthermore, skeletal rearrangement following cyclization is not generally observed in the unperturbed catalytic pathways of these enzymes, unlike their oxidosqualene cyclase counterparts. The lower basicity of a carbon–carbon double bond relative to the epoxide group suggests that the squalene cyclases provide a catalytic acid at least as strong as that used to initiate the cyclization of oxidosqualene. Consistent with this hypothesis, the bacterial squalene cyclases accept oxidosqualene and many of its analogues as substrates for cyclization.^[9] While no chemical studies on squalene cyclases have elucidated the mechanism of A- and B-ring formation, it is tempting to assume, in analogy with the oxidosqualene cyclases, that A-ring closure is concerted with olefin protonation since anchimeric assistance may significantly accelerate protonation of the relatively nonbasic 2,3-olefin.

In contrast with the oxidosqualene cyclases, substrate analogue studies suggesting a five-membered C-ring intermediate followed by ring expansion during the cyclization of

squalene by squalene–hopene cyclase have only emerged very recently. Squalene–hopene cyclase from *Alicyclobacillus acidocaldarius* processes a truncated alcohol **40** into a 6-6-5 product consistent with trapping of a Markovnikov cation generated from five-membered C-ring closure (Figure 13).^[37]

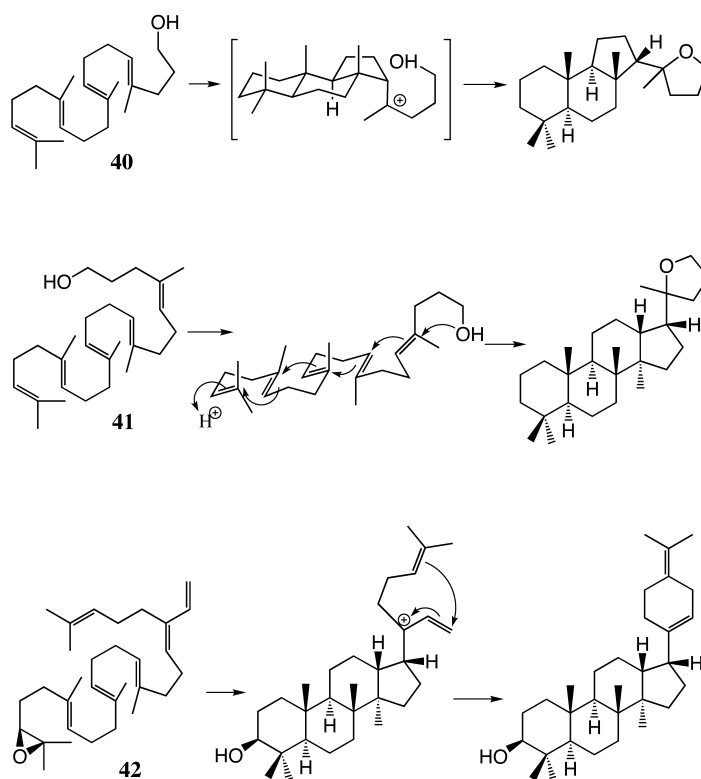


Figure 13. Evidence for five-membered D-ring formation during squalene cyclization.^[38, 39] In each case, squalene–hopene cyclase from *A. acidocaldarius* was used.

No 6-6-6 products corresponding to trapping of an anti-Markovnikov cation arising from six-membered C-ring formation were detected. The prospects of a Markovnikov versus anti-Markovnikov mechanism for C-ring closure are discussed further in light of recent structural biology and site-directed mutagenesis studies (see below).

2.2.2. D-Ring Formation

Chemical studies strongly suggest that D-ring cyclization catalyzed by squalene cyclases proceeds through a five-membered ring closure to afford a Markovnikov cation followed by ring expansion to yield a 6-6-6-6 system in a mechanism similar to C-ring closure catalyzed by the oxidosqualene–lanosterol cyclases. Incubation of a truncated squalene analogue **41** with squalene–hopene cyclase leads to almost quantitative formation of a 6-6-6-5 product which may arise from trapping of an intermediate C18 Markovnikov carbocation (Figure 13).^[38] Similarly, incubation of 29-methylidene-oxidosqualene (**42**) with squalene–hopene cyclase provides a 6-6-6-5 product (Figure 13) and concomitant irreversible inactivation of the cyclase (see below).^[39] Cycli-

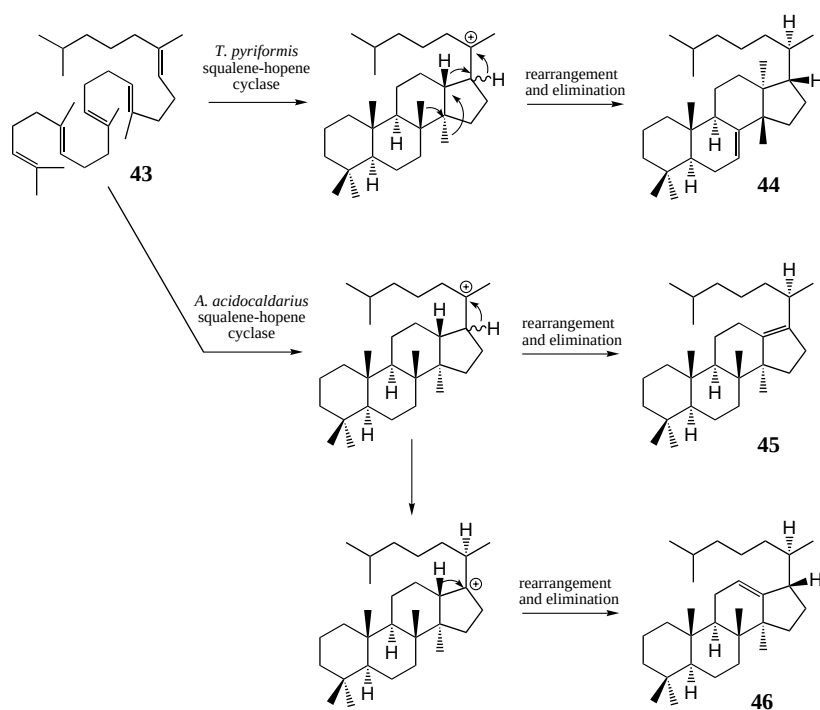


Figure 14. Additional evidence for five-membered D-ring formation during squalene cyclization.^[40, 41]

zation of 2,3-dihydrosqualene (**43**) also leads to trapping of a 6-6-6-5 intermediate yielding three products (**44–46**) from two different squalene cyclases (Figure 14).^[40, 41] Importantly, 6-6-6-5 compounds (**47–51**) have also been observed as minor products in the natural turnover of squalene with squalene–hopene cyclase from *A. acidocaldarius* (Figure 15).^[42] This finding represents the first evidence for five-membered D-ring closure that is free from any bias which could be introduced by stereochemical perturbations arising from substrate analogues or enzyme active-site mutants.

2.2.3. E-Ring Formation, Rearrangement, and Elimination

The importance of generating a Markovnikov carbocation during enzyme-catalyzed five-membered E-ring closure is exemplified by the finding that squalene analogues **52** and **53**, which lack one terminal methyl group and therefore cannot form a tertiary carbocation upon E-ring cyclization, yield tetrahymanol-like 6-6-6-6-6 products **54** and **55** upon incubation with squalene–hopene cyclase (Figure 16).^[43] The structural elucidation of tetracyclic compounds **44–46** arising from incubation of 2,3-dihydrosqualene (**43**) with *Tetrahymena pyriformis* and *A. acidocaldarius* squalene cyclases indicate the occurrence of hydride and methyl group rearrangements in these products (Figure 14).^[9] Moreover, the collection of side products arising from the action of *A. acidocaldarius* squalene–hopene cyclase on its natural substrate also includes a number of partially rearranged tetracycles **49–51** (Figure 15).^[42] These findings are consistent with the oxido-squalene cyclase model described above, in which skeletal rearrangement occurs spontaneously in the absence of base or water to quench carbocations. The partitioning between

hopene (**3**; 80 %) and the water-quenched diplopterol (**4**; 20 %) during the natural action of squalene–hopene cyclases suggests significant water accessibility to the termination region of the active site.^[44]

3. The Reaction Pathway: Biological Studies

Molecular and structural biologists have joined chemists in probing the mechanism of enzyme-catalyzed triterpene formation. The last seven years have witnessed the cloning and sequence elucidation of at least 15 putative triterpene cyclases: Those from *Homo sapiens*,^[45] *Rattus norvegicus*,^[46, 47] *Candida albicans*,^[48, 49] *Saccharomyces cerevisiae*,^[50, 51] *Schizosaccharomyces pombe*,^[52] *Arabidopsis thaliana*,^[53] *Panax ginseng*,^[54] *Alicyclobacillus acidocaldarius*,^[55] *Zymomonas mobilis*,^[56] *Bradyrhizobium japonicum*,^[57] *Methylococcus capsulatus*,^[58] *Rhodopseudomonas palustris*,^[58] *Rhizobium*,^[59] *Synechocystis*,^[60] and *Bacillus subtilis*.^[61] In 1997 the first three-dimensional structure of a triterpene cyclase (squalene–hopene cyclase from *A. acidocaldarius*) was solved.^[62–64] This structure, together with additional biological studies, has produced a wealth of mechanistic insight into both squalene and oxidosqualene cyclases.

3.1. The Squalene–Hopene Cyclase Structure

Squalene–hopene cyclase is a homodimeric enzyme with 631 amino acids (71.5 kD) per subunit.^[55, 63, 65] The peptide chain of each subunit is organized in two mainly α -helical domains, which together form a dumbbell-shaped molecule of 50 Å diameter and 70 Å height (Figure 17). Domain 1 is a regular (α/α)₆ barrel, while domain 2 assumes a similar but less regular α -barrel fold. The structural alignment of both domains indicates that domain 2 may be derived from domain 1 by an early gene duplication.^[66] The two domains are connected by long loops which together enclose a large central cavity of 1200 Å³ (Figure 17). A second cavity of 400 Å³ (the “upper cavity”) is located in the center of the (α/α)₆ barrel in domain 1.^[64]

The structure suggests that the active site is localized in the enzyme’s central cavity, where the competitive inhibitor *N,N*-dimethyldodecylamine-*N*-oxide (LDAO)^[67] is bound (Figure 18).^[63] This view is confirmed by an abundance of biochemical and structural evidence. Residues Asp376 and Asp377, which contact the upper part of the central cavity, have been shown by site-directed mutagenesis to be essential for catalysis in squalene–hopene cyclase.^[68] Mutations at nearby residues Asp374 and His451 also lower the catalytic efficiency of the enzyme.^[69] Asp456 of *S. cerevisiae* oxido-squalene–lanosterol cyclase corresponds to Asp376 by se-

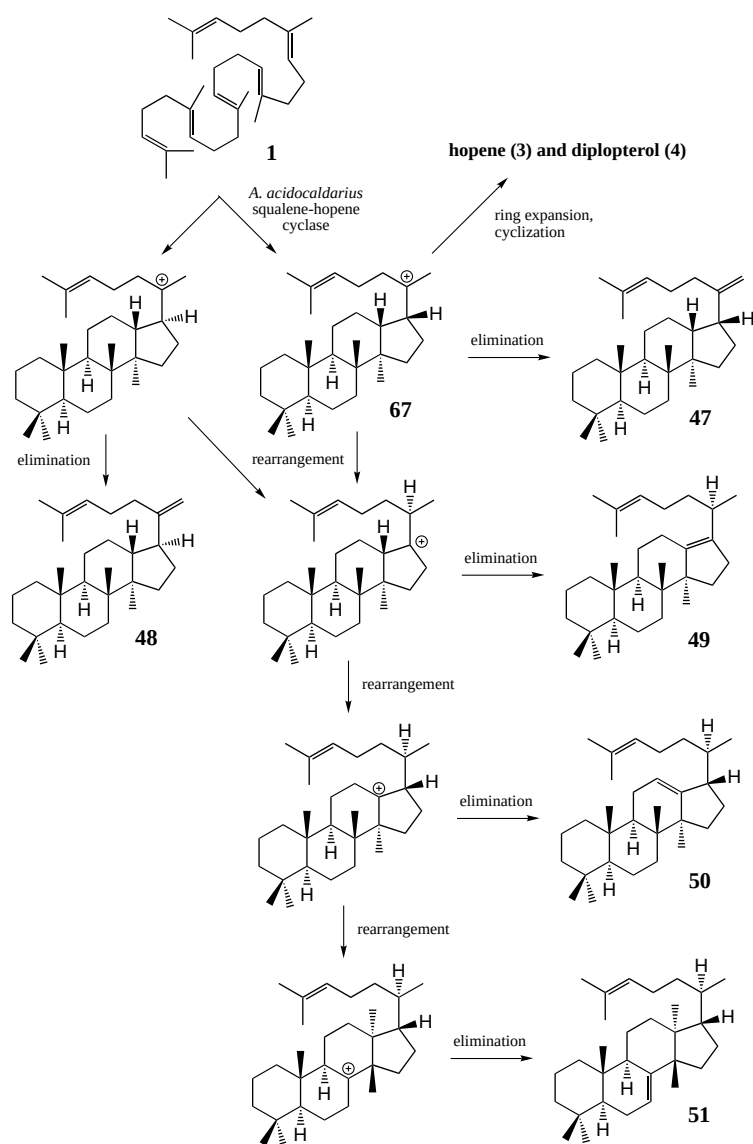


Figure 15. Formation of minor side products in the natural reaction catalyzed by squalene-hopene cyclase.^[42]

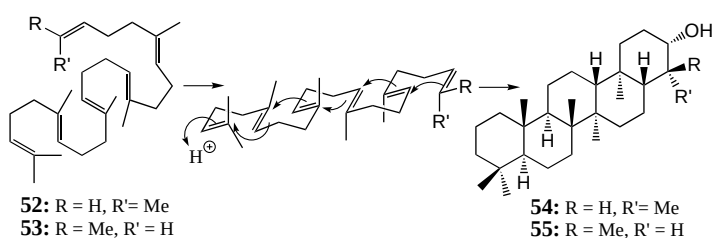


Figure 16. Substrate analogues that yield tetrahymanol-like 6-6-6-6 products upon cyclization with squalene-hopene cyclase.

quence alignment and is also required for enzyme activity.^[18] Additional evidence for placing the active site of squalene-hopene cyclase within the central cavity emerges from the mutagenesis of residues lining this cavity, which results in the formation of altered products.^[38, 69–73] Furthermore, several mechanism-based inhibitors of oxidosqualene-lanosterol cyclases covalently label amino acids corresponding to

central-cavity residues of squalene-hopene cyclase.^[18, 74] Finally, the topological position of the active site with respect to the (α/α)-barrel folds is equivalent to the position of active sites commonly found in other (α/α)-barrel proteins.^[63]

Squalene and oxidosqualene cyclases share a unique sequence repeat (referred to as a “QW repeat”) with the consensus sequence: a-b-x₂₋₃-c-d-x₃-Gln-x₂₋₅-Gly-x-Trp, where a = Lys or Arg, b = Gly or Ala, c = Phe, Tyr, or Trp, and x = any residue.^[75] This repeat typically occurs five times in oxidosqualene cyclases and up to eight times in the squalene cyclases. The high conservation of this repeat throughout the triterpene cyclases has led to hypothesis that the conserved Trp may be involved in the stabilization of intermediate carbocations,^[76] consistent with earlier active-site models which proposed a structurally defined^[9] arrangement of aromatic amino acids to stabilize carbocationic intermediates.^[50, 77, 78] Such an arrangement has also been suggested to induce the different transition state conformations in squalene versus oxidosqualene cyclases.^[79] The structure of squalene-hopene cyclase, however, demonstrates that these QW-repeats are not located in the active-site cavity but rather at the surface of the enzyme (Figure 17) where the conserved amino acid residues of the repeats form an intricate network of hydrogen-bonding and hydrophobic interactions that connect the α helices of the (α/α)₆ barrel of domain 1.^[63, 64] It has been proposed that this arrangement might stabilize the triterpene cyclases against the reaction enthalpy released during cyclization.^[63]

3.2. Membrane Binding

Cellular localization and solubilization characteristics suggest that the triterpene cyclases are integral membrane proteins.^[44, 80] The three-dimensional structure of squalene-hopene cyclase identifies the enzyme as a monotopic membrane protein^[81] that interacts with the nonpolar portion of the phospholipid bilayer without protruding through the membrane (Figure 19).^[64] The membrane-binding region forms a flat nonpolar surface of 1600 Å², the center of which is an opening to an extended channel connecting the active-site cavity with the interior of the phospholipid bilayer.^[63, 64] This arrangement most likely provides a path for the exchange of squalene (1) and the products hopene (3) and diplopterol (4) between this cavity and the nonpolar section of the lipid bilayer where these nonpolar compounds are expected to reside.

The large size and rigidity of the cyclization products together with the hydrophobicity of the active site raise the interesting possibility that triterpene cyclases may undergo relative domain motions during product release. No indication for this, however, has yet been observed in the squalene-hopene cyclase crystal structures. An analysis of

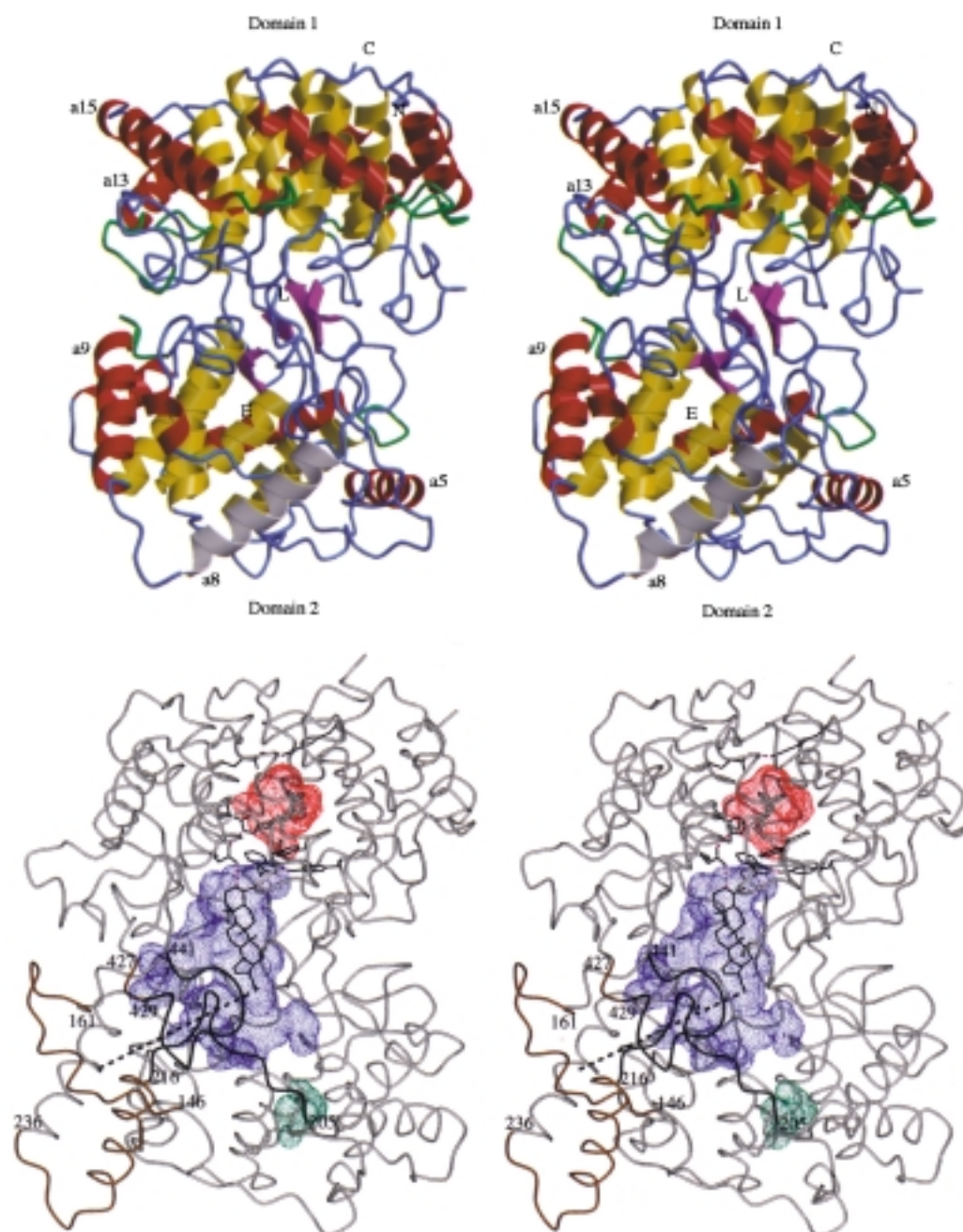


Figure 17. Overall structure of *A. acidocaldarius* squalene-hopene cyclase. Upper stereo view: Ribbon representation of one subunit of the dimeric enzyme with labels at the termini of the peptide chain (N and C), the position of the competitive inhibitor LDAO (L), and the entrance of the active-site channel (E). Color coding: inner and outer helices of the (α/α) barrels are yellow and red, respectively, the β structure is purple, the QW motifs are green, and helix α -8 in the suggested membrane-binding region is white. Lower stereo view: Ca chain trace of the cyclase. The three main cavities^[103] within the structure are colored: The active-site cavity is blue, the upper water-filled cavity is red, and a smaller water-filled cavity in domain 2 is green. Highlighted are the membrane-binding region (yellow; residues 146–161, 216–236, and 427–429) and mobile loops (dark; residues 205–216 and 429–441) that presumably open for the substrate entrance through a channel (dashed line) that connects the membrane binding region with the active site. The picture contains a modeled molecule of the product hopene (see text).

crystallographic B-factors in the enzyme structure suggests that the gating loops of the entrance channel are mobile enough to permit the passage of the bulky and rigid nonpolar products to and from the nonpolar section of the membrane.^[63, 64] In the absence of additional structural data, it remains to be determined if or how the enzyme avoids energetically unfavorable scenarios between turnovers, such as the uptake of water into the nonpolar 1200 Å³ central cavity.

The membrane-binding regions are oriented approximately in parallel within the dimer of squalene-hopene cyclase (Figure 19a). Such an arrangement is also observed in dimeric prostaglandin-H(2) synthases I and II, which are the only other structurally characterized monotopic membrane proteins (Figure 19c).^[82–84] In the squalene-hopene cyclase structure, detergent ligands are bound in a nonpolar pocket at the dimer interface. This pocket may accommodate lipid molecules in vivo and it has been suggested that lipid binding

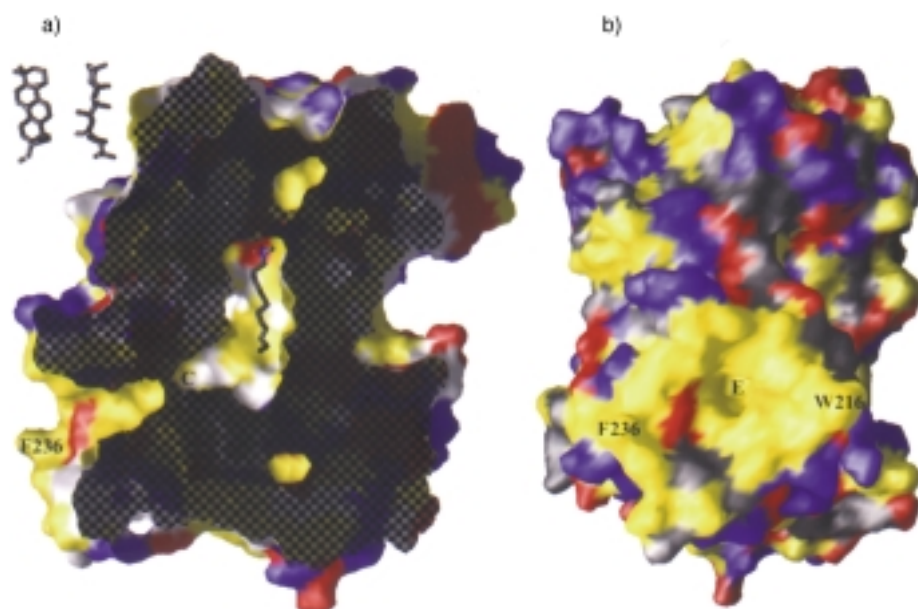


Figure 18. Surface representation^[104] of one subunit of *A. acidocaldarius* squalene-hopene cyclase with nonpolar areas (yellow), positively charged areas (blue), and negatively charged areas (red). a) The surface cut (cutting plane checkered) presents a view of the active-site cavity with the bound inhibitor LDAO. The nonpolar entrance channel runs into the extended nonpolar region on the left, which is presumably responsible for integral membrane binding. The channel constriction (C) seems to be closed but is flexible enough to permit substrate passage. In the upper left area, hopane is depicted from two views. b) View of the nonpolar membrane-binding surface centered around the channel entrance (E), rotated by 90° around a vertical axis from (a). A ring of positively charged Arg and Lys residues (blue) around this nonpolar plateau may interact with the membrane's polar phospholipid head groups.

to these pockets may shield the polar dimer interface from water and, thus, link dimer assembly with the insertion of squalene hopene-cyclase into the membrane.^[64]

3.3. The Active Site

The active-site cavity of squalene-hopene cyclase consists of an extended central nonpolar section and two smaller polar patches at the top (around Asp376) and bottom (around Glu45) of the cavity (Figure 20).^[63] Several of the residues lining this cavity are conserved between squalene and oxidosqualene cyclases (Figure 21). The central section contains a chain of conserved aromatic amino acids that face the cavity and are likely to stabilize cationic intermediates. The top and bottom sections of the cavity are formed by polar hydrogen-bonding networks around Asp376 and Glu45, which were suggested to participate in the initial olefin or epoxide protonation and in the final deprotonation step, respectively.^[63]

The assignment of the catalytic acid to one of the polar patches is a prerequisite for further mechanistic discussion. A structure-function examination of these enzymes allows this assignment.^[63] The protonation steps catalyzed by squalene and oxidosqualene cyclases occur in the same portion of the substrate, while the deprotonation steps occur in different portions of the product skeleton. Since the mutation of the corresponding residues Asp376 (*A. acidocaldarius* squalene-hopene cyclase) and Asp456 (*S. cerevisiae* oxidosqualene-lanosterol cyclase) in the polar patch at the top of the

active-site cavity inactivates these two enzymes,^[18, 68] and since the inhibitor LDAO binds with its polar head at this position,^[63] it is likely that these residues serve as the proton donor. Consistent with this hypothesis, residues lining the active-site cavity show a gradient in conservation between squalene and oxidosqualene cyclases with high conservation near Asp376 at the top and low conservation near Glu45 at the bottom of the cavity (Figure 21). In view of the similar initiation steps in squalene and oxidosqualene cyclases compared to their diverse termination steps, this evidence supports the role of Asp376 and Asp456 as the catalytic acids that initiate the cyclizations of squalene and oxidosqualene, respectively.^[63]

3.4. Mechanism-Based Suicide Inhibitors

The suggested function of Asp376 (*A. acidocaldarius* squalene-hopene cyclase) or Asp456 (*S. cerevisiae* oxidosqualene-lanosterol cyclase) described above is further supported by studies with substrate analogues capable of covalently labeling the enzyme. Tryptic digestion and Edman sequencing of the resulting labeled protein indicate that cations generated at increasing distances from the site of protonation interact with active-site residues at increasing distances from the catalytic aspartate. Incubation of the *S. cerevisiae* enzyme with tritiated 6-demethyloxidosqualene (**56**), 10-demethyloxidosqualene (**57**), or 10,15-didemethyloxidosqualene (**58**) is expected to produce reactive mono- and bicyclic cations. These incubations result in labeling one or more residues between positions 486–512 (Figure 22),^[18] among which Tyr510 corresponds to active-site residue Tyr420 of the *A. acidocaldarius* squalene-hopene cyclase (Figure 21). The cyclizations of 20-oxa-oxidosqualene (**10**) and of the two truncated dienes **59** and **60** are expected to produce reactive cations in the vicinity of the D ring (Figure 20). These compounds label *S. cerevisiae* oxidosqualene-lanosterol cyclase at His234,^[18] which corresponds to Trp169 of squalene-hopene cyclase, a residue more distal to Asp376 than Tyr420 (Figure 21). Similarly, *A. acidocaldarius* squalene-hopene cyclase is labeled with 29-methylideneoxidosqualene (**42**)^[39] at a peptide section containing Glu45 located at the bottom of the active site (Figure 20); this is the most likely target for the covalent attack, thereby supporting participation of this residue in the terminal deprotonation step.^[85]

In contradiction with these studies and the derived substrate orientation, 29-methylideneoxidosqualene (**42**) labels rat liver oxidosqualene-lanosterol cyclase at Asp456 (*S. cere-*

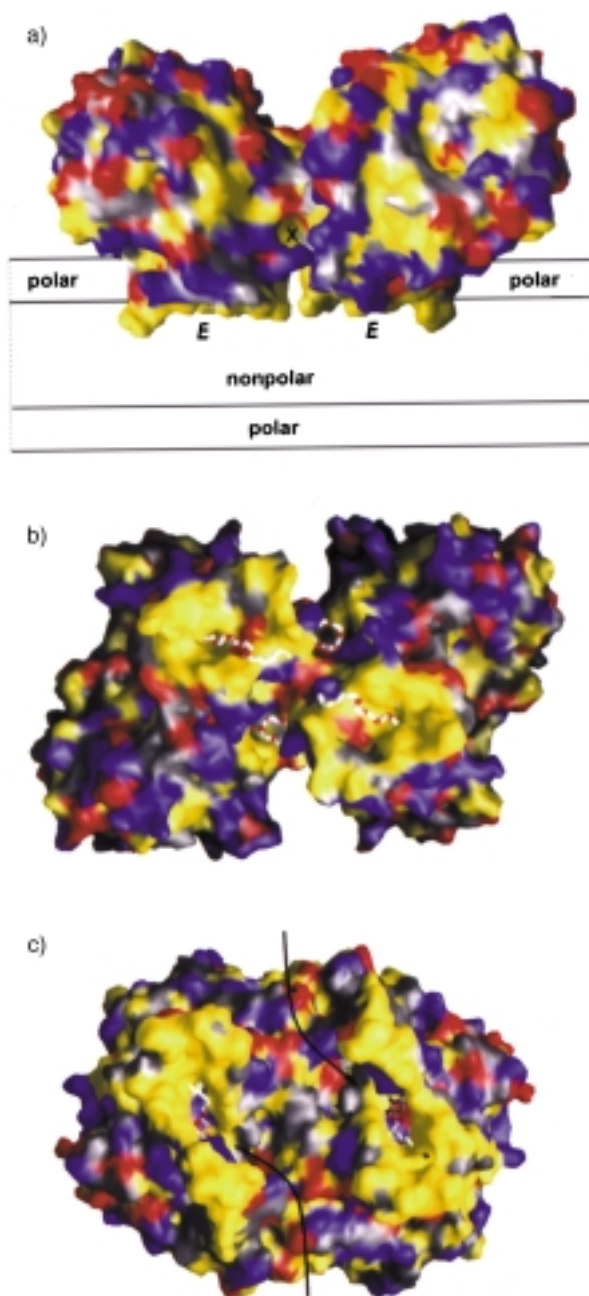


Figure 19. Surface representations^[104] of squalene-hopene cyclase from *A. acidocaldarius* (upper two views) and the only other structurally characterized monotopic membrane proteins, prostaglandin-H(2) synthases I and II (lower view).^[82–84] The surfaces are color coded with nonpolar regions (yellow), positive regions (blue), and negative region (red). a) Model proposed for the monotopic membrane binding of squalene-hopene cyclase. The nonpolar regions of the protomers are oriented in parallel. This arrangement allows the channel entrances (E) to reach into the nonpolar part of the membrane where squalene is dissolved. A nonpolar pocket, binding a detergent molecule at the dimer interface, is marked (X). b) View from the membrane onto the nonpolar regions of the squalene-hopene cyclase dimer. Several molecules of the crystallization detergent *n*-octyltetraoxyethylene (white) are bound in the high-resolution crystal structure.^[64] c) View from the membrane onto the horseshoe-shaped membrane-binding regions of dimeric prostaglandin-H(2) synthase I, with two molecules of the crystallization detergent β-D-octylglucoside bound at the active-site entrance. The arrows indicate a pathway for the polar headgroup of the substrate arachidonic acid toward the active site entrance. Such a passage is not required in squalene-hopene cyclase since its nonpolar substrate does not intercalate between the polar headgroups of the lipid bilayer.

visiae numbering), which suggests that this residue is involved in the stabilization of the protosterol cation (Figure 19).^[74] Interestingly, this compound labeled several vertebrate oxidosqualene cyclases but did not label the yeast or plant enzymes.^[46] This finding may indicate unusual conformational flexibility or a nonnatural binding orientation of 29-methylideneoxidosqualene in the rat liver enzyme.

3.5. Cation Stabilization

Together with the three-dimensional structure and previously described enzyme-labeling experiments, recent mutagenesis results allow for the first time a detailed discussion of specific π -cation interactions in the triterpene cyclases. Structure-based mutagenesis of conserved residues in the active site of *A. acidocaldarius* squalene-hopene cyclase can alter the partitioning between the main products (hopene and diplopterol) and the side products of unusual structure. Replacement of Asp377 with Cys or Asn results in the formation of monocyclic compound **61** (Figure 23), suggesting that this residue may stabilize the C10 cation.^[37] Similarly, mutation of Tyr420 to Ala leads to the production of significant amounts of bicyclic triterpenes **62** and **63** and minor amounts of tricyclic compound **64** (Figure 23).^[71] The bicyclic compounds may arise from premature quenching of the C10 B-ring cation (C8 in hopene numbering, see Figure 20), which may be stabilized in wild-type squalene-hopene cyclase by its proximity to Tyr420 (Figure 24). The bicyclic compounds **63** and **65** produced from mutation of Phe365 to Ala (Figure 23) probably arise by a similar mechanism,^[72] which implicates Phe365 in the stabilization of the bicyclic C8 cation as well (Figures 20 and 24). Replacement of Tyr609 with Phe similarly resulted in the production of bicyclic triterpene **62**, which suggests that this residue is also involved in the stabilization or positioning of intermediate carbocations.^[73] This mutant also produced tetracyclic compounds **47–51** at a significantly higher level than the wild-type enzyme.

Mutation of Trp169 in the center of the active-site cavity (Figure 20) to the less electron-rich residues Phe and His results in increasing amounts of 6-6-6-5 fused ring compound **47** (Figure 15).^[38] While wild-type squalene-hopene cyclase produces 0.6 % of **47**, the Trp169Phe mutant produces 5 % of this compound, while the Trp169His variant forms 29 % of this compound. Interestingly, mutation of Phe601Ala, which is located close to Trp169 (Figure 20), results in a significant increase in production of **47**, accompanied by lower yields of two 6-6-5 tricyclic compounds **64** and **66** (Figures 15 and 23).^[37, 70] These findings suggest that residues Phe601 and Trp169 may stabilize cation **67** (Figure 15) by π -cation interactions and that Phe601 may interact with a 6-6-5 Markovnikov cation intermediate (Figures 20 and 24). The loss or the perturbation of these interactions may result in the premature quenching of tricyclic and tetracyclic Markovnikov cations to form 6-6-6-5 product **47** and 6-6-5 compounds **64** and **65**.

Surprisingly, mutation of Trp489, which is adjacent to Tyr420 in the upper part of the active site (Figure 20), to Phe

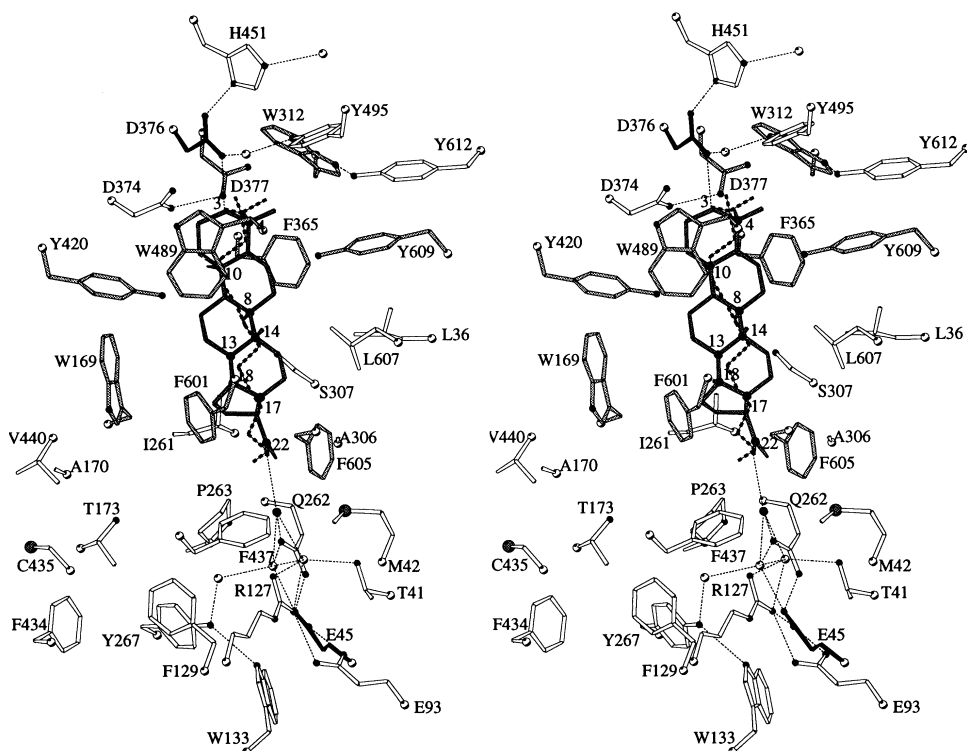


Figure 20. Stereoview of the active site of *A. acidocaldarius* squalene–hopene cyclase with the inhibitor LDAO (dotted) and a modeled hopene (gray). Hopene was modeled in the wild-type structure,^[63] whereas the water molecules (open circles) and the neighboring amino acids are from the high-resolution structure of mutant Asp376Cys.^[64] Asp376 and Glu45 in the polar patches at the top and bottom of the cavity are shown in black. The expected cation positions are depicted as black circles on the hopene model and the suggested position for the reactive water leading to diplopterol is depicted next to residue Gln262. Residues that are suggested or implicated for cation stabilization are highlighted in gray.

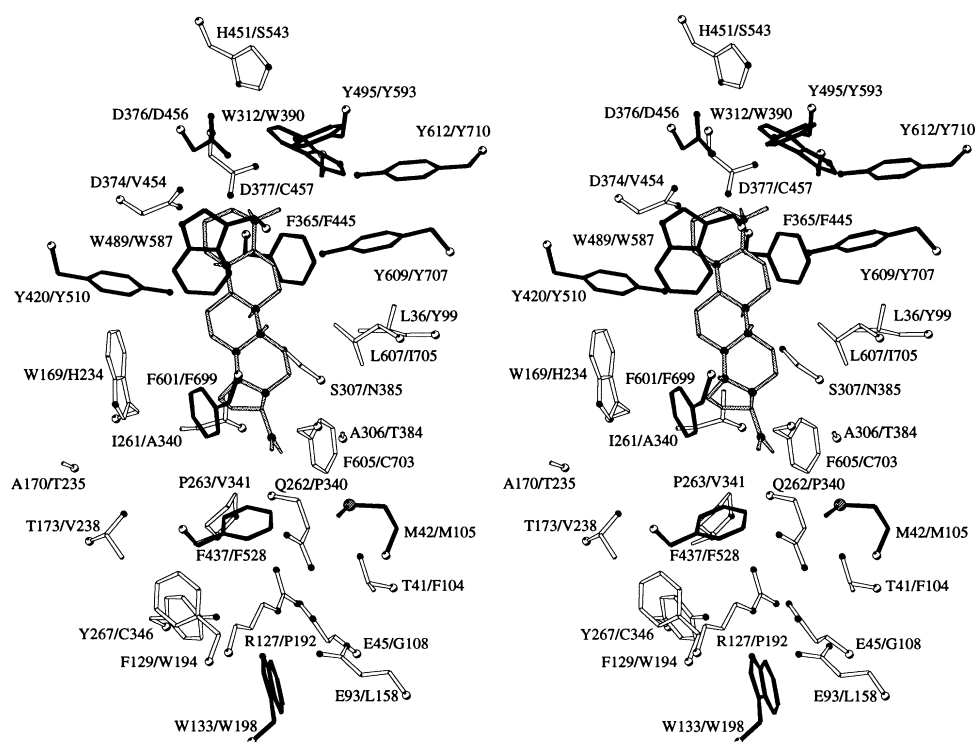


Figure 21. Alignment of *A. acidocaldarius* squalene–hopene cyclase and *S. cerevisiae* oxidosqualene–lanosterol cyclase mapped onto the active-site structure of squalene–hopene cyclase. The residue numbers on the right correspond to the squalene–hopene cyclase and those on the left to oxidosqualene–lanosterol cyclase. Identical residues are depicted in black. The hopene model (gray) and the expected cation positions (black dots) are taken from Figure 20. The alignment was taken from a multiple sequence alignment generated with the program CLUSTAL.^[105] The figure suggests a gradient in homology of active-site residues ranging from low homology at the bottom of the active-site cavity (around Glu45) to higher homology at the top of the cavity (around Asp376).

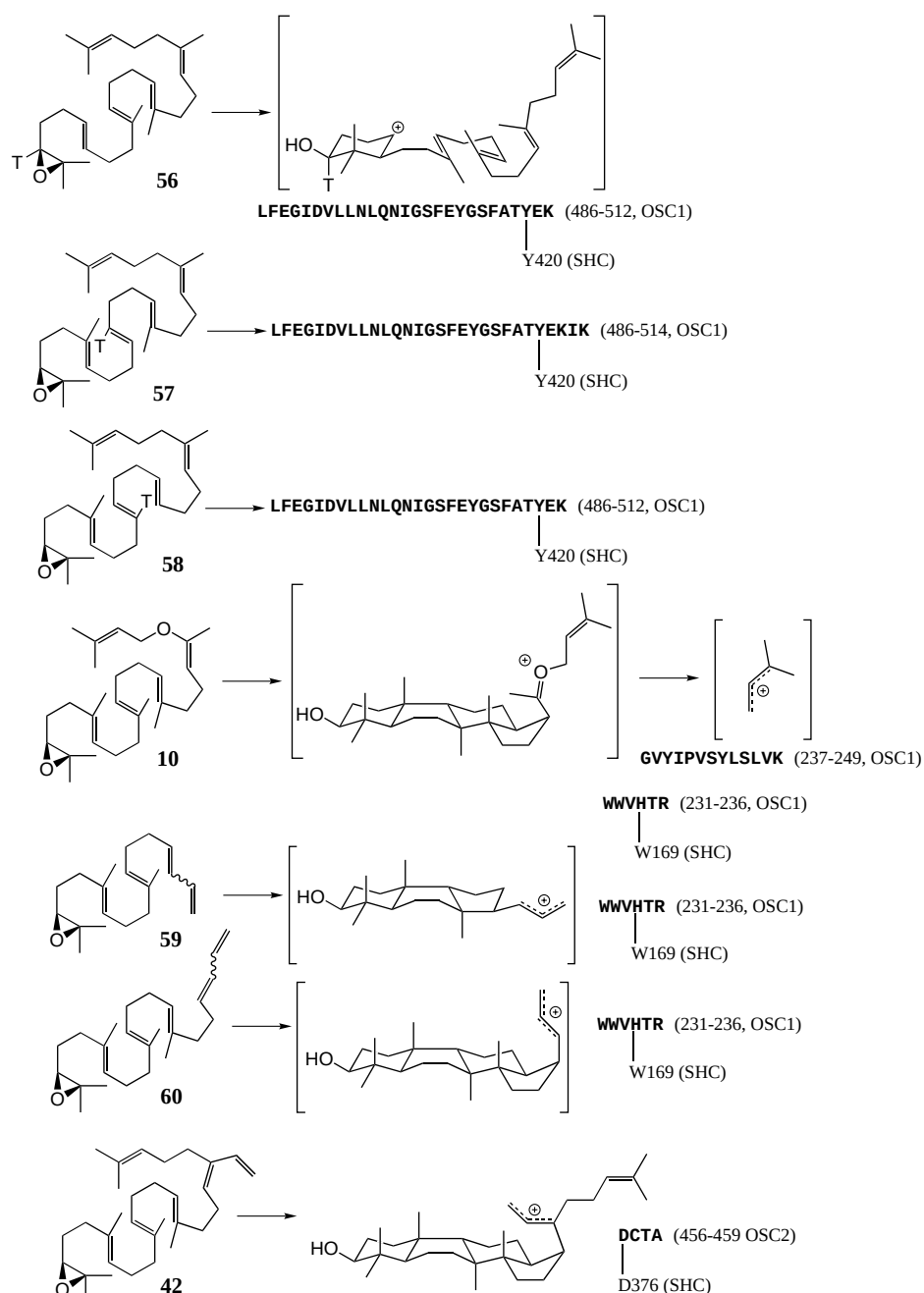


Figure 22. Labeling of oxidosqualene cyclases with suicide inhibitors.^[17, 18, 41] OSC1 = Oxidosqualene cyclase from *S. cerevisiae*, OSC2 = rat liver oxidosqualene cyclase, SHC = squalene–hopene cyclase from *A. acidocaldarius*.

also results in significantly increased production of **47** in contradiction with the model proposed in Figure 24.^[38] Mutants Trp489Val and Trp489Leu, however, possess no activity,^[38] while Trp489Ala has decreased catalytic activity without altered product distribution.^[70] Since the placement of Trp489 within the structure (Figure 20) suggests that this residue is not well positioned to interact with cation **67** (Figure 15), these results may be explained if nonisosteric replacements of Trp489 displace the C19 cation (squalene numbering) away from stabilizing aromatic residues in the lower part of the active-site cavity, such as Trp 169 and Phe 601 (see below).

4. The Enzymatic Mechanism: A Unified View

A comparison of the chemical studies on uncatalyzed versus cyclase-catalyzed processes reveals that the elementary chemical steps required for triterpene formation can take place in non-enzymatic reactions following predictable chemical principles. The roles of the cyclase enzymes are, therefore: 1) Presenting a Brønsted acid of sufficient strength to initiate cyclization; 2) enforcing stereochemistry of the transition state wherever a nonchair-like conformation is required; 3) shielding cationic intermediates from premature quenching by enzyme nucleophiles or by water long enough for cyclizations and rearrangements to proceed along one controlled pathway; and 4) accelerating the reaction by stabilizing carbocations with an electron-rich environment. Below we present a unified mechanism that is consistent with the present body of chemical and structural findings (Schemes 1 and 2).

4.1. Suitability of the Cyclase Structure as a Scaffold for Mechanistic Interpretation

The crystal structure of *A. acidocaldarius* squalene–hopene cyclase was determined at 2.8 Å resolution (wild-type)^[63] and at 2.0 Å resolution (inactive mutant Asp376Cys).^[64] The estimated co-ordinate errors of about 0.4 and 0.2 Å, respectively, for these

structures allow for the identification of hydrogen-bonding networks and the localization of tightly bound water molecules. In light of the current absence of a high-resolution structure of a triterpene cyclase complexed with a transition state analogue, hopene has been tentatively modeled into the active site of squalene–hopene cyclase following the substrate orientation discussed in Section 3.3.^[64]

After manual placement of a hopene model in the active site, 36 binding modes were generated by rotation around the C3–C17 axis of hopene in 10° increments. The van der Waals energy of all resulting positions was minimized computationally using XPLORE.^[86] No protein movement was permitted

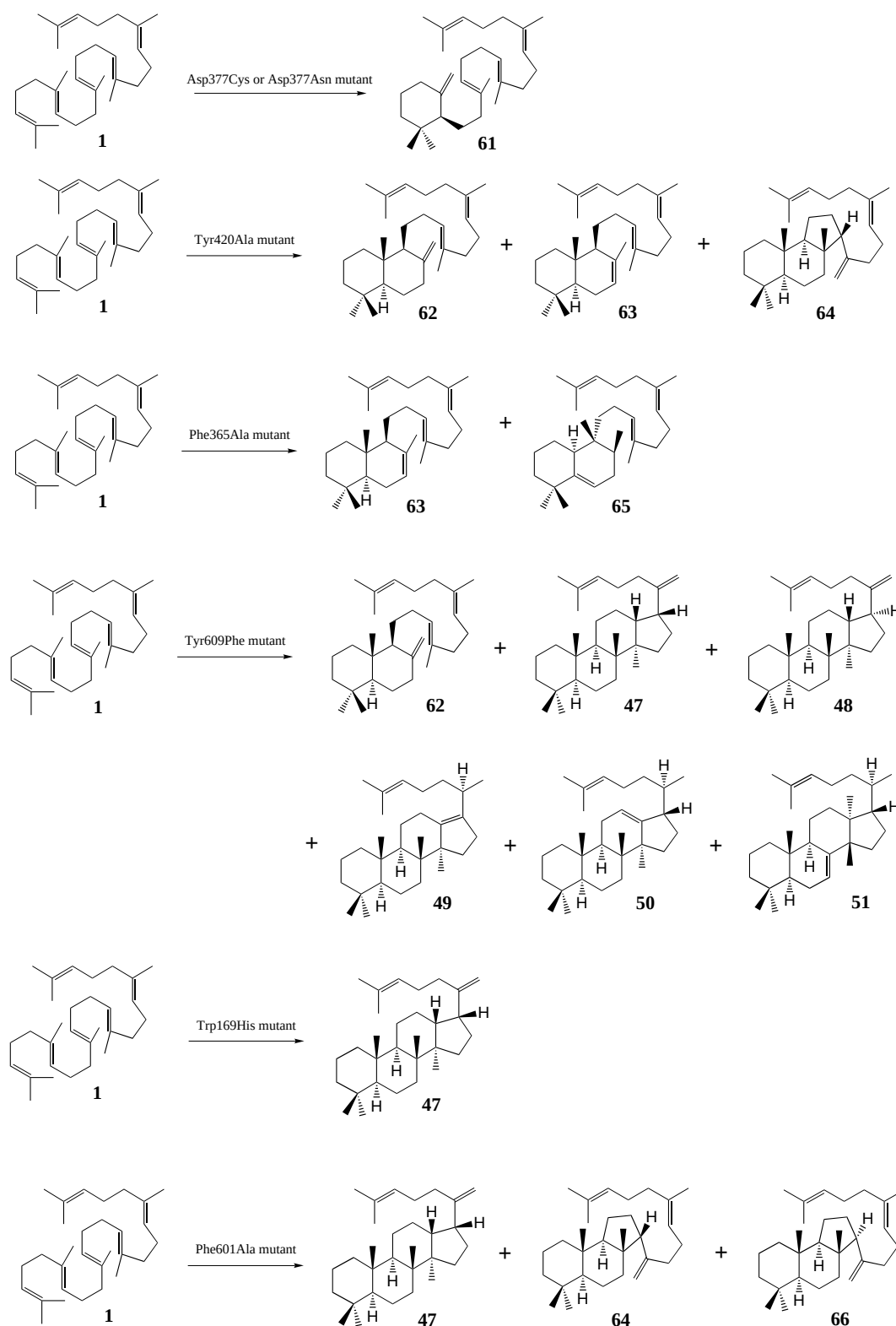


Figure 23. Partially cyclized products resulting from the incubation of squalene with squalene–hopene cyclases bearing mutations at putative cation-stabilizing residues.^[37, 38, 70–73]

during the minimization and hopene was treated as a rigid body. All low-energy binding conformations clustered tightly around the most favorable one shown in Figure 20. A second minimization round allowed for rotation around the C21–C22 bond of hopene but resulted in no significant changes. The

resulting model shows no residual clashes between protein and hopene atoms and serves as a starting basis for further mechanistic discussion. The A ring of the modeled hopene molecule is in good agreement with the binding mode of the observed inhibitor LDAO (Figure 20), with its C3 atom at

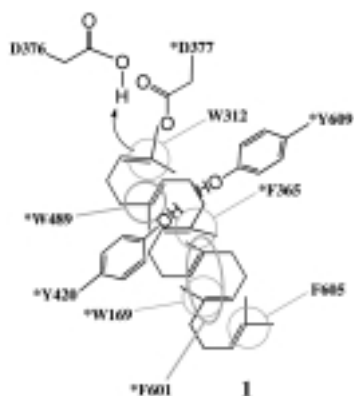
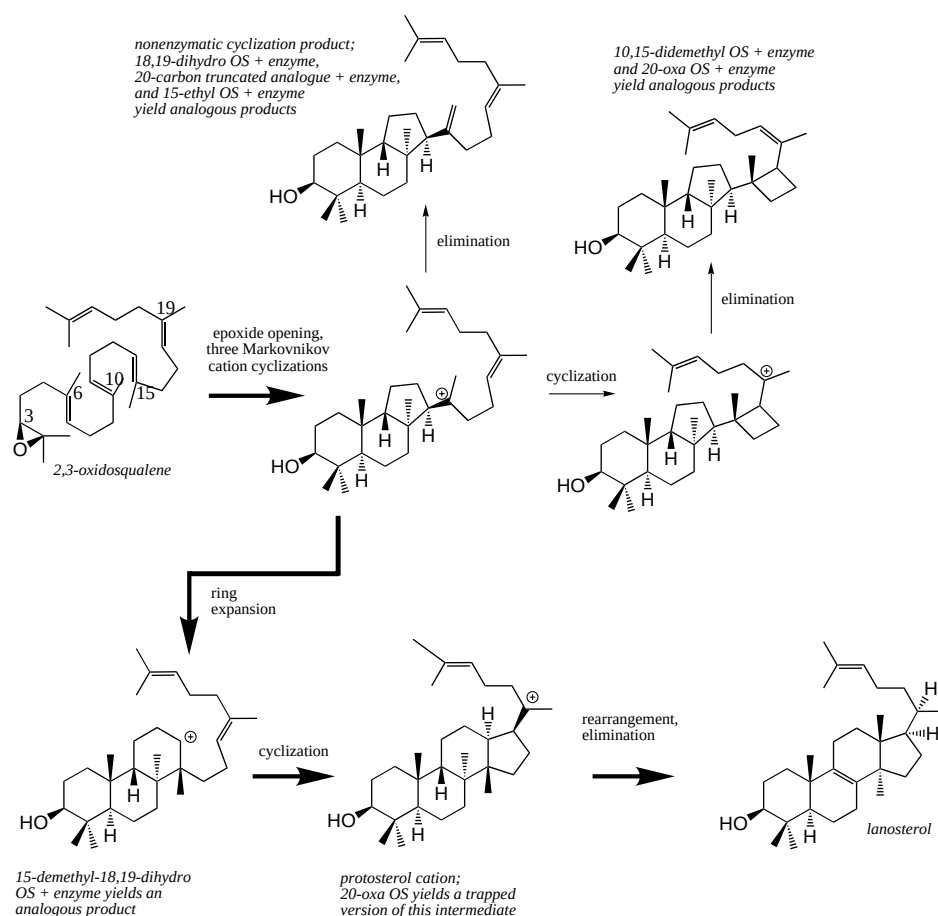


Figure 24. Proposed cation-stabilization interactions during squalene cyclization. Interactions supported by mutagenesis data are marked with an asterisk (see also Figure 20).



Scheme 1. The enzyme-catalyzed cyclization of oxidosqualene into lanosterol. This appears to occur through epoxide protonation by an acidic group of the enzyme which leads to epoxide opening; this is concerted with one, two, or three Markovnikov cation cyclizations to a tricyclic intermediate. The ring widening of the C ring and the Markovnikov cyclization of the intermediate complete the protosterol skeleton which is converted into lanosterol by a series of hydride and methyl group transfers. Products from an elimination, a direct cyclization, or a ring expansion of the key intermediate are obtained from enzymatic reactions with a number of substrate analogues. OS = oxidosqualene.

3.0 Å distance from the carbonyl oxygen of Asp376. The lower part of the model places the C22–C29 double bond of hopene, which is uniquely formed by squalene–hopene cyclases, in proximity to Phe605, a residue uniquely conserved among squalene–hopene cyclases. These findings suggest that

this preliminary working model may reflect the approximate location of the initiation and termination steps of the reaction.^[64]

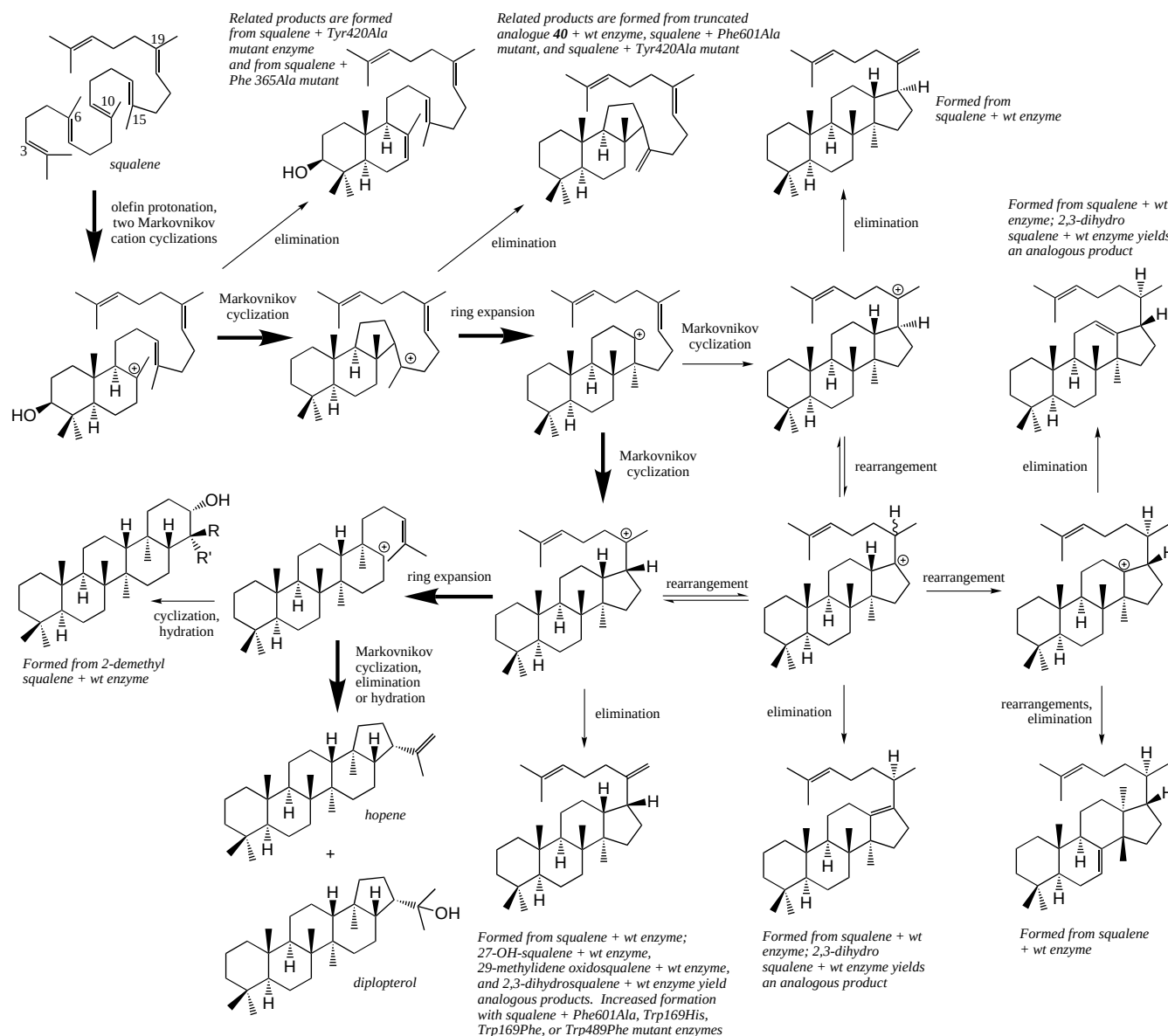
4.2. Initiation

4.2.1. Squalene–Hopene Cyclase

The following mechanism has been proposed for the initial protonation.^[63, 64] The catalytic acid Asp376 lies 3.0 Å from the C3 atom of the modeled hopene, in agreement with the expected geometry for protonation (Figures 20 and 25). Asp376 and His451 are hydrogen bonded at 2.8 Å distance. Since a strong Brønsted acid is required for olefin protonation, it is likely that both residues are protonated prior to

initiation. An additional hydrogen bond to an ordered water molecule, which connects Asp376 to the side chain hydroxyl of Tyr495, may further enhance the acidity of Asp376 (Figures 20 and 25); the lowered activity of the Tyr495Phe mutant^[73] is consistent with this hypothesis. A net negative charge on the tightly hydrogen-bonded Asp374:Asp377 pair (2.6 Å distance) has been suggested to accommodate the positive charge on the Asp376:His451 pair, located in the otherwise uncharged core of the protein structure, prior to proton transfer. Interestingly, Asp376 is clearly oriented in the enzyme crystal structure to donate its *anti* proton to squalene. Consistent with the evolution of squalene–hopene cyclase to provide a strong acid for olefin protonation, carboxylic acid protons in the *anti* conformation have been estimated to be 10⁴ times more acidic than those in the *syn* conformation.^[87] The Asp376:His451 pair loses its charge following proton transfer to squalene, leaving the remaining negative charge on the Asp374:Asp377 pair for stabilization of the initial cationic intermediates.^[63] In support of this hypothesis, mutation of Asp377

to Cys or Asn leads to the formation of monocyclic product **61** (Figure 23), suggesting that this residue stabilizes the C10 cation during cyclization.^[69] Lowered catalytic rates upon mutation of His451 and Asp374 further support their roles in the initial protonation.^[68, 69] The reprotonation of Asp376



Scheme 2. The enzyme-catalyzed cyclization of squalene to hopene. An olefin protonation caused by an acidic enzyme group begins the transformation. This protonation can be concerted with one or two Markovnikov cation cyclizations. The C and D rings probably arise from ring closure of the five-membered Markovnikov cations, which for their parts allow ring expansion. A final Markovnikov cyclization completes the hopene skeleton. Products have been isolated from premature cyclizations or eliminations for almost all of the putative intermediates. The products can be side products from the natural reaction of squalene by the wild-type enzyme, from the transformation of substrate analogues, or from the transformation of squalene with a squalene–hopene cyclase mutant. wt = wild-type.

can occur through Tyr495-OH, which in turn can transfer protons from disordered water in the solvent-accessible upper cavity of squalene–hopene cyclase (Figures 17 and 25).^[64] This conclusion is consistent with the significant loss of catalytic activity arising from replacement of Tyr495 with Phe.^[73]

4.2.2. Oxidosqualene–Lanosterol Cyclase

While a hydrogen-bonded Asp456:His146 pair has been suggested to serve as the catalytic acid for the initial protonation in *S. cerevisiae* oxidosqualene–lanosterol cyclase,^[18] the structural data on *A. acidocaldarius* squalene–hopene cyclase, in combination with a sequence alignment, suggest that His146 is in domain 2 of the enzyme and is

therefore distal from the putative catalytic acid Asp456. Since epoxides are more readily protonated than olefins, an isolated aspartic acid may suffice as the catalytic acid for the oxidosqualene cyclases. This view is also consistent with the fact that the hydrogen-bonded Asp374:Asp377 pair proposed to compensate the charge on the catalytic Asp:His pair in squalene–hopene cyclase is conserved within the squalene cyclase family but is not present among oxidosqualene cyclases.^[63]

4.3. Ring Formation

The chemical and theoretical studies described above indicate that A- and, possibly, B-ring formation are concerted with the initial epoxide or olefin protonation and probably

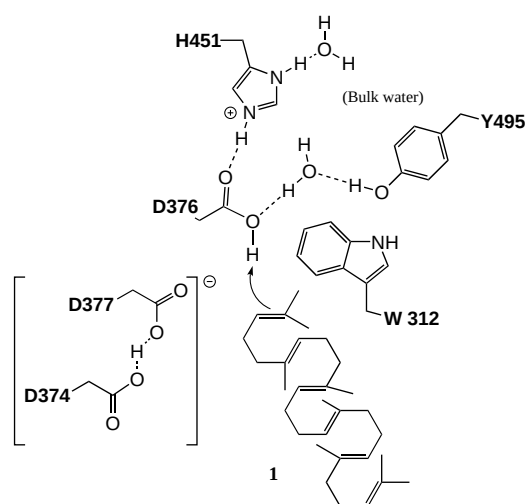


Figure 25. Proposed mechanism for the initial protonation during squalene cyclization.^[64]

facilitate the initiation of the cyclization cascade. Based on the structure of squalene–hopene cyclase (Figure 20), it has been proposed that Trp312, Trp489, and Phe365 stabilize the carbocations forming at C2, C6, and C10 of squalene (C4, C10, and C8 according to hopene numbering, see Figure 24), respectively.^[64] Residues Tyr420, Tyr609, and Tyr612 are oriented with their hydroxyl groups pointing towards the active-site cavity and may provide additional negative electrostatic potential during early cyclization events.^[64, 78] Indeed, mutation of Tyr612 to Phe results in a significant loss of catalytic activity.^[73]

The increased formation of bicyclic products **62** and **63** resulting from the Tyr420Ala squalene–hopene cyclase mutant agrees with such a role for Tyr420 (Figures 23 and 24).^[71] Similarly, the formation of bicyclic product **62** arising from mutation of Tyr609 to Phe also supports the above model.^[73] The production of compounds **63** and **65** by mutant Phe365Ala (Figure 23) additionally points towards a cation– π interaction between Phe365 and the C8 cation (hopene numbering, Figure 20).^[71] Consistent with these suggestions, Trp312, Trp489, Phe365, Tyr609, and Tyr612 are conserved among squalene and oxidosqualene cyclases. These amino acids, thus, provide a hydrophobic, non-nucleophilic, and electron-dense environment for promoting the initial cyclization steps (see above).

It is likely that C-ring formation in the case of oxidosqualene cyclase proceeds by a five-membered cyclization followed by a ring expansion (see above). Perhaps this five-membered intermediate is kinetically favored over the six-membered alternative for Markovnikov reasons, while subsequent cyclization steps thermodynamically favor products with a six-membered C ring. Indeed, further Markovnikov cyclization of the 6-6-5 cation would lead to a strained 6-6-5-4 product (see Scheme 1). In analogy to the oxidosqualene cyclases, it is reasonable to expect a ring-widening step in the C-ring formation of squalene–hopene cyclase as the kinetically favored pathway. Demonstrating the possibility of such a cyclization pathway within squalene–hopene cyclase, the Tyr420Ala mutant of this enzyme forms 6-6-5 triene **64** as a

minor product,^[71] and 6-6-5 products **64** and **66** were recently isolated from the Phe601Ala mutant of squalene–hopene cyclase^[37] (Figure 23). Finally, the enzyme has been shown to process truncated alcohol **40** into a 6-6-5 product consistent with trapping of the Markovnikov cation generated from five-membered C-ring closure (Figure 13).^[37]

The isolation of varying amounts of 6-6-6-5 products in the reaction of wild-type and mutant squalene–hopene cyclases points strongly toward a five-membered closure and ring expansion for D-ring formation catalyzed by squalene–hopene cyclase.^[38, 42, 70] A five-membered D-ring intermediate may be favored by 1,3-diaxial repulsion between the C15 and C19 methyl groups of squalene in the six-membered D-ring alternative; this difference, however, does not apply to hopene C-ring formation. Based on the data of Abe et al.^[37, 38] and Merkofer et al.,^[70] we propose that Trp169 and Phe601 stabilize cation **67** (Figures 15 and 20) which arises from five-membered D-ring cyclization (see above). The hopene model, derived by energy minimization prior to recent mutagenesis results, gratifyingly places Trp169 and Phe601 in close proximity to the expected positions of the C19 cation (C18 in hopene numbering) in **67** (Figures 20 and 24).^[64] Taken together, these observations suggest that a 6-6-6-5 tetracycle is an on-pathway intermediate of hopene cyclization rather than simply a side product of the reaction.

Phe605 seems well positioned to stabilize the terminal cation resulting from E-ring formation in squalene–hopene cyclase (Figure 20).^[64] This hypothesis is supported by the conservation of Phe605 among all known squalene cyclases (all of which form pentacyclic products) as well as the absence of this residue in the oxidosqualene–lanosterol cyclases (all of which form only four rings).^[64]

Assuming that the C20 protosterol cation formed by oxidosqualene–lanosterol cyclases binds to the conserved aromatic residues in a manner similar to that of the 6-6-6-5 intermediate formed by squalene–hopene cyclase, Trp232 and/or His234, as well as Phe699, may serve to stabilize the C20 protosterol cation in the *S. cerevisiae* oxidosqualene cyclase (Figure 21). Among these residues, Trp232 is conserved within the oxidosqualene cyclases only, whereas Phe699 corresponds to Phe601 of squalene–hopene cyclase and is, therefore, conserved among both classes of enzymes. This view is consistent with the enzyme-labeling and mutagenesis experiments on the *S. cerevisiae* cyclase described above.^[18]

4.4. Rearrangement and Elimination

No methyl or hydride rearrangement occurs on the pathway to hopene, although the proposed 6-6-6-5 intermediate **67** (Figure 20) undergoes rearrangements to yield small amounts of minor products.^[42] Termination occurs by elimination to the main product hopene (80%) or by addition of water to diplopterol (20%).^[44] In the structure of squalene–hopene cyclase determined at a resolution of 2.0 Å, a network of well-defined water molecules exists in the lower part of the active-site cavity (Figure 20).^[64] It has been proposed that these bound waters are polarized by residues in the extended

hydrogen-bonding network around Glu45 at the bottom of the active-site cavity.^[64] The position of Glu45 within the squalene–hopene cyclase structure together with its conservation among the squalene cyclases suggest that a polarized water molecule may abstract a proton or attack the E-ring cation, leading to hopene or diplopterol formation. All partially cyclized products isolated from the action of squalene–hopene cyclases on a wide variety of substrate analogues contain olefins resulting from proton elimination, rather than hydroxyl groups resulting from cation hydration (see above). This finding suggests that the water network around Glu45 may be the only location in which active-site water is available for cation quenching.

Substrate analogue studies with the oxidosqualene cyclases described above suggest that the enzyme's role during rearrangement is most likely to simply shield intermediate carbocations from addition of water or elimination by base, thereby allowing the hydride and methyl group migrations to proceed down a thermodynamically favorable and kinetically facile cascade. A homology-based structural alignment of *S. cerevisiae* oxidosqualene cyclase and *A. acidocaldarius* squalene–hopene cyclase (Figure 21) suggests that His234, an essential and conserved residue within the oxidosqualene–lanosterol cyclases,^[18] may be well positioned to serve as the catalytic base in the oxidosqualene cyclases.

5. Future Directions

5.1. Structural Biology

The first structural elucidation of a triterpene cyclase, in conjunction with the wealth of recent chemical and biochemical data, has advanced our understanding of the mechanism of triterpene cyclization to a new level. An in-depth and detailed understanding of the roles of cyclases in substrate prefolding and cation stabilization clearly requires the determination of additional triterpene cyclase structures. Given the large quantity of chemical data that is now available for the oxidosqualene–lanosterol cyclases together with their potential medical importance, the structural determination of an oxidosqualene cyclase is a major priority in this field. These efforts will, hopefully, be assisted by the rapid development of new techniques in membrane protein expression and crystallization.^[88, 89] While the human and *Candida albicans* oxidosqualene cyclases are highly attractive targets for the development of anticholesteremic and antifungal drugs, our understanding of the enzymatic mechanism would also be facilitated by the structure of more tractable bacterial or fungal cyclases.

5.2. Structure-Based Mutagenesis

Recent successes in structure-based mutagenesis and the careful analysis of resulting altered product distributions have added greatly to our understanding of the mechanism of triterpene cyclases. The characterization of further mutants will undoubtedly continue to enhance our understanding of

these enzymes. For clarity, squalene–hopene cyclase residue numbering will be used throughout this section, although analogous experiments on oxidosqualene cyclases are also relevant.

Mutations Leu36Tyr and Leu607Ile for squalene–hopene cyclase and the corresponding mutations Tyr99Leu and Ile705Leu for oxidosqualene–lanosterol cyclase may provide insight into the role of these residues in the determination of B-ring configuration or in ring widening during C-ring formation. The repeated observation of multiple partially cyclized products arising from many of the site-directed mutants described above reflects the general problem of separating specific π -cation interactions from simple steric effects that may arise from displacement of the forming product in the active-site cavity or from subtle changes in the structure of the active site. This problem is particularly evident in the partially contradictory reports of Sato et al.^[38] and Merkofer et al.^[70] related to D-ring formation in squalene–hopene cyclase. Isosteric replacements using unnatural amino acid mutagenesis^[90] may resolve such contradictions. Replacement of Trp489, Phe601, and Trp169 with fluorinated analogues of these amino acids would provide series of relatively isosteric side chains with varying levels of electron richness and, thus, varying abilities to stabilize cations. A correlation between electron richness and product distributions arising from trapping of putative 6-6-6-5 intermediates would support specific π -cation interactions. The need to develop in vitro expression of these enzymes in the presence of lipids and to analyze very minor products arising from these mutant enzymes, together with the low turnover number of squalene–hopene cyclase (0.3 s^{-1}), however, will make this a challenging endeavor. Generation of a monomeric squalene–hopene cyclase by mutations in the suggested dimer interface may clarify whether dimer formation is required for enzymatic activity and membrane binding.

5.3. Directed Evolution

The relaxed substrate and product specificities of mutant squalene–hopene cyclases point to the possibility of generating novel triterpenes by the directed evolution of these enzymes.^[91, 92] In support of this hypothesis, an Ile481Val mutant oxidosqualene–cycloartenol cyclase, which can produce lanosterol, was recently isolated by spontaneous mutation and genetic selection in a yeast strain unable to produce lanosterol.^[93] A terpene-cyclase catalytic antibody that catalyzes the cyclization of an unnatural sesquiterpene to a series of unsaturated *trans*-decalins^[94] also structurally mimics the active site of a natural sesquiterpene cyclase.^[95–97] Even though no catalytic antibody with triterpene cyclase activity has been described at present,^[98] the development of methods to generate such catalysts may open new routes to unnatural triterpenes.^[99, 100]

Since many squalene cyclases are known to accept oxidosqualene as a substrate,^[9] it may be possible to apply directed-evolution strategies to squalene–hopene (or other non-lanosterol forming) cyclases.^[101, 102] The oxidosqualene–lanosterol cyclase deficient yeast strain *ERG7* provides an

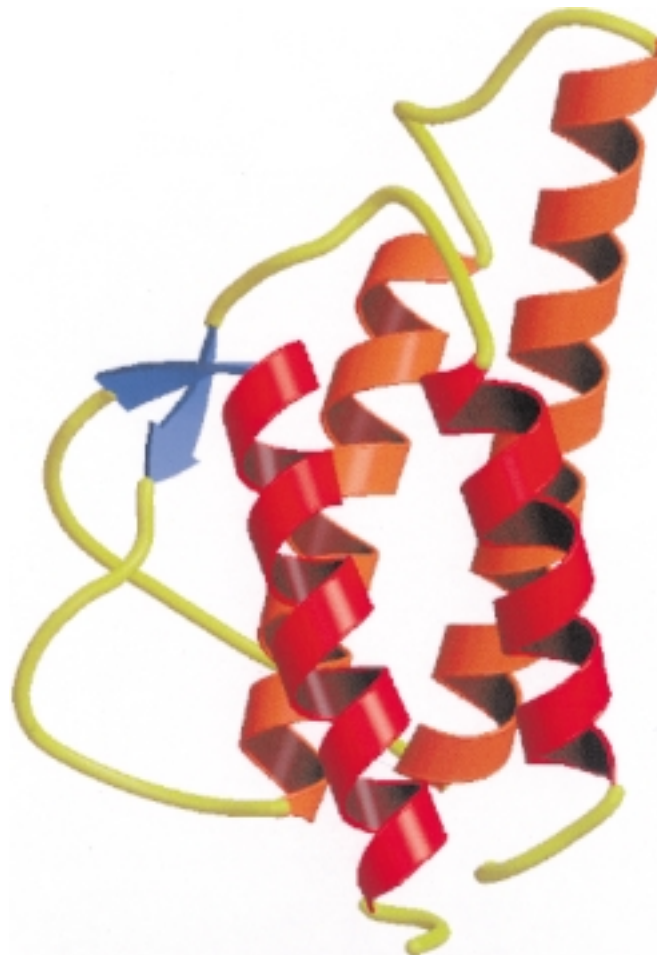
opportunity to select in vivo for lanosterol formation.^[53] The structure of an evolved squalene–hopene cyclase mutant capable of lanosterol formation may yield valuable information on the structural requirements for different transition state geometries and backbone rearrangements unbiased by natural evolutionary drift. More generally, the generation and use of mutant organisms known to be deficient in the production of other essential triterpenes provides the opportunity to apply genetic selections towards the artificial evolution of cyclase enzymes with new product specificities. The rigorous analysis of mutations responsible for altered product specificities may provide important insights into the roles of residues both near and distant from the active site in defining the course of these mechanistically complex yet extremely elegant enzyme-catalyzed cyclizations.

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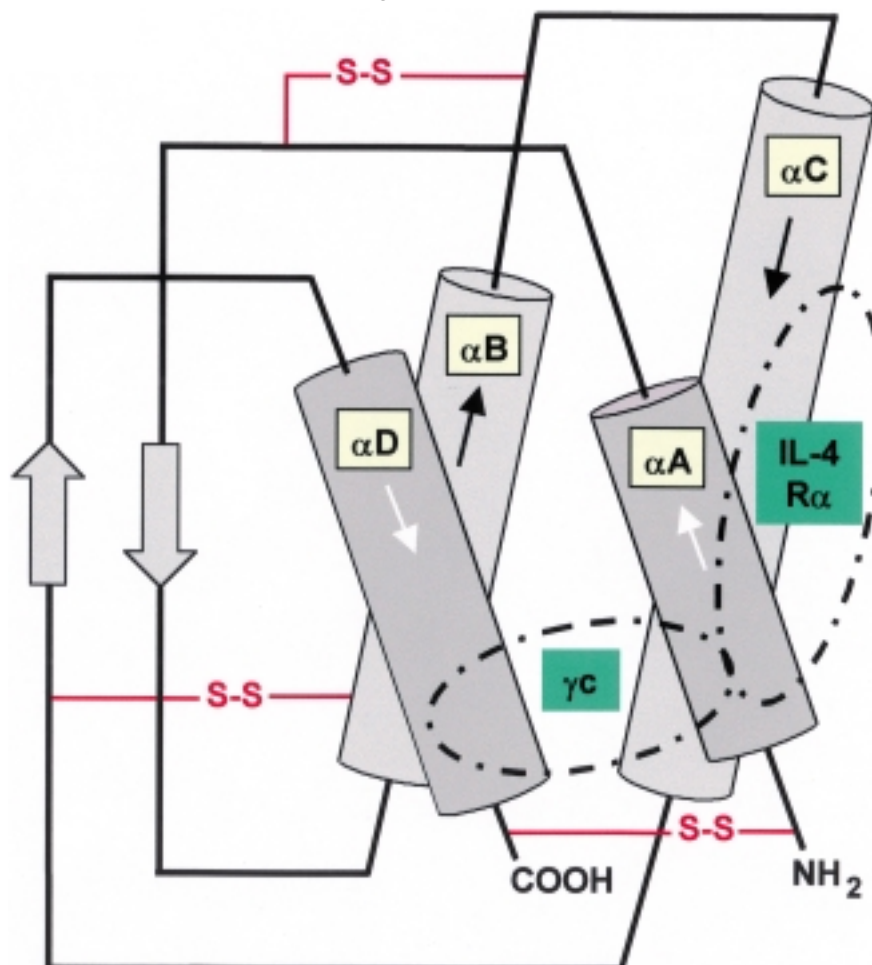
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A

The structure of the pro-allergic protein hormone interleukin-4 has been known since the early 1990s (A). Through the analysis of the interaction of its receptor chains (B) it became possible to modify the molecule into an antiallergic mediator.

B

The Interleukin-4-Receptor: From Recognition Mechanism to Pharmacological Target Structure

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Organic synthesis of hormone derivatives is an established route to yield pharmacologically active agents. Until recently this has only been feasible for small organic compounds, but nowadays it is also possible to produce antagonists for larger protein hormones. In particular, the interleukin-4-receptor was a well-suited target for this approach since it plays a pivotal

role in the release and progression of allergic diseases. Accordingly, a strong interest and a high medical need is associated with the development of inhibitors. The structural elucidation of the ligand/receptor complex and an improved understanding of the mechanisms concerning receptor binding and activation allow for the rational design of variants that inhibit interleu-

kin-4. Since it is possible to specifically inhibit the interleukin-4-receptor system in this way, a completely new approach to the development of new drugs against allergy and asthma has been established.

Keywords: allergy • drug research • gene technology • hormones • interleukin-4

1. Emasculated Hormones

In 1988 James Black presented the concept of “emasculated hormones” in his Nobel prize lecture: hormone derivatives, that retain binding affinity to their receptors, but which are unable to activate them.^[1] From this idea he developed beta blockers and antihistamines which are particularly impressive examples for the realization of this concept.

As a consequence of the spectrum of methods in genetic engineering and biotechnology that is available today the same principle can now be transferred to protein hormones. Here we present how directed mutagenesis of interleukin-4 (IL-4) leads to the development of a specific antagonist. The IL-4-receptor system represents a particularly lucky example of such an approach since the signaling of two protein hormones which are essential for the development of allergic diseases and asthma is triggered by using the IL-4 receptor (see Sections 2 and 3). Moreover, an understanding of the structure and recognition mechanism of this receptor has led in a direct way to the development of an “emasculated” IL-4-hormone.

The IL-4-receptor is a prototype for receptors being activated by oligomerization—mostly dimerization—of receptor chains in the plasma membrane of the cell. As a consequence, the signal of the hormone is transmitted into the cell.^[2,3] The attributes of the IL-4-receptor—heterodimeric structure, binding with high affinity only to the α chain, little coupling between the receptor chains—offer a direct way to construct antagonistic IL-4 variants which retain the high-affinity binding, but no longer mediate the dimerization of the receptor chains. A refined analysis of the mutants results in selective antagonists, which only affect special receptor subtypes and therefore only particular cells, as well as super-antagonists, which show effects at decreased concentrations.

Antagonistic IL-4 variants are of pivotal interest in proof-of-principle studies to evaluate the IL-4 system as a fundamental new target in antiallergic therapy. Furthermore, it seems to be a realistic concept that these antagonistic proteins themselves could be developed as therapeutic agents. It will be a scientific challenge for the future to develop specific low molecular weight α -chain blockers based on the acquired knowledge of the recognition mechanism in this receptor system.

2. Allergy Arises from a Misguided Immune System

Allergy is a hyperreaction of the immune system against compounds that are tolerated by the immune system of most individuals. Nonimmunological hyperreactions caused, for

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example, by heat or UV light are not counted as allergic reactions. As allergy is restricted to the hyperresponsiveness of the immune system it can be separated from toxicity. Particular allergens, such as bee venom, can be quite toxic, but this quality is not causal for their allergic potential. Accordingly, the allergic reaction can be considered as a misguided defense mechanism against compounds that have no harmful potential without this reaction.

Atopy is a genetic predisposition to develop allergic sensitivity against suitable stimuli. Up to 5% of the population have the potential to develop atopy. They exhibit an increased risk for developing allergies, often suffer from multiple allergies, and are extremely susceptible to develop allergic asthma.^[4] Atopy originates from mutations in genes associated with allergies. They have been intensively searched in connection with asthma, and it has been found that a variety of genes can be responsible for the development of asthma.^[5] The Asthma Gene Database, established and maintained by the GSF-Research Center for Environment and Health in Munich, (<http://cooke.gsf.de/asthmagen/main.cfm>) offers a continuously updated list of the current

status of research in this field. Interestingly, IL-4 and the IL-4 receptor α chain are found among the genes at risk from asthma.

IL-4 is specifically responsible for the development of immediate-type allergic reactions.^[6–8] This type is characterized by a prompt occurrence of symptoms against the allergen after exposition. Pre-sensitized individuals can develop edemas within minutes. In addition, symptoms such as widening of capillaries, stimulation of pain receptors, and—in particular in asthma—contraction of bronchial smooth muscles are developed. The immediate-type allergic reactions comprise allergic rhinitis (hay fever), allergies against house dust, animal hair, and insect venoms, as well as allergic asthma. Allergies with a delayed development of symptoms, such as allergy against nickel, do not belong to the immediate-type allergic reactions and can therefore not be attributed to IL-4. Allergies that depend on IL-4 use the same set of molecules and cells as used for the defense of helminthic macroparasites. Accordingly, immediate-type allergic reactions are considered as a misguided activation of the cellular parasite defense system.

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Albert Duschl studied Biology at the University of Giessen from 1977 to 1984. In 1986 he completed his Dissertation on bacterial chloride transporting systems in the department of Prof. G. Wagner at the Botanical Institute I. From 1987 to 1989 he was a post-doc in the department of Prof. J. K. Lanyi at the University of California in Irvine and in 1990 in the department of Prof. D. Oesterhelt at the Max-Planck-Institute for Biochemistry in Martinsried. Since 1990 he has been an assistant of Prof. W. Sebald at the Institute for Physiological Chemistry at the Theodor-Boveri-Institute for Biosciences at the University of Wuerzburg. In 1997 he completed his Habilitation on "Molecular Mechanisms of the Immunomodulating Cytokine IL-4". The focus of his research are signal transduction and gene expression triggered by interleukins and in vitro and in vivo models for allergic diseases.

The progression of the allergic disease is shown schematically in Figure 1. An antigen is absorbed by antigen-presenting cells, subsequently processed, and finally presented by the MHC-II complex to T helper cells. When this stimulus triggers differentiation to a Th2 cell (type 2 T helper cells), the antigen will be treated as an allergen. For Th2 differentiation to occur it is necessary that the T cell is stimulated with IL-4 during its activation. Mast cells and subsets of T cells are considered as a potential source for early IL-4.^[9] Since Th2 cells and other cytokines also produce IL-4, autocrine amplification is triggered.

Two of the Th2 cytokines, namely IL-4 and IL-13, induce class switching of the antibodies in activated B cells to the IgE-type.^[10, 11] The resulting allergen-specific IgE antibodies bind to surface receptors of mast cells, basophiles, and eosinophiles and thereby induce their sensitization to the antigen. Any new contact with the allergen will trigger an immediate allergic reaction: the allergen binds to cell-associated IgE, and leads to degranulation of the effector cells. In the case of hay fever it is mainly the mast cells in the mucous membranes of the eyes and the respiratory tract that degranulate. Compounds released thereby, such as histamine, serotonin, and prostaglandins finally cause the typical symptoms. A variety of further pathological conditions can be observed in asthma: increased production of mucus in the bronchi, increased reactivity of bronchial muscles, and production of an inflammatory infiltrate in the tissue that is dominated by eosinophiles.^[12]

IL-4 and IL-13 are not restricted to the sensitization phase, but they also contribute to reactions during the effector phase.

They increase the activity of mast cells^[13], basophiles,^[14] and eosinophiles^[15], and they lead to differentiation and hypertrophy of mucus-producing goblet cells.^[16, 17] Furthermore, they are involved in the production of an eosinophilic infiltrate by induction of specific adhesion molecules^[18] and chemokines.^[19]

The Th2 differentiation taking place at the beginning of the reaction cascade is the one and only reaction that is solely triggered by IL-4. It is particularly important to note that IL-13 is unable to induce Th2 differentiation.^[20] However, IL-13 seems to be the dominating cytokine during the subsequent progression of the disease, since it is produced for longer periods and in higher amounts than IL-4. IL-13 can be found in large quantities in the asthmatic lung, while IL-4 is hardly detectable. Hence, IL-13 is considered as the more important cytokine during the effector phase.^[21–24]

3. IL-4R α —The Molecular Switch that Triggers Allergy

The reason for the large similarity in the effects of IL-4 and IL-13 is the use of a common receptor chain, IL-4R α . Transgenic knock-out mice without an IL-4R α gene do not show any measurable reaction upon stimulation of IL-4 or IL-13; hence both cytokines are dependent on IL-4R α .^[17, 25–28] It is known from binding studies that IL-4 can directly bind to IL-4R α .^[29–31] Subsequently, depending on the cell type, a second receptor chain will be recruited to initiate signaling: either γ_c ^[32, 33] or IL-13R $\alpha 1$ ^[34–37] (Figure 2). In contrast, IL-13

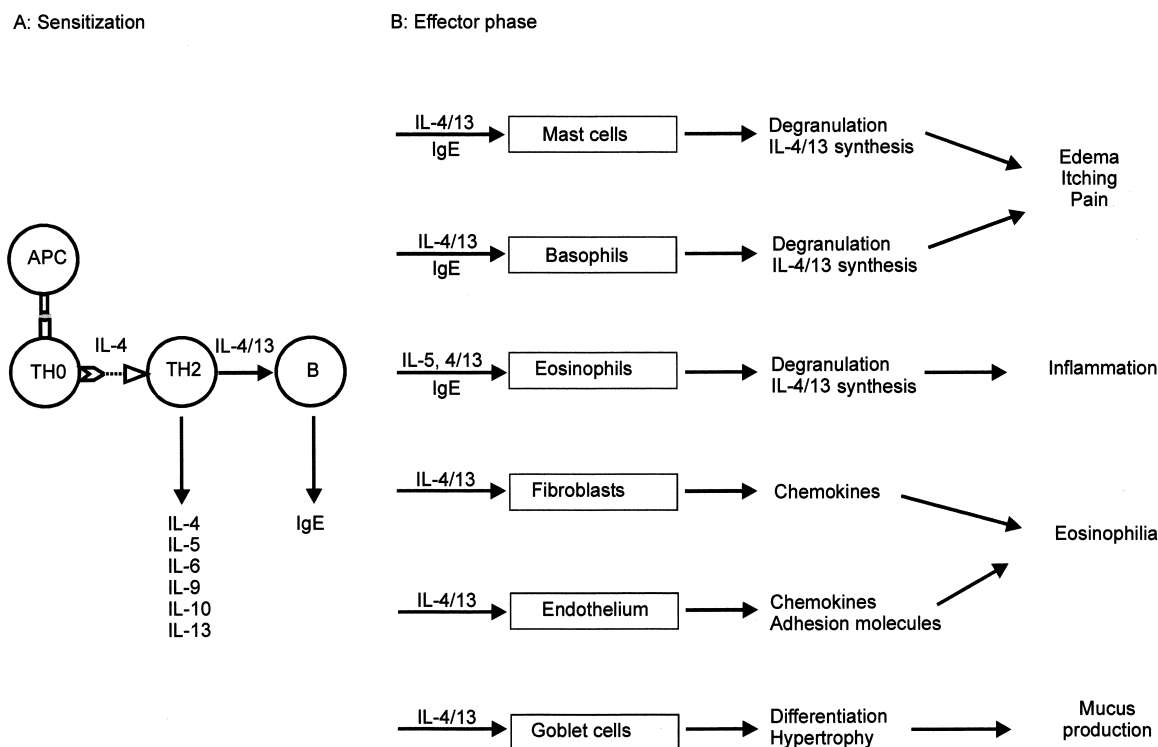


Figure 1. Schematic representation of sensitization (A) and effector phase (B) of the immediate-type allergic reaction. IL-4 and IL-13 are involved in both processes, since they have activating and/or differentiating effects on the involved cell types. APC = antigen presenting cell, TH0 = T helper cell type 0, TH2 = T helper cell type 2, B = B cell, IgE = immunoglobulin E.

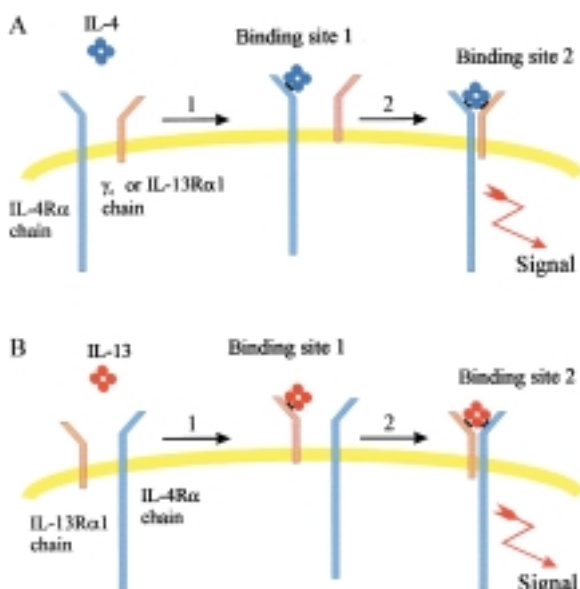


Figure 2. An identical heterodimeric receptor comprising IL-4Rα and IL-13Rα1 is used by both IL-4 (A) and IL-13 (B). The sequence of dimerization however is different (see text). The complex of IL-4 and IL-4Rα within the membrane can either recruit γ_c or IL-13Rα1. As far as is known, the resulting signal is identical.

binds first to IL-13Rα1 and subsequently recruits IL-4Rα. Despite the differing sequence of association to the receptor chain IL-4 and IL-13 both use the same IL-4Rα/IL-13Rα1 heterodimeric receptor complex.^[20, 38]

Within the allergic process IL-4 can be regarded as the master switch, which triggers all following processes. Therefore, the IL-4 system is regarded as an ideal target for the prevention of allergy. Transgenic mice without a functional gene for IL-4 are not able to develop allergic reactions and the above-mentioned transgenic mice without a functional IL-4Rα gene can also not develop allergic reactions.^[39] In contrast, transgenic mice with elevated IL-4 expression develop symptoms similar to allergic reactions even in the absence of allergens.^[40–43] The administration of IL-4 into the lungs of asthmatic patients induced an asthma attack,^[44] while corresponding experiments using IL-13 in mice led to a very high production of mucus and other asthmatic symptoms.^[17, 21, 22, 24] This result demonstrates the pivotal importance of IL-4 and IL-13 for the induction and progression of allergic reactions and accordingly reveals that inhibition of the IL-4/IL-13-system may be highly beneficial in antiallergic and antiasthmatic therapy.

3.1. Selecting the Target Molecule

A particularly suitable target for IL-4/IL-13 inhibition seems to be IL-4Rα. The receptor is essential for both cytokines and as a consequence both cytokines will be inhibited. Inhibiting the ligands directly, however, would only affect one of the two cytokines. Moreover, as the sequences of IL-4 and IL-13 only exhibit 25% sequence homology there is almost no chance of developing bispecific inhibitors. Soluble variants of the receptors merely recognize one of the cytokines: separated IL-4Rα solely binds IL-4, separated

IL-13Rα1 solely binds IL-13, and separated γ_c does not bind to either of the ligands.

Of the receptor chains both IL-13Rα1 and γ_c are unattractive targets, since their disengagement would not result in complete and simultaneous neutralization of both ligands. In particular, γ_c is not regarded as a pharmaceutical target at all. This receptor chain is shared between IL-2, IL-7, IL-9, and IL-15, and accordingly inhibition of γ_c will create interference with a variety of different regulation systems.^[45] The genetic loss of γ_c leads to the severe immune deficiency syndrome X-SCID (X-chromosome-linked severe combined immunodeficiency), where the afflicted individual suffers from a severe breakdown of the immune system as a result of the inability to develop T cells in the absence of IL-7 signals.^[45–48] In contrast, transgenic mice who lack IL-4Rα seem to cope well with the situation: these animals exhibit normal immune competency and have no difficulties in bacterial and viral defense.^[25–28] Thus IL-4Rα can be identified as a key protein in the allergic reaction cascade and therefore as a preferential target for pharmaceutical intervention.

IL-4- and IL-13-dependent signaling is primarily arranged by IL-4Rα. A variety of signal-transducing factors can bind to IL-4Rα to mediate the downstream signal.^[8] A large protein complex comprising IRS-2 (insulin receptor substrate-2) and proteins coupling to IRS-2 seem to be responsible for cell proliferation and inhibition of apoptosis.^[49] A second signaling pathway involves the activation of kinases belonging to the Jak family and subsequent phosphorylation of Stat6.^[50] The transcription factor Stat6 forms a homodimer after phosphorylation and, together with other transcription factors, regulates gene expression during cell differentiation. This process includes amplification of the IL-4 production during Th2 differentiation^[51], class switching of B cells to IgE^[52–54], expression of the IgE receptor CD23 on B cells and monocytes^[55, 56], and induction of the antiinflammatory protein IL-1RA in macrophages.^[57]

If these signaling pathways are considered in the search for attractive pharmaceutical targets, the pathway using IRS-2 is likely to be dismissed, since the control of cell proliferation and apoptosis by IL-4/IL-13 induction can be neglected in the context of allergy. In contrast, cell differentiation is of considerable importance. However, within the Jak/Stat pathway the Jak kinases seem to be less promising, since they are activated by numerous other receptors as well. Therefore, it seems to be almost impossible to achieve inhibition without serious side effects.^[58, 59] In contrast to the Jak family, Stat6 is—at least as far as cytokine signaling is considered—specific for the signal transmission of IL-4 and IL-13, and therefore can be regarded as a promising target molecule.^[58, 59]

Inhibition of IL-4Rα or Stat6 opens up a new perspective for the therapy of allergic diseases. Until now it has only been possible to either try to achieve a desensitization against a defined allergen by using a specific immunotherapy or to influence the course of the effector phase during an acute state of the disease by application of certain compounds such as antihistamines, cromoglicinic acid, nedocromil, or corticosteroids. The neutralization of the effects of IL-4/IL-13, however, would cause a specific immunosuppression of the allergic system.

4. Structure Elucidation of the IL-4/IL-4R α Binding Interface

A rational understanding of the inhibition in the IL-4/IL-13 system requires a structural understanding of its components. From the structural biologists point of view the cytokine-mediated, sequential association of different receptor chains requires a sharply tuned coordination of molecular recognition events between the cytokine and the extracellular domains of the interacting receptor chains. The binding affinity of the IL-4R α chain is of pivotal importance in regard to its intervention in the IL-4 system. A loss of its binding affinity would result in an effective inhibition of IL-4 and IL-13.

Molecular recognition of the ligand within the IL-4R α chain is solely mediated by its 207 amino acid residues that comprise the extracellular domain. This domain, hereafter referred to as the IL-4 binding protein (IL4-BP), can be expressed as a separate protein and exhibits the same binding affinity, specificity, and association constant of IL-4 binding as the entire receptor α chain ($K_d \approx 150$ pM, $K_{on} \approx 10^7$ M $^{-1}$ s $^{-1}$).^[60, 61] The X-ray crystal structure of the binary complex of IL4-BP and IL-4 has recently been determined.^[62] Prerequisite was the establishment of IL4-BP expression in *E. coli* and subsequent refolding of the protein into its functional structure.^[63] The knowledge of the structure and spatial arrangement of the binding partners and their molecular interactions in the binary complex together with the mutation and quantitative interaction analysis^[64] enables a better understanding of the molecular recognition between IL-4 and IL-4R α . This knowledge sheds further light on receptor/ligand interactions in the cytokine superfamily and promotes the development of suitable inhibitors.

4.1. The Cytokine Receptor Superfamily

The cytokine receptors are pooled in a superfamily, since they possess conserved sequence motifs—the so called “cytokine receptor homology (CRH) region”—in their extracellular domains.^[65] The CRH, which comprises approximately 200 amino acid residues, contains the ligand-binding determinants of the receptor and is organized in two protein domains, which are covalently connected by a short linker segment. In addition, the CRH in most receptors is characterized by four conserved cysteine residues in the N-terminal domain

and a strictly conserved Trp-Ser-Xaa-Trp-Ser sequence motif in the C-terminal domain. Receptors with these characteristics, such as IL-4R α , are referred to class I cytokine receptors. The closely related class II receptors are characterized by a CRH with four different conserved cysteine residues and the lack of the Trp-Ser-Xaa-Trp-Ser motive.^[65]

4.2. The Structure of IL4-BP

The interleukin-4 binding protein exhibits an L-shaped structure and consists of domains D1 (amino acid residues 1–91) and D2 (amino acid residues 97–197), which are covalently connected by a short helical segment (Figure 3). Both domains belong to the immunoglobulin superfamily and show structural similarity to the fibronectin type III module.^[66, 67] Their folding is characterized by a sandwichlike structure. It comprises seven antiparallel β strands organized in a three-stranded (strand β A, β B, β E) and a four-stranded β -pleated sheet (strand β G, β F, β C, β C') and are twisted by approximately 40° relative to each other. The folding topology of domain D1 belongs to subtype “h”^[66] of the immunoglobulin family where the β -strand C' first interacts with β -strand C and then its direct continuation—now designated β -strand D—interacts with β -strand E of the other sheet. The domain contains six cysteine residues, which are engaged in three disulfide bridges, namely Cys⁹–Cys¹⁹, Cys⁴⁹–Cys⁶¹, and Cys²⁹–Cys⁵⁹. The first two are both conserved in class I CRHs^[65],

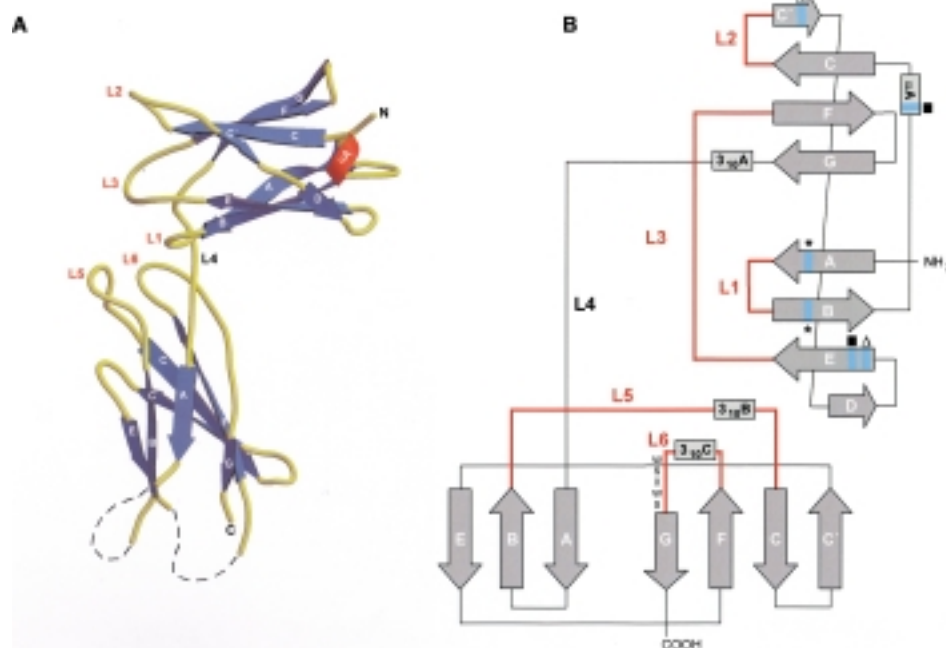


Figure 3. Structure of the extracellular domain of IL-4R α (IL4-BP). A) Ribbon-type representation of IL4-BP. α helices are depicted in red, β strands in blue, and connecting loops in yellow. Amino and carboxy termini are marked with N and C, respectively. The β strands are labeled according to ref. [66] and the loops orientated towards the ligand labeled according to ref. [76]. B) Topologic diagram of the structure of IL4-BP. The folding topology of both domains belongs to the immunoglobulin family and both exhibit structural similarity to the fibronectin type III module.^[79] Secondary structural elements are depicted as arrows (β strands) and boxes ($\alpha/3_{10}$ helices). The positions of the six cysteine residues engaged in the three disulfide bridges (Cys⁹–Cys¹⁹, Cys²⁹–Cys⁵⁹, and Cys⁴⁹–Cys⁶¹) are marked as blue bars. The position of the conserved WSXWS motif in D2, which precedes strand β G, is also indicated. The five loops, which contribute to ligand binding (L1–L3, L5, and L6) are labeled in red.

while the third only occurs in IL-4R α . Domain D2 exhibits standard fibronectin type III topology ("s"-type of the immunoglobulin superfamily).^[66] It does not contain any disulfide bridges and is further characterized by the conserved Trp-Ser-Xaa-Trp-Ser motif, which is situated in a bulge that precedes strand β G (Figure 3). The orientation of the domains relative to each other within the binding protein is characterized by a relatively open total structure. The C-terminal edge of domain D1 and the N-terminal edge of domain D2 are both oriented towards bulk solvent and not to each other. This arrangement creates a domain–domain angle of about 90° and a comparably small domain–domain interface, which has important consequences for ligand binding since the loops AB_{D1} (marked as L1 in Figure 3), CC'_{D1} (=L2), DF_{D1} (=L3), BC_{D2} (=L5), C'E_{D2} (not marked in Figure 3), and FG_{D2} (=L6) are thereby situated in the vicinity of the linking segment and are largely accessible to bulk solvent.

4.3. The Structure of IL-4

Mature human IL-4 is a monomeric protein comprising 129 amino acid residues, whose structure has been independently elucidated by NMR spectroscopy and X-ray crystallography.^[68–71] The molecule consists of four α helices, hereafter referred to as α A, α B, α C, and α D, which are arranged in a bundlelike fashion (Figure 4). Helices α A and α B are oriented upwards within this arrangement, while helices α C and α D are oriented downwards. This unusual arrangement is referred to as an "up-up-down-down" topology. In this arrangement each helix is in an antiparallel orientation to both neighboring helices and there are two long connecting segments situated after helices α A and α C that both cover the whole molecule. In addition, there is a short connecting segment situated after helix α B which joins two sequential helices. The two long connecting segments furthermore contain two short β strands.

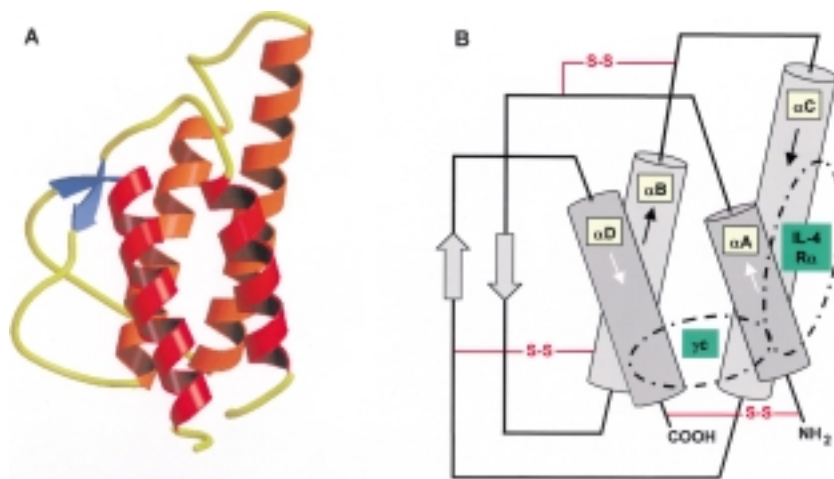


Figure 4. Structure of interleukin-4 (IL-4). A) Ribbon-type representation of IL-4. α helices are depicted in red (α helices α A and α D in the foreground) and orange (α helices α B and α C in the background), β strands in blue, and connecting loops in yellow. B) Topologic diagram of the structure of IL-4. Secondary structural elements are depicted as cylinders (α helices) and arrows (β strands). The positions of the six cysteine residues engaged in the three disulfide bridges (Cys³–Cys¹²⁷, Cys²⁴–Cys⁶⁵, and Cys⁴⁶–Cys⁹⁹) are labeled in red. The positions of the binding sites on both receptor chains are indicated as dashed lines: the binding epitope of the receptor α chain on the helix AC-face and the binding epitope of the γ_c chain on the helix AD-face.

These build a small antiparallel β sheet, which is packed against helices α B and α D. The molecule contains six cysteine residues which are engaged in three disulfide bridges, namely Cys³–Cys¹²⁷, Cys²⁴–Cys⁶⁵, and Cys⁴⁶–Cys⁹⁹. Such a structure, hereafter referred to as a "four-helix bundle", can also be found in other cytokines. Two variants can be discriminated: the short-chain four-helix-bundle cytokine family, which comprises IL-4 as well as IL-2, -3, -5, -7, -9, -13, -15, and GM-CSF, and the long-chain four-helix-bundle cytokine family with growth hormone, prolactin, G-CSF, erythropoietin, IL-6, IL-11, oncostatin M, LIF, and CNTF.^[72] Members of the short-chain family can be characterized by chain lengths of 105–145 amino acid residues, short α helices (about 15 amino acid residues) and an angle of about 35° between the helix pairs A/D and B/C. For members of the long-chain family it is typical to have between 160 and 200 amino acid residues, long α helices (about 25 amino acid residues) and a stronger alignment of the helical axes (angle of about 20° between helix pairs A/D and B/C).

IL-4 exhibits one binding site for each of its interacting receptor chains. The functional epitope of IL-4 that determines its high-affinity binding to the receptor α chain is localized on the surface built up by helices α A and α C (hereafter referred to as "helix AC-face", Figure 4).^[64, 73] The epitope responsible for the binding of γ_c is located on the surface created by helices α A and α D ("helix AD-face").^[73, 74]

Investigations with IL-4 mutants on cells which express the type-II IL-4 receptor have led to the conclusion that the binding interfaces of IL-13R α 1 and γ_c overlap on the IL-4 molecule.^[75, 76] IL4-BP binds through the above-mentioned loops to the helix AC-surface of IL-4. The quarternary structure of the complex is characterized by an almost perpendicular arrangement of the molecular main axes. The binding loops of IL4-BP are arranged in a stacklike fashion and are approximately colinear to the helical axes of the IL-4 helices (Figure 5).

4.4. The IL-4/IL4-BP Complex

The structure of receptor-bound IL-4 is very similar to that of free IL-4. The crystallographic analysis^[62] however shows a slightly changed relative conformation of the IL-4 helices. Whereas the arrangement of the helical axes of helices α B and α C remains unchanged, the axes of helices α A and α D are twisted towards each other. The most significant structural changes occur in the region of the functional binding epitope of γ_c ^[74], where reduced intramolecular Ca–Ca distances of the determining amino acid residues (Thr¹¹, Asn¹⁵, and Tyr¹²⁴) are observed. The distances between the pairs Ile¹¹–Tyr¹²⁴ and Asn¹⁵–Tyr¹²⁴ are decreased by 1.0 and 1.5 Å, respectively. Since γ_c does not bind free IL-4 at submicromolar concentrations, the conformational changes of IL-4

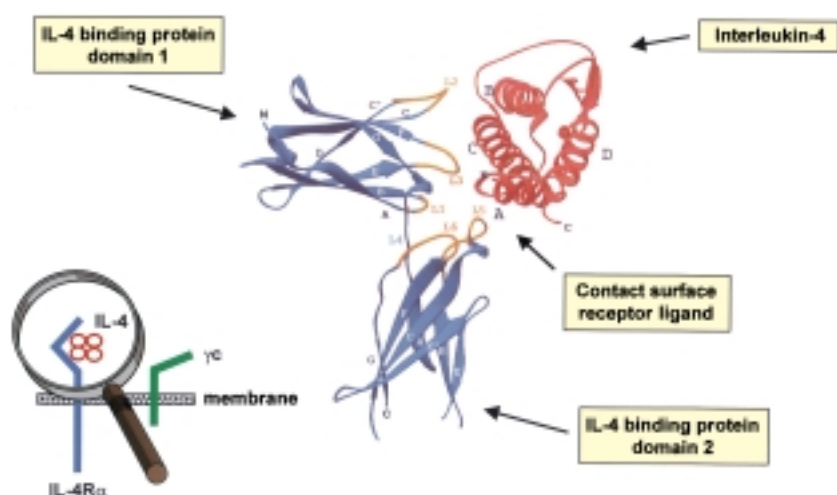


Figure 5. Structure of the IL-4/IL4-BP complex. Ribbon-type representation of the IL-4/IL4-BP-complex (IL4: red, IL4-BP: blue/orange, see also the small scheme). Amino and carboxy termini are labeled with N and C, respectively; the helices and strands are labeled according to ref. [66]. The five loops of IL4-BP which are involved in interactions with IL-4 are highlighted in yellow and are labeled according to ref. [76]. They are arranged in a stacklike fashion comprising four levels in the sequence L2, L3, L1, and L5/L6. Loop L4, which is depicted in blue, connects both domains and has no interaction with IL-4 but is included for consistency.

after binding to IL-4R α can be of functional importance.^[74] Conformational changes in IL-4 triggered by binding to IL-4R α can therefore be prerequisite for an association of γ_c to the binary complex and therefore for signal transduction.

The structural binding epitope comprises 18 amino acid residues on each of the binding partners, which are distributed over multiple sequence segments. The contact areas deviate strikingly from planarity and it is important to note the polar character of the complementary binding surfaces (Figure 6A). Amino acid residues that contribute to IL-4 binding are dispersed over three helices and mainly comprise polar and charged amino acids. The receptor epitope, however, consists of amino acids from five loops and has a midline of hydrophobic side chains with patches of tyrosine and serine residues on one side and a patch of aspartic residues on the other side (Figure 6A). The structural binding epitope as a whole has a surface area of about 800 Å² on each binding partner (measured as the decrease in the solvent-accessible surface^[77] upon complex formation). The covered surface is similar in size to that buried in other cytokine/receptor interactions, such as the topologically equivalent low-affinity interac-

tion between the growth hormone and its binding protein.^[78] This observation indicates that a high-affinity interaction can also be generated from a likewise small contact area and that its size is not directly related to binding affinity.

4.5. High Resolution Interaction Analysis

The analysis of the binding of IL-4 to the receptor on whole cells or to soluble IL-4BP results in a dissociation constant K_d of about 100 pM (Figure 7). This high affinity is the result of a quite fast association ($k_a = 1 - 2 \times 10^7$) and a rather slow dissociation ($k_d = 2 \times 10^{-3}$) process. The analysis of the binding of mutations together with quantitative interaction analysis allows the contribution of each contact residue to the free energy of binding to be estimated. Therefore, an alanine-substituted variant of each contact residue

in IL-4 and IL4-BP was created by site-directed mutagenesis and corresponds to the replacement of the original amino acid side chain with a methyl group. Most of the alanine variants of the contact residues in IL-4 and IL4-BP could be obtained with >96% yield and their binding affinity analyzed after expression in *E. coli*, renaturation, and purification

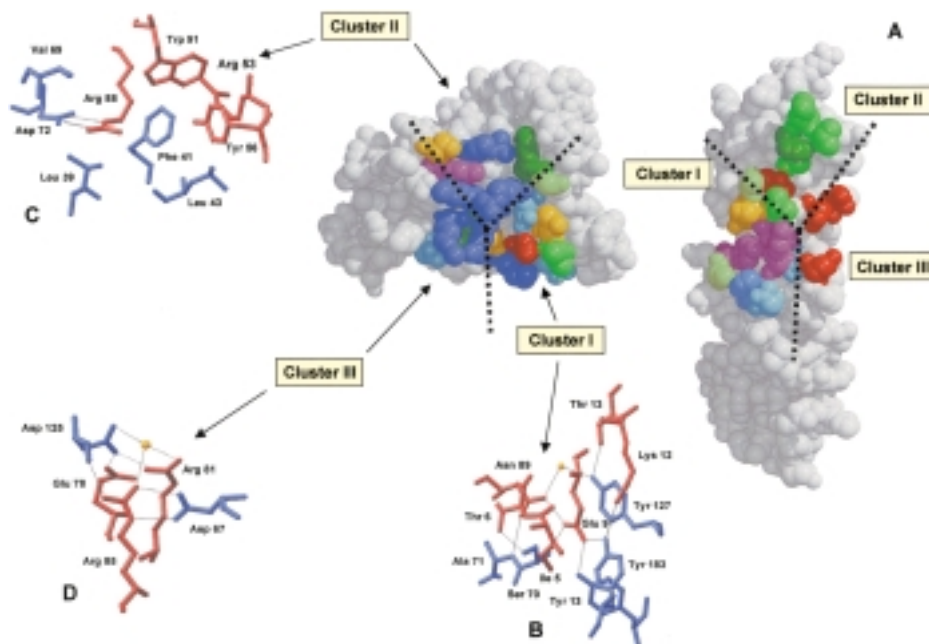


Figure 6. The structural epitope of the IL-4/IL4-BP complex. A) Open-book view of the complementary structural epitopes of IL-4 (left) and IL4-BP (right). Amino acid residues that form interactions between the receptor and ligand are highlighted and colored according to their physicochemical properties: red: negatively charged (Asp, Glu); dark blue: positively charged (Arg, Lys); light blue: His; cyan: Gln/Asn; magenta: Tyr; orange: Ser/Thr; and green: hydrophobic. B) – D) Detailed views of the IL-4/IL4-BP contact. IL-4-Glu⁹ forms the center of a network of hydrogen bonds and is surrounded by hydrophobic side chains ("cluster I", B). IL-4-Arg⁸⁸ and IL4-BP-Asp⁷² build a central salt bridge and are surrounded by aromatic and aliphatic moieties ("cluster II", C). Electrostatic interactions dominate in "cluster III" (D).

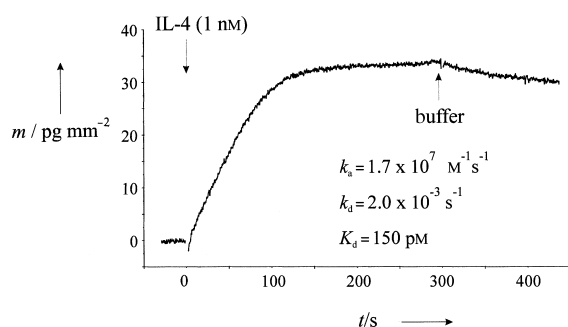


Figure 7. The binding of IL-4 to IL4-BP is extraordinarily fast. In contrast, the dissociation of the complex is rather slow. The graph obtained by analysis of a biosensor shows the kinetics for the association of 1 nM of IL-4 to IL4-BP, which is immobilized on the biosensor.^[61] In the presence of buffer the complex decomposes at a half-life rate $\tau_{1/2}$ of about 6 min. The rate constants of association (k_a) and dissociation of the complex (k_d) as well as the calculated dissociation constant K_d are also indicated.

Substitution of the IL-4 side chains Glu⁹ and Arg⁸⁸ leads to a loss of affinity (K_a) by a factor of 300 to 1000. This loss corresponds to a loss of free binding energy ($\Delta\Delta G_o$) by 3–4.5 kcal mol⁻¹. Clearly, Glu⁹ and Arg⁸⁸ are the main determinants of binding. Five additional side chains, namely Ile⁵, Thr¹³, Asn⁸⁹, Trp⁹¹, and Arg⁵³ are secondary determinants of binding, whose substitution by alanine results in a loss of binding affinity by a factor of 3 to 10 ($\Delta\Delta G_o = 0.4$ –1 kcal mol⁻¹). The other contact residues of IL-4 do not directly contribute to binding. A smaller contribution to the association constant (K_a), summing to a factor of 10 in total, is conveyed by the positively charged amino acid side chains of IL-4. This global charge contribution from six positively charged side chains results in a preliminary orientation of the complementary charged proteins during association (electrostatic steering).^[79]

Mutagenesis of the receptor α chain completes the energy profile of the IL-4 binding epitope. The highest loss in binding affinity is obtained after substitution of Asp⁷², Tyr¹³, and Tyr¹⁸³ with alanine. The negatively charged residue Asp⁷² seems to form an electrostatic interaction with the side chain of Arg⁸⁸ in IL-4. The structure reveals that a perfect spatial orientation of their carboxylate and guanidino groups is adopted. The residues Tyr¹³ and Tyr¹⁸³ of the receptor employ their phenolic hydroxyl groups to form hydrogen bonds to the side chain of IL-4-Glu⁹. Additional interactions are contributed from the carbonyl moiety of Ser⁷⁰ in the receptor and the carbamide group of residue Asn⁸⁹ in IL-4. The structure reveals the participating atoms have a perfect “sp² geometry”. Interestingly, secondary determinants of the receptor, which lead to a loss of binding affinity by a factor of 10 to 50, are hydrophobic residues, such as Tyr¹²⁵, Val⁹⁰, Phe⁴⁵, and others. Presumably, this outer mantle of hydrophobic side chains (“avocado cluster”) leads to an intensified binding of the polar, main determinants. Thus, the arrangement of these binding epitopes exhibits a mosaic structure and is mainly polar. This observation is contrary to individual hydrophobic interactions (“hot spot of hydrophobic binding energy”) that have been described for the high-affinity binding in the growth hormone/receptor complex.^[78]

4.6. The Mechanism of Protein/Protein Interaction: The Three-Cluster Model

The contact surface between IL-4 and the binding protein exhibits a mosaiclike structure comprising three distinct groups of interacting amino acid residues (hereafter referred to as “cluster”); Figure 6A). Two of these clusters are arranged as “avocado clusters”, which means that they reveal an eye-catching amphiphilic structure with an outer mantle of hydrophobic side chains arranged around an inner core of polar groups. Cluster I is arranged around Glu⁹ of IL-4. Its carboxylate group accepts three bonds from residues Tyr¹³, Tyr¹⁸³, and Ser⁷⁰ of the binding protein (Figure 6A and 6B). The geometrically perfect arrangement seems to be preorientated by an intermolecular bond to IL-4 Asn⁸⁹ and is further completed by interactions between Thr⁶, Lys¹², Thr¹³, and Asn⁸⁹ of IL-4 and Ser⁷⁰, Tyr¹⁸³, Tyr¹²⁷, and Ala⁷¹ of IL4-BP. A single water molecule is locked in the contact area and forms a hydrogen-bonding interaction between IL-4-Asn⁸⁹ and IL4-BP-Tyr¹²⁷. This network of polar interactions is surrounded by aromatic and aliphatic side chains: IL-4-Glu⁹ has van der Waals contacts with Ile⁵, Lys¹², and Thr¹³ of IL-4, packs flat against the side chain of IL-4-BP-Tyr¹²⁷, and its carboxylate group is encased by the side chains of Tyr¹³, Tyr¹⁸³, Val⁶⁹, and Ala⁷⁰ of the binding protein. Cluster II (Figure 6A and 6C) is assembled around the central salt bridge that links IL-4-Arg⁸⁸ and IL4-BP-Asp⁷². The guanidino and the carboxylate group are orientated in the same plane and align edge to edge through two hydrogen bonds. Asp⁷² seems to be completely buried at the base of a cleft in the surface of the binding protein. IL-4 Arg⁸⁸, however, completely stands out from the surface of IL-4 and is situated between the side chains of Val⁶⁹ and Phe⁴¹ in the complex. Cluster III deviates from the previous design principle and exhibits a completely different architecture that is dominated by electrostatic interactions (Figure 6A and 6D). However, the interacting residues Arg⁸¹, Arg⁸⁵, and Gln⁷⁸ of IL-4 and Asp⁶⁶, Asp⁶⁷, and Asp¹²⁵ of the binding protein cannot establish strong interactions, since their binding geometries deviate significantly from standard sp²-type interactions.

Altogether the crystal structure analysis^[62] and the mutational analysis of contact residues of IL-4^[64] yields a mechanistic model of the interaction of IL-4 with the extracellular domain of its high-affinity receptor. The main determinants of the binding affinity of IL-4 are the residues Glu⁹ and Arg⁸⁸, which are the central residues embedded in clusters I and II. Both residues are equally important for structure and function and together are responsible for a large portion of the whole binding affinity. Mutations of Glu⁹ and Arg⁸⁸ result in a loss of binding affinity of two to three orders of magnitude. Mutations of peripheral amino acid residues, however, only lead to a loss of binding affinity of one order of magnitude. This result is in accordance with their subordinate structural importance. In contrast, the structure suggests a different role for cluster III: this cluster seems to have only minor functional significance in the stabilization of the complex, but does, however, promote the extraordinarily fast formation of the complex by electrostatic steering. Hence, all mutations in cluster III of IL-4 show hardly any effect on binding, but influence their rate of association.

4.7. The Binding of the Second Receptor Chain (γ_c or IL-13R α 1)

The high affinity of the interaction of IL-4 with the receptor α chain, as described in the preceding discussion, is now well understood. Both the structural epitope (contact surface) and the functional epitope (energy profile/map of the binding residues) have been characterized. The kinetic and thermodynamic parameters of binding resulting from IL-4 binding to the soluble receptor domain and from IL-4 binding to the receptor on whole cells are equal. This is not unexpected, since in both experiments IL-4 binds out of solution.

However, less well understood and much more difficult to analyze is the interaction with the second receptor chain, which may be either γ_c or IL-13R α 1. The second activation step of the IL-4 receptor—and as far as is known of other cytokine receptors—proceeds by the binding of the second receptor chain to the complex of the ligand and high-affinity receptor chain which is located in the plasma membrane. IL-4 does not bind to γ_c (or to IL-13R α 1) on whole cells up to concentrations of 100 nM if the α chain is not simultaneously present. However, IL-4R α is already fully saturated at these IL-4 concentrations ($K_d \approx 100$ pM). For this reason, γ_c and IL-13R α 1 are called low-affinity binding partners of IL-4.

The formation of the ternary complex in the presence of IL-4 can be demonstrated on a biosensor by using soluble ectodomains of the α chain and the γ_c chain (Figure 7). The γ_c ectodomain binds to the complex of IL-4 and immobilized IL4-BP with an affinity constant of $3 \times 10^5 \text{ M}^{-1}$, while the affinity between free IL-4 and the γ_c ectodomain is only reduced by a factor of 50. The increased affinity to the IL-4/IL4-BP complex could originate from a small conformational change in the IL-4 complex or from rather weak interactions between both ectodomains.

The dissociation constant of 3.5 μM for the dissociation of the γ_c ectodomain from the ternary complex is far beyond the physiological concentration of IL-4. Nevertheless, IL-4 is already active at subnanomolar concentrations. It is therefore conclusive that an IL-4-dependent dimerization and transactivation of the chains takes place. Certain possibilities may be considered.

Direct interactions between the α and the γ_c ectodomain are clearly very low as reflected by a K_d value of 5 μM . However, direct interactions could occur between the receptor chains in the cytoplasmatic domains or in the transmembrane regions. Then IL-4 would only act as the necessary trigger for this receptor–receptor binding. In cells exhibiting high expression levels such ligand-independent receptor activation, which hints to direct interaction between the receptor chains, has been demonstrated for other receptors. The expres-

sion levels of IL-4 receptor chains are often low, but it cannot be excluded that additional concentration takes place in membrane microdomains such as the “rafts”.^[80]

The probability of productive collisions among membrane proteins is significantly higher than among dissolved molecules. A density of 1000 receptor α chains homogeneously distributed on a cell surface area of 100 μm^2 exhibits molecular distances corresponding to a 50 nM solution. Therefore, IL-4 is concentrated by orders of magnitude upon receptor binding. Since the degrees of freedom in diffusion and in particular in rotation are restricted in the membrane productive collisions will happen more frequently. As a result of these membrane effects the binding of the ligand to the second chain is sufficient for receptor activation despite its low affinity for the second receptor chain.

4.8. From Theory to Practice: IL-4 Receptor Antagonists

The high-affinity binding to the receptor α chain is responsible for almost the entire binding affinity of the whole IL-4 receptor. Clearly, there are only limited interactions between both receptor chains, because otherwise γ_c affinity would make a stronger contribution to the binding of IL-4 to the entire receptor. Since the required interaction between IL-4 and γ_c is only weak, it is not sufficient to replace the involved interacting residues by the neutral amino acid alanine. Such variants will still keep a significant agonistic activity. Therefore, repulsive residues must be inserted into the γ_c -binding epitope of IL-4 to obtain complete antagonists effectively.

The substitution of Ile¹¹, Asn¹⁵, and in particular of Tyr¹²⁴ in IL-4 with alanine identified these residues as the main determinants of γ_c binding. Although the corresponding alanine variants exhibit a 5 to 100-fold decreased binding to the γ_c ectodomain, their biological activity is hardly impaired: their ED₅₀ values—the dose at which the target cell is half maximally stimulated—are unchanged and their maximal signals are reduced to 50 % at best. Substitution of Tyr¹²⁴ with a variety of amino acid residues yields partial agonists to which cells respond with different efficiencies (Figure 8). Individual variants yield between 0.5 and 90 % activity of

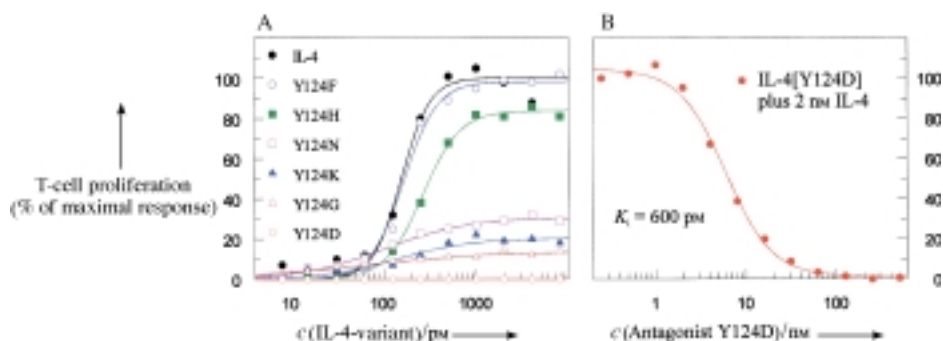


Figure 8. IL-4 mutant proteins are partial agonists or antagonists if the low-affinity binding epitope for γ_c or IL-13R α 1 is impaired or completely blocked.^[73] A) The exchange of IL-4 Tyr¹²⁴ for Phe, His, Asn, Lys, Gly, or Asp creates partial agonists. Their ED₅₀ values remains almost unchanged relative to that of IL-4. B) The variant Tyr¹²⁴Asp is a competitive inhibitor of IL-4 in a T-cell proliferation test and exhibits an inhibiting constant K_i of 600 pM.^[73]

the wild-type, but none of them really shows complete inactivity. However, a double substitution of Arg¹²¹ and Tyr¹²⁴ by two aspartate residues forms a completely inactive variant of IL-4.^[81] The K_d value for the binding of this variant to the α chain and its IC₅₀ value are elevated relative to the wild-type by a factor of two to three to 200–300 pM. The aspartate residues in the γ_c -binding epitope of IL-4 prevent association with the γ_c chain and therefore inhibit dimerization of the receptor chain through electrostatic repulsion.

Up until now the IL-4 binding epitope for IL-13 α 1 has not been determined. Investigations with different variants of IL-4 on cells, using either type 1 or type 2 receptors, show an overlap of the binding epitopes of IL-13R α 1 and γ_c .^[75, 76] However, their repulsion epitopes are distinct from each other, so that selective agonists can be created. The IL-4 variants Y124E or Y124D hardly exhibit any activity with γ_c , but they show considerable activity with IL-13R α 1. Likewise, the variants R121D or R121E are still active with γ_c , but almost inactive with IL-13R α 1.

5. Preclinical and Clinical Application of IL-4 Antagonists

Antagonistic IL-4 mutants, in particular the complete antagonist R121D/Y124D, show their potential as inhibiting compounds in a variety of cell culture systems. These variants suppress cellular reactions to IL-4 and IL-13, such as phosphorylation of signal transduction proteins, regulation of gene expression, and cytokine-dependent cell proliferation. These experiments also demonstrate the specificity of these variants as inhibitors, since other cytokines are not impaired.

In order to evaluate their effectiveness in a mouse model an analogous variant of murine IL-4 had to be established, since IL-4 is specific to each species. This murine IL-4 variant, Q116D/Y119D, is a complete antagonist for murine IL-4 and IL-13 in vitro.^[82] When applied in vivo in a murine allergy model against a protein antigen it demonstrated the suppression of allergic reactions.^[39] Moreover, when the antigen and IL-4 antagonist were simultaneously applied the mice did not develop measurable levels of specific IgE and IgG1 antibodies, which are both associated with allergic reactions in the mouse. The intradermal or intravenous application of the antigen remains without any symptoms, although all animals which had not been treated with the IL-4 antagonist developed strong allergic reactions including lethal shocks. Thus, the murine IL-4 antagonist Q116D/Y119D protected treated animals from developing an allergy. The potential to suppress already established allergies by inhibition of IL-4R α is currently under examination.

Monkeys (*Macaca fascicularis*) were used to evaluate IL-4 antagonists in asthma.^[83] Here, the human IL-4 antagonistic variant could be used. Also in this case, the antagonist, which was applied before sensitization, prevented the development of symptoms. Moreover, a therapeutic effect could be established: the application of the IL-4 antagonist in asthmatic animals resulted in a decreased bronchial hyperreactivity and a lower rate of infiltration of eosinophiles into the respiratory tract. Neither in these experiments nor during the

above mentioned mouse studies could toxicities or other harmful side effects of the IL-4 variants be observed.

The favorable in vitro and in vivo tests, and in particular the therapeutic effects observed in the asthmatic monkey model, resulted in clinical development of the IL-4 R121D/Y124D variant. Since spring 1999 the effectiveness of IL-4 R121D/Y124D in humans has been evaluated in phase 1 clinical trials conducted by Bayer AG; however, results from these studies are not yet available.

6. IL-4 Antagonists: Paradigma or Lucky Strike?

Cytokine receptors are activated by homo- or heterodimerization of receptor chains within the plasma membrane. Some receptors such as the IL-2 or the IL-6 receptor employ a third chain to increase the affinity of ligand binding. The ligand primarily binds to the first chain with high affinity in both homodimeric as well as heterodimeric receptors. Subsequently, the intermediate complex formed recruits the low-affinity chain. The question arises, whether it is feasible to create inhibiting variants for each cytokine receptor by following the principle established with the IL-4 receptor antagonist, namely to block the low-affinity binding epitope of the ligand by mutation.

Presumably this is not possible. Problems with the binding affinity of the corresponding antagonistic variants of other cytokines—even in structurally similar systems—can be predicted. Most cytokine receptors bind their ligands with $K_d = 100\text{--}500$ pM. However, this total affinity of the receptor complex is often not only provided by the high-affinity chain, as occurs within the IL-4 receptor complex. The high-affinity chain usually binds up to a 100 times weaker than the entire receptor. Only by association with the low-affinity chain is the whole binding strength achieved. This binding strength can even vary within receptor families which employ shared receptor chains (gp130, γ_c , or β_c).

GM-CSF binds to its α chain with $K_d = 2\text{--}12$ nM, IL-3 binds its α chain with $K_d = 20\text{--}100$ nM, and IL-5 binds its α chain with $K_d = 1.2\text{--}2$ nM. By addition of the common β chain to each of these receptors, the affinity for their ligands increases to 100–500 pM. The increase in K_d amounts to a factor of 3 for IL-5, to a factor of 100 for GM-CSF, and to almost 1000 for IL-3. Presumably, the increase for GM-CSF and IL-5 originates from a direct interaction of the corresponding α chain with β_c , where the extent of coupling is different.

Similar differences can be found within the γ_c family. Until now it has been impossible to develop useful antagonists for IL-2, since the binding affinity of IL-2 for IL-2R β and even for a complex of IL-2R β and γ_c is unsatisfactorily low. An antagonistic variant would exhibit a correspondingly low binding affinity and therefore would be rather ineffective in displacing the stronger binding wild-type ligand from its receptor. However, a possible solution to this dilemma may be the creation of superagonists, whose affinity for the first chain is increased. Mutagenesis of several cytokines such as hGH, IL-6, and IL-4 yielded variants that exhibit stronger binding affinity to the high-affinity receptor chain than the corresponding wild-type ligand. A combination of superagonistic

and antagonistic mutations may therefore allow the design of antagonistic cytokine variants that employ the inhibiting principle of the IL-4 antagonist.

7. Perspectives: Miniproteins, Pepidomimetica, and Low Molecular Weight Ligands

A rational approach for the production of an IL-4 “mini-protein” arises from the characterized binding epitopes. The high-affinity binding epitope of IL-4 for the α chain is located on two antiparallel α helices. The transfer of the IL-4 binding residues to the parallel α helices of a leucin zipper (Gal4) yielded synthetic peptides which bind to the α chain with a micromolar K_d value.^[83]

From a pharmacological point of view small mimetics for IL-4 that bind IL-4R α with high affinity and effectively compete with IL-4 and IL-13 are desirable. In principle, the production of synthetic analogues seems to be possible. A variety of different approaches has already yielded small ligands for other cytokine receptors that can be chemically synthesized. Dimeric peptides for erythropoietin and thrombopoietin have already been identified from peptide libraries. They comprise approximately 20 amino acid residues, which dimerize by forming disulfide bridges and exhibit agonistic activity down to nanomolar concentrations. Agonistic ligands for G-CSF have been discovered by high throughput screening. These results show that small high-affinity ligands can be created for the large and relatively flat contact surfaces of the cytokine receptors.

Looking ahead there is a further possible approach for a structure-based ligand development arising from the structure of the IL-4/IL-4-BP contact. In regard to the mosaic structure with two independently binding clusters (“avocado-cluster” I and II) it may be possible to find low-affinity ligands for each of the two clusters independently and subsequently link them to form a high-affinity ligand.

8. Summary and Outlook

The development of an antagonistic IL-4 variant shows that the idea of “emasculated hormones” can be successfully transferred to protein hormones. Decisive in the success was both the structural elucidation of the binding surface and the understanding of the mechanism of receptor activation. It is as yet unclear how far the results gained in the IL-4 system can be transferred to structurally similar hormone/receptor systems. The strong interaction between IL-4R α and its ligand—independent of other binding partners—is quite unique among the cytokines. Presumably, it may be possible within ligand/receptor systems that are characterized by weaker binding interactions to create variants that combine super-agonistic and antagonistic features in one molecule, that is, that bind to their high-affinity chains with increased affinity but do not trigger downstream signaling because of impaired

low-affinity binding epitopes. Therefore, development of antagonistic protein ligands for other protein hormones that employ the IL-4-like mode of action may be possible in the future.

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A New Role for Polyketides

Jürgen Rohr*

Recent studies have shown that natural products are still not replaceable in the broad field of drug discovery and development.^[1] Polyketides, which are produced by various organisms such as bacteria, fungi, or plants, form perhaps the most interesting subgroup of natural products, since they exhibit the largest diversity in chemical structures and biological activities. Between 5000 and 10000 polyketides are known and about 1 % (!) of these possess drug activity, which is five times as many as the average. Pharmaceutically important polyketide drugs include antibiotics, cancer chemotherapeutics, immunosuppressants, cholesterol lowering agents, and antifungals; their sales exceed 15 billion dollars. Thus, an enormous value is attached by the pharmaceutical industry to polyketide compounds. More and more new polyketides are created through the developing advanced techniques of combinatorial biosynthesis,^[2] and new directions can be observed, such as attempts to link the biosyntheses of modular polyketides and nonribosomal peptides^[3] and the attachment of important deoxysugar moieties to various polyketide-derived aglycones.^[4]

Recently, a previously totally unknown role of polyketides has been discovered by Small and co-workers, in the laboratories of the National Institutes of Health in Hamilton, Montana, USA; this is in the role of a toxin which is required for virulence in context with Buruli ulcers caused by *Mycobacterium ulcerans*.^[5] The latter is a chronic and progressive necrotizing ulcer for which no medical treatment exists. The discovery might provide a target for future therapeutic strategies to treat this severe human skin disease which primarily occurs in Australia and Africa. In addition to this potential direct benefit of the research, there is evidence that this polyketide toxin might represent the first of a family of virulence factors associated with other, even more dangerous mycobacterial diseases, such as leprosy or tuberculosis. However, the presence of a toxin has been suggested only in the context of Buruli ulcers,^[6] since this is unique among the mycobacterial diseases because of its small acute inflammatory response.

Although a toxin in the culture supernatant of *M. ulcerans* was reported to cause a cytopathic effect on mouse fibro-

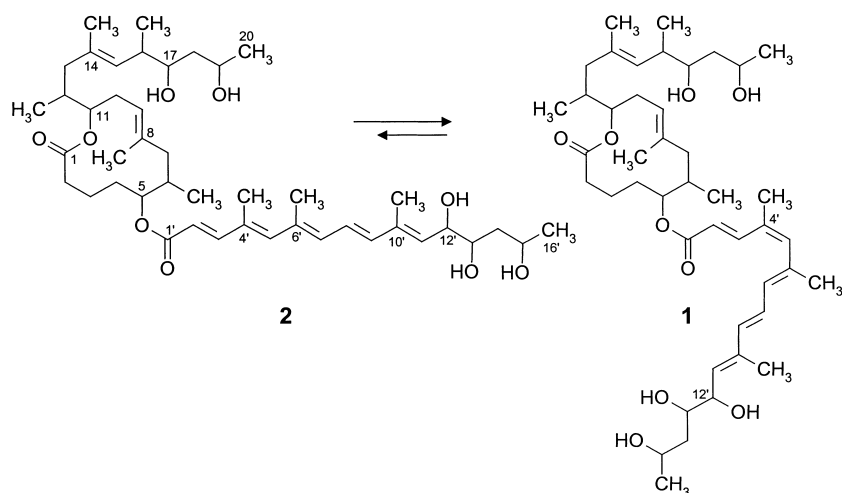
blasts,^[6] and immunosuppressant properties of the filtrate of *M. ulcerans* were even detected,^[7] it seemed to be impossible to isolate and characterize the compound associated with this effect. More recently, a method for the isolation of the toxin from intact bacteria was developed, which allowed its partial purification, and evidence for a lipophilic compound was published.^[8, 9] Then Small and co-workers succeeded in isolating and identifying two polyketides, unusual macrolides with two long side chains, as the toxic principals. They were subsequently called mycolactones A (**1**) and B (**2**), and it was proven through intradermal injections of the purified mycolactones into the backs of guinea pigs that the mycolactones are directly responsible for the necrosis associated with the Buruli ulcer.^[5, 10]

The chemical structures and physicochemical data of the mycolactones A (**1**) and B (**2**) were published about six months later (Scheme 1).^[11] The suggested structures reveal that these compounds are constructed from two type 1 polyketide chains, a decaketide (consisting of five acetate and five propionate units), in which the last five building blocks fold into the twelve-membered lactone moiety, and an octaketide (consisting of five acetate and three propionate units), which forms a hexadecanoic side chain linked through an ester bond to the lactone moiety (Scheme 2).

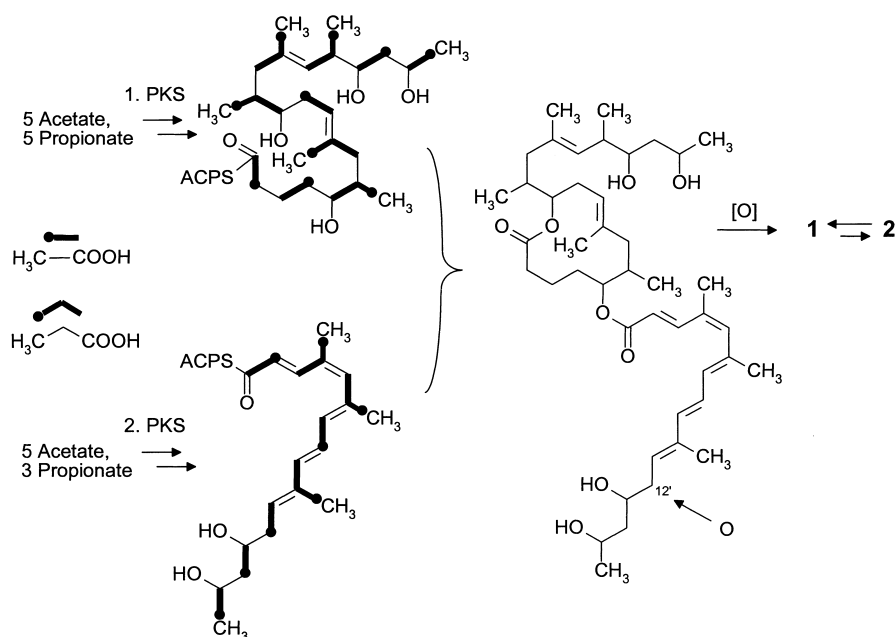
It is assumed that the structures of mycolactones A and B differ only in their ester side chains, in which the 4',5' double bond is *Z*-configured in **1**, and *E*-configured in **2**. Both structures seem to be in an equilibrium, since separation and reanalysis (HPLC, NMR spectroscopy) always yields the same mixture (**1**:**2** = 3:2). This *E/Z*-isomerization obviously complicated the structure elucidation,^[11] however, it might have been avoided with light exclusion, since it is likely to be light-induced. Although the reported structures of **1** and **2** show an *E*-configured 8,9 double bond, the authors assign a *Z* configuration in their text as well as in their deduced IUPAC name.^[11] The indicated nonobservation of an NOE correlation between the 22-CH₃ methyl protons and 9-H also supports an *E* rather than a *Z* configuration of this double bond. Thus, the missing stereochemistry of the ten chiral centers and the questionable configuration of the 8,9 double bond make the structure elucidation incomplete; however, there is no doubt about the macrolide structures and, thus, about their polyketide origin.

The structures of the mycolactones indicate an unusual biosynthesis. Two independent type 1 polyketide synthases (PKS) are required, since both PKS-derived moieties differ

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Scheme 1. Suggested structures for the two mycolactones A (**1**) and B (**2**), which seem to be in equilibrium.



Scheme 2. Plausible biosynthetic hypothesis for the formation of the mycolactones which involves two different type 1 polyketide synthases (PKS) and only one post-PKS enzyme (a monooxygenase).

not only in chain length and sequence of the building blocks (AAPPAPPAA and AAPAPPA for the decaketide- and octaketide-derived chains, respectively; A = acetate, P = propionate), but also in the degree of reduction. It is known that modular type 1 PKS^[12] can catalyze a different degree of reduction after each condensation step, either: no reduction at all, only ketoreduction (KR), KR followed by dehydration (DH), or complete reduction to a saturated CH₂ group through KR, DH, and enoyl reduction (ER). Here we find either KR, or KR-DH, or KR-DH-ER reduction cycles, in a different sequence for the octaketide- and the decaketide-derived moieties of the mycolactones. Besides the PKS reactions, post-PKS modifications, such as glycosyl transfer steps or introductions of additional oxygen atoms through oxygenases, are typical for most known polyketides. Here, post-PKS enzymes seem to be scarcely involved. Only the

oxygen at C-12' necessitates an oxygenase reaction due to its position in the molecule (Scheme 2). Gene sequence analysis studies revealed a close relationship between *M. ulcerans*, *M. tuberculosis*, and *M. marinum* genes.^[13a] The genes encoding the mycolactone biosynthesis have not been discovered or described yet, however, type 1 PKS genes were found in the course of the *M. tuberculosis* genome project,^[13b] and, therefore, it is speculated that polyketides may also play a role in tissue destruction and immunological modulation characteristic of tuberculosis or leprosy.^[5]

In conclusion, the mycolactones possess interesting novel structures due to an unusual biosynthesis requiring a cooperation of two PKS. More important, the mycolactones are the first discovered macrolides from *Mycobacterium*, and the first identified virulence determinants of a bacterial human pathogen, which means an entirely new role for bioactive polyketides.

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D-Serine as a Modulator in the Nervous System

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D-Amino acids occur rarely in nature. The genetic process only has codes, which consist of nucleotide triplets, for the 20 “standard” L-amino acids. On the other hand, D-amino acids do exist in the cell wall of gram negative bacteria, in numerous peptide antibiotics, and in other natural systems. Unlike the L-amino acids, they are not taken up with food or synthesized stereospecifically. They are formed by racemization from their L congeners, a process which is enzymatically catalyzed by racemases.

Unexpectedly, two Japanese laboratories reported some time ago the existence of D-serine and D-aspartate in mammals as well, in the brains of rats and humans.^[1,2] Over the years, evidence accumulated for the fact that D-serine in mammalian brains is not an artefact, but is, rather, an important signalling molecule.^[3] This hypothesis was recently strengthened by the findings of Snyder's group at the Johns Hopkins University in Baltimore.^[4] They succeeded in cloning and sequencing a serine racemase from a mammalian brain. This protein represents a novel enzyme, possibly a first member of a whole family of enzymes, without any significant homology to other known amino acid racemases from lower organisms (with the exception of a short consensus sequence form-

ing the binding site for pyridoxal phosphate). The enzyme is 339 amino acids long, has a calculated relative molecular mass of 36.3 kDa, and operates with the coenzyme pyridoxal phosphate (Figure 1).

Several amino acids, such as L-glutamate, glycine, and γ -aminobutyric acid (GABA) serve in the nervous system as neurotransmitters by transmitting nerve impulses from cell to cell. D-Serine does not seem to be such a transmitter, but rather a “neuromodulator”, an essential regulator at synapses which use L-glutamate as an excitatory transmitter. D-Serine is not released from neurons, like a “normal” transmitter, but from astrocytes, a type of glia cells. In these cells Snyder and his co-workers localized the serine racemase.

To appreciate this discovery one has to take a closer look at the neurochemistry of glutamatergic synapses. L-Glutamate is

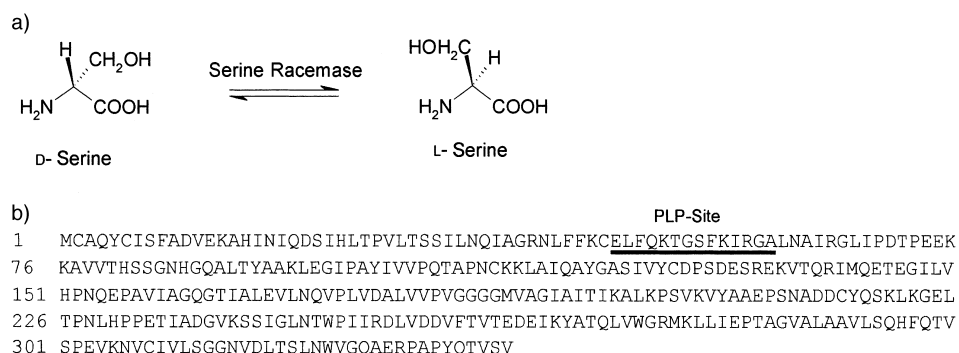


Figure 1. a) The reaction catalyzed by serine racemase. b) The amino acid sequence of serine racemase (single letter codes). The binding site for the coenzyme pyridoxal phosphate, close to the N terminus, is underlined.

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the most important excitatory transmitter in the central nervous system (CNS) of vertebrates. (Insects use L-glutamate peripherally, that is, at their nerve–muscle synapses.) L-Glutamate is released presynaptically and triggers an effect postsynaptically, after it has bound to receptor proteins. Glutamate receptors comprise a large and complex group of

receptors.^[5] For our purpose primarily one subtype of these is of interest, the so-called NMDA receptors.^[6] NMDA stands for *N*-methyl-D-aspartate, a nonnatural molecule which activates specifically this subtype of glutamate receptors. NMDA receptors are of special interest because they play a key role in the CNS with processes involved in learning and memory, with the regulation of growth and programmed death (apoptosis) of neurons, but also with pathological phenomena like strokes, hypoxia, ischemia, neurodegenerative diseases, epilepsy, and schizophrenia.

NMDA receptors are “ligand-gated ion channels”: after binding the neurotransmitter (L-glutamate) the receptor protein forms a cation-selective channel in the postsynaptic membrane, through which primarily calcium ions diffuse into the cell. Interestingly, L-glutamate alone is insufficient for activation of this ion channel. Electrophysiologists observed long ago that glycine is required as a coactivator.^[7] Besides the binding site for L-glutamate the receptor protein contains another site for glycine. (This site was recently localized by recombinant DNA techniques in the primary structure of the receptor.^[8]) From the very beginning it was not really clear whether glycine or another molecule functions as the physiological coactivator in vivo. Johnson and Ascher, who discovered the glycine effect,^[7] speculated that the glycine concentration in the brain is sufficiently high to saturate the glycine binding sites. Others questioned this and postulated either the existence of another endogenous ligand or a locally upregulated glycine concentration. Now, D-serine could be the hypothetical endogenous ligand for the coactivator site.

This interpretation is supported by several experimental results. D-Serine is able to replace glycine in the experimental system; it is about three times as powerful as glycine. With serine-specific antibodies, Snyder and co-workers were able to show that there is a close correlation between the concentrations of D-serine and NMDA receptors in mammalian brains.^[3] Removal of D-serine by means of a D-amino acid oxidase reduces the possibility of activating the NMDA receptor considerably. D-Serine occurs in high concentrations in astrocytes; its release from which can be triggered by glutamate and specific ligands of the other members of the receptor family, the so-called non-NMDA receptors. The last, the most important piece of the puzzle is the proof that a serine racemase exists in those astrocytes.

The picture is now complete (Figure 2). Normally, excitatory impulse transmission goes through non-NMDA receptors. Only with very strong, long-lasting excitation, such as with considerable presynaptic release of L-glutamate, are the NMDA receptors activated too. As soon as the released glutamate secures it from neighboring astrocytes, the coactivator D-serine is supplied. The activation of the NMDA receptors then has the consequences listed above.

However, the physiological function of this coactivation remains unclear. The NMDA receptor is regulated by a variety of mechanisms. For example, its ion channel is blocked by a magnesium ion. This block is released when a second nerve impulse arrives at a neighboring synapse of the same cell, resulting in a depolarisation of the membrane. This too is a “coactivation”, in this case by a second nerve impulse. This

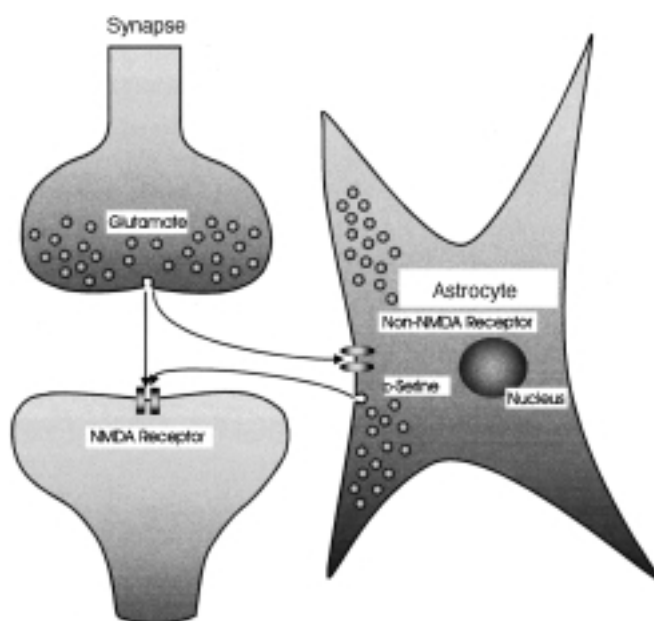


Figure 2. Schematic representation of a glutamatergic synapse, consisting of a nerve ending (top) with its glutamate-containing vesicles and the postsynaptic cell (below) with its NMDA receptors. (The non-NMDA receptors, which are also present here, have been omitted to simplify the figure. The vesicular exocytosis of D-serine from astrocytes and the D-serine uptake system have not yet been shown experimentally.) Next to the synapse, an astrocyte is depicted; this cooperates with the synapse in multiple ways. Amongst others, non-NMDA receptors are found here which regulate D-serine exocytosis after activation by L-glutamate. Only with this D-serine can the NMDA receptors of the postsynaptic cell be activated.

mechanism makes sense when two nerve impulses have to be coordinated, for example, in the case of associative learning. A comparable “purpose” still has to be found for the coactivation by D-serine.

Already, the discovery of the neuromodulatory effect of D-serine is of great practical importance. By considering the medical implications of the NMDA receptors, novel drug targets can now be envisaged. Inhibitors of NMDA receptors and their ion channels are applied as neuroprotective agents against strokes, ischemia, and epilepsy, receptor activators are developed for schizophrenia. With D-serine known to be a NMDA-receptor regulator, new leads for novel targets are at hand. Possible drug targets are enzymes of serine metabolism (including the serine racemase), serine transporters, key proteins of D-serine exocytosis machinery, or the D-serine binding site on the receptor itself.

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537. Adolf Baeyer und Victor Villiger: Einwirkung des Caro'schen Reagens auf Ketone.

[Vorläufige Mittheilung aus dem chemischen Laboratorium der Akademie der Wissenschaften zu München.]

(Eingegangen am 27. December.)

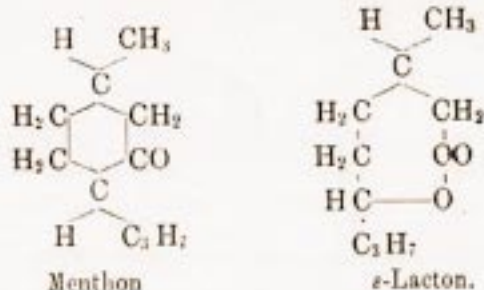
Caro¹⁾ hat im vergangenen Jahre gefunden, dass die Salze der Überschwefelsäure durch Zusammenbringen mit concentrirter Schwefelsäure eine noch unbekannte Veränderung erleiden, indem die nachher folgende Lösung Anilin glatt oxydirt. Nachdem die Oxydation des Anilins nach Tschirner²⁾ gelang es uns, die Oxydation des Anilins noch weiter zu mässigen und bei der Bildung des Phenylhydrazins festzuhalten.

Mit Versuchen über die Sprengung der ringförmigen Ketone in der Terpengruppe beschäftigt, wurden wir auf diese Beobachtungen aufmerksam und fanden, dass das Caro'sche Reagens ein ebenso specifisch auf Ketone wirkendes Mittel ist, welches gestattet, eine Reihe von bisher unansführbaren Aufgaben spielend leicht zu lösen.

Im Folgenden veröffentlichen wir die ersten Resultate dieser Untersuchung mit freundlicher Erlaubnis des Hrn. Caro und hoffen in kürzester Zeit die noch vorhandenen Lücken ausfüllen zu können.

Das Caro'sche Reagens ist ein Oxydationsmittel, welches in der Art seiner Wirkung dem Hydroxylamin ähnlich ist. Aethylalkohol davon nicht merklich angegriffen, während die meisten Ketone oxydirt werden. Diese Aehnlichkeit ist noch weiter in der Oxydationsproducte der Ketone, die Oxime entsprechen, die von Beckmann entdeckten Umlagerung der Oxime entspricht.

So werden Menthon und Tetrahydrocarvon von dem Reagens glatt in die zugehörigen ϵ -Lactone übergeführt, von denen das erste in der vorhergehenden Abhandlung beschrieben worden ist.

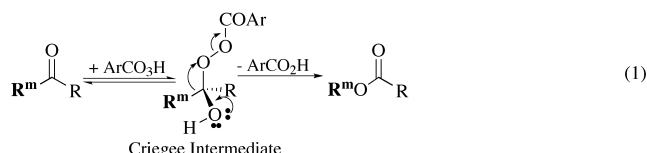


The small and greedy fluorine atom is used to demonstrate the primary stereoelectronic effect in the Baeyer-Villiger reaction. Find out more on the following pages.

A Demonstration of the Primary Stereoelectronic Effect in the Baeyer–Villiger Oxidation of α -Fluorocyclohexanones**

Cathleen M. Crudden,* Austin C. Chen, and Larry A. Calhoun

In 1899, Baeyer and Villiger first reported their method for converting ketones into esters [Eq. (1)].^[1] In the 100 years since Baeyer and Villiger's discovery, the reaction that now



bears their names has become a mainstay of organic synthesis.^[2] The popularity of this reaction is due mainly to the uniqueness of the transformation it affects. However, the stereospecificity and predictable regiochemistry of the Baeyer–Villiger reaction are also key features. With few exceptions, the ketonic substituent that can best stabilize a partial positive charge (R^m in [Eq. (1)]) migrates preferentially.^[3]

The stereoelectronic components of this reaction have been discussed in a handful of publications by a select few groups.^[4–7] The requirement for correct antiperiplanar alignment of the migrating substituent (R^m) and the O–O bond of the leaving group is termed the primary stereoelectronic effect, and antiperiplanar alignment of a lone pair on oxygen with the migrating group is the secondary stereoelectronic effect (Figure 1). Considering the importance of the Baeyer–Villiger reaction in synthesis, and the greater importance of predicting the product of the reaction a priori, the lack of conclusive evidence for these stereoelectronic effects is somewhat surprising.

In 1980, Noyori described the oxidation of a rigid bicyclic substrate that is the only clear demonstration of the secondary stereoelectronic effect (Figure 2).^[5] In this system, unfavorable steric interactions between the hydroxyl proton and the

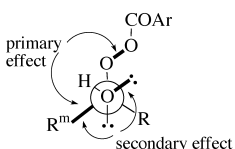


Figure 1. Primary and secondary stereoelectronic effects in the Baeyer–Villiger reaction.

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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.

bicyclic substrate were responsible for restricting rotation around the C–O bond.

With respect to the primary stereoelectronic effect, Chandrasekhar and Roy have designed the best system to date (Scheme 1).^[6, 8]

In their scheme, the conformation of the O–O bond of the peroxyester is fixed since the Criegee intermediate is generated intramolecularly. Thus, treatment of 2-oxocyclohexane acetic acid (**1**) with peracetic acid is postulated to yield

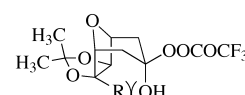
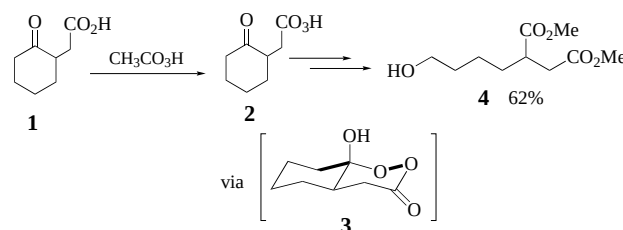


Figure 2. Model for the secondary stereoelectronic effect.

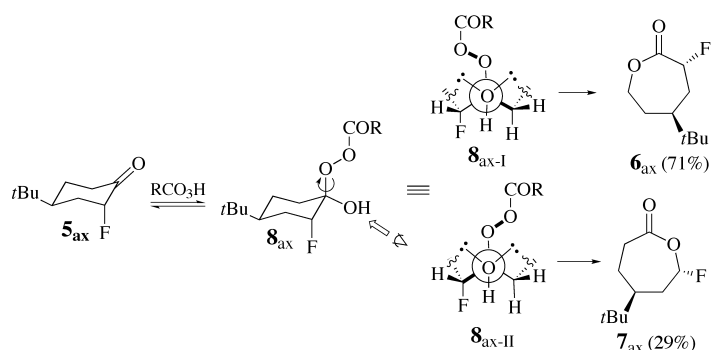


Scheme 1. Chandrasekhar model for the primary stereoelectronic effect.

2-oxocyclohexane peracetic acid (**2**). Intramolecular attack of this peroxyacid on the ketone is proposed to generate bicyclic intermediate **3**, in which only one of the ring bonds is properly aligned for migration. The product they obtained was derived from migration of the properly aligned bond, leading to the conclusion that stereoelectronic alignment was more important than migratory aptitudes. Unfortunately, the immediate Baeyer–Villiger product could not be isolated as it was converted into **4**, which was obtained in only 62% yield. Although Chandrasekhar and Roy present strong evidence for the primary stereoelectronic effect in the Baeyer–Villiger reaction, the poor mass balance they obtained weakens their otherwise ingenious experiments.^[9]

We report herein our study of the Baeyer–Villiger oxidation of α -fluoroketones, which we believe provides the most convincing evidence of the primary stereoelectronic effect reported thus far. The key to our study is that the conformation of the peroxyester bond is biased through dipole/dipole interactions with the α -fluorine substituent. An added benefit of the fluoroketone substrates is that the small size and high electronegativity^[10] of fluorine permit the migratory aptitudes of the two groups to be differentiated without adding any steric biases.^[11]

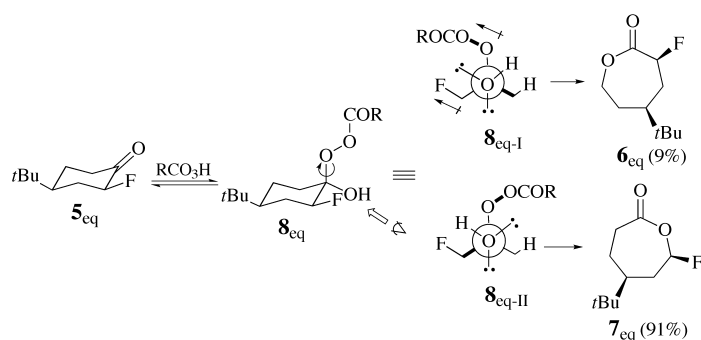
The substrates for our study, *trans*- and *cis*-4-*tert*-butyl-2-fluorocyclohexanone, **5_{ax}** and **5_{eq}**, were synthesized in two steps using the electrophilic fluorinating agent Selectfluor.^[12] Treatment of the enol silyl ether of 4-*tert*-butylcyclohexanone with Selectfluor led to a mixture of **5_{ax}** and **5_{eq}** that could be separated by repeated chromatography.^[13] Subjection of these fluoroketones to Baeyer–Villiger oxidation with recrystallized mCPBA in the presence of NaHCO₃ gave fluorolactones **6** and **7**. As predicted by expected migratory aptitudes,^[14] ketone **5_{ax}** underwent oxidation preferentially at the non-fluorinated carbon to generate lactone **6_{ax}** as the major product (Scheme 2).^[15] The lactone derived from oxidation of



Scheme 2. Stereoelectronic analysis of Criegee intermediate 8_{ax} resulting from axial attack of mCPBA on ketone 5_{ax} .

the fluorine-bearing carbon (7_{ax}) accounted for the remaining material (mass balance 90–98% on repeat runs in CHCl_3).

Exposure of the isomeric fluoroketone 5_{eq} to the same conditions gave dramatically different results. In violation of accepted migratory aptitude rules,^[14] 5_{eq} yielded lactone 7_{eq} resulting from migration of the fluorine-bearing carbon with 91:9 regioselectivity (Scheme 3). Due to the delicate nature of the fluorolactone 7_{eq} , careful workup conditions had to be employed to prevent its decomposition. Under optimized conditions, high mass balances were obtained (up to 99%), and the mass ratios of the isolated lactones were within a few percent of those observed by NMR analysis of the crude reaction mixtures.^[16]



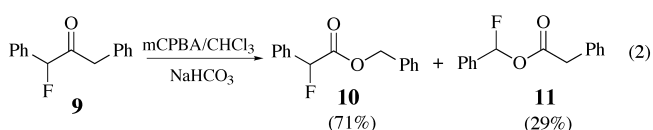
Scheme 3. Stereoelectronic analysis of Criegee intermediate 8_{eq} resulting from axial attack of mCPBA on ketone 5_{eq} .

The observed differences in the regiochemistry of oxidation can be explained by the primary stereoelectronic effect. The Criegee intermediates resulting from axial attack of mCPBA on fluoroketones 5_{ax} and 5_{eq} are shown in Schemes 2 and 3 (for clarity, the ring is omitted and the properly aligned bonds are highlighted). Two conformations of Criegee intermediate 8_{ax} which result from rotation about the C–O bond are shown in Scheme 2. In the two reactive conformations, 8_{ax-I} and 8_{ax-II} , the O–O bond of the peroxyester is properly aligned with the CH_2 or the CHF substituent, respectively. There appears to be little energetic difference between these conformers, so the observed lactone should be the product of relative migratory aptitudes and, as expected, the nonfluorinated carbon migrates preferentially.^[14]

In the case of the isomeric fluoroketone 5_{eq} , conformer 8_{eq-I} of the Criegee intermediate is predicted to have a dipole/

dipole interaction between the peroxyester and the fluorine atom. This dipole would be expected to be most significant in the transition state of the reaction as it involves O–O cleavage. Therefore, the most favorable conformation for Criegee intermediate 8_{eq} would be 8_{eq-II} in which the CHF group is stereoelectronically activated for rearrangement. Since the product distribution greatly favors 7_{eq} over 6_{eq} (82% selectivity), this leads to the conclusion that in this system, correct stereoelectronic alignment is more important than intrinsic migratory aptitudes.^[17]

In order to determine the effect of fluorine on relative migratory aptitudes under our exact conditions, we examined the conformationally unrestricted fluoroketone 9 .^[18] When 9 was reacted with mCPBA under our standard conditions, a 71:29 mixture of products derived from oxidation at the CH_2 and CHF substituents, respectively, was obtained [Eq. (2)], which is virtually identical to the ratio observed with 5_{ax} .



In order to determine whether the dipole/dipole interaction^[19] is the primary factor in the regioselectivity of the oxidation of 5_{eq} , we carried out the Baeyer–Villiger reaction in solvents of varying polarity. Since dipole moments are known to be stabilized by polar solvents,^[20a] we would expect to see a decrease in selectivity for the reaction of 5_{eq} as the solvent polarity increased, while the reaction of 5_{ax} should be unaffected. As shown in Table 1, this is exactly what is observed. The selectivity for conversion of 5_{eq} to 7_{eq} drops from 9.3:1 in CHCl_3 to 5.1:1 in sulfolane (tetrahydrothiophenedioxide), while no change is observed in the reaction of 5_{ax} in these solvents. When a less polar solvent is employed (CCl_4), the selectivity for the oxidation of 5_{eq} increases to

Table 1. Relationship between solvent polarity and selectivity for oxidation of 5_{ax} and 5_{eq} .^[a]

Ketone	Solvent	Solvent polarity ^[b]	$\text{CH}_2\text{:CHF}$ migration (ratio $6\text{:}7$) ^[c]	Mass balance [%] ^[d]
5_{eq}	CCl_4	0.052	1:13.6	85
5_{eq}	CHCl_3	0.259	1:9.3	94
5_{eq}	CH_2Cl_2	0.309	1:7.2	87
5_{eq}	CH_3CN	0.460	1:5.5	99
5_{eq}	sulfolane ^[e]	0.410	1:5.1	— ^[f]
5_{ax}	CCl_4	0.052	1.3:1	82
5_{ax}	CHCl_3	0.259	2.4:1	99
5_{ax}	CH_2Cl_2	0.309	2.5:1	86
5_{ax}	CH_3CN	0.460	2.6:1	72
5_{ax}	sulfolane ^[e]	0.410	2.5:1	— ^[f]

[a] Reaction conditions: 5_{ax} or 5_{eq} (2.6 mmol), mCPBA (3 mmol, 99%), and NaHCO_3 (2.6 mmol) were mixed in solvent (0.2 M in substrate) at RT for 3–20 h. [b] E_T^N (an empirical parameter used to describe solvent polarity relative to tetramethylsilane (0) and water (1)) is used as a measure of solvent polarity.^[20] [c] As measured by ^1H NMR of the crude reaction mixture, which matches isolated ratios within experimental error. [d] Combined isolated yields of **6** and **7** after chromatography, in spectroscopically pure form. [e] Sulfolane = tetrahydrothiophenedioxide. [f] Not determined due to difficulties related to removal of the high-boiling solvent.

13.6:1. The relationship between the polarity of the solvent and the selectivity of the oxidation of **5_{eq}** is displayed graphically in Figure 3. Within experimental error, a linear relationship is observed. If the reaction was carried out in a solvent of high enough polarity to eliminate the effects of the dipole on the conformation of the O–O bond, we would expect that the selectivity would be related only to the intrinsic migratory aptitudes of the system. Under such conditions, the ratio of **7:6** should be equal to that observed in the **5_{ax}** series, namely 1:2.5.

While Schemes 2 and 3 consider only the Criegee intermediates derived from axial attack, it is important to note that

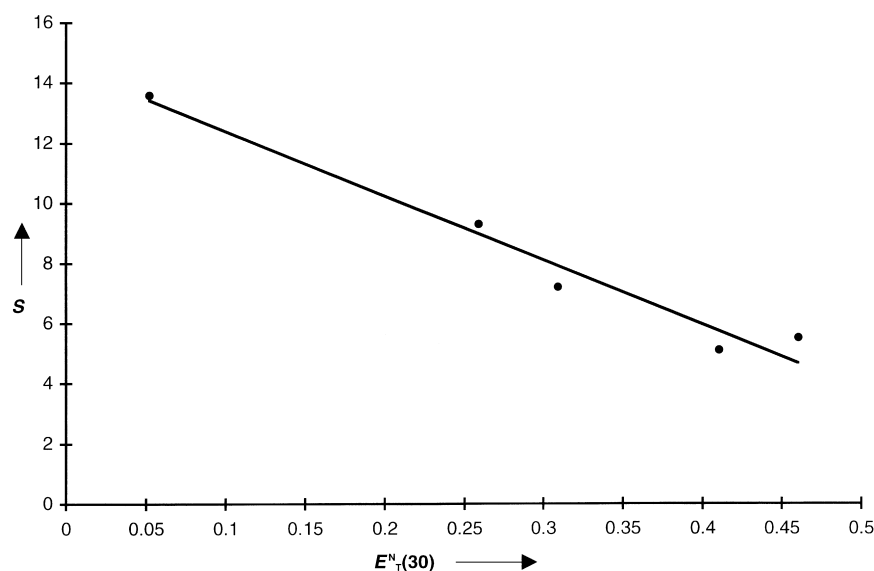


Figure 3. Solvent polarity ($E_N(30)$)^[20] versus selectivity (S) for **7_{eq}** over **6_{eq}**.

the Criegee intermediates produced by equatorial attack lead to the same conclusions. These intermediates are shown in Figure 4. In the case of **8_{eq}**, the analysis is simple. The C–F and

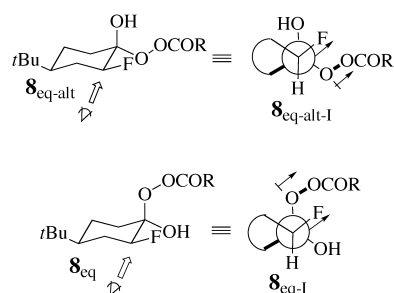
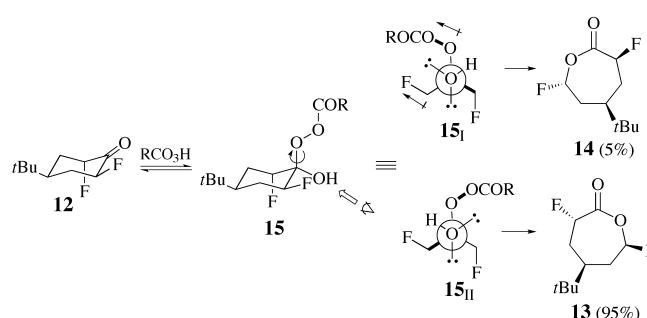


Figure 4. Stereoelectronic analysis of Criegee intermediate **8_{eq-alt}** resulting from equatorial attack of mCPBA on ketone **5_{eq}**. (Note only conformers that activate the CH₂ are shown.)

C–OO substituents have the same gauche relationship regardless of whether they are derived from equatorial attack of the peroxyacid (**8_{eq-alt-I}**) or axial attack (**8_{eq-I}**). When the O–O bond is correctly positioned to activate the CH₂ substituent, a syn-pentane-type arrangement is observed in both cases, and an unfavorable dipole is introduced.^[21]

Finally, substrate **12** was chosen as a more stringent test of the stereoelectronic effect (Scheme 4). If any selectivity is



Scheme 4. Stereoelectronic analysis of Criegee intermediate **15** resulting from axial attack of mCPBA on ketone **12**.

observed, it will be directly related to the spatial orientation of the fluorine atoms, since both substituents are CHF groups. We expect compound **13** (from migration of the equatorial fluorine-substituted carbon) to be the major product, since the conformer leading to this product has the offending dipole minimized (Scheme 4). In the event, treatment of **12** with mCPBA under our standard conditions yielded a 95:5 ratio of **13:14**.^[22]

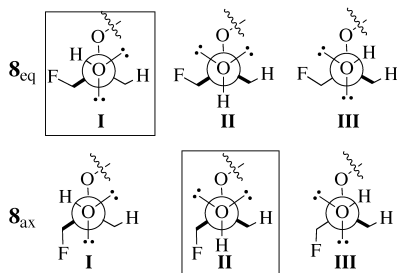
In conclusion, we have demonstrated that the Baeyer–Villiger oxidation of fluoroketone **5_{eq}** yields fluorolactone **7_{eq}** as the major product resulting from migration of the CHF substituent, contrary to accepted migratory aptitudes. On the other hand, **5_{ax}** reacts as expected to give the major product resulting from migration of the CH₂ substituent, with the

same regioselectivity as is observed in a simple acyclic monofluoroketone. The unusual behavior of **5_{eq}** can be explained by invoking the stereoelectronic effect. Migration occurs primarily from the conformer in which the O–O bond of the peroxyester is oriented to minimize the overall dipole. The observation of a significant solvent effect on regioselectivity supports this argument. Furthermore, Baeyer–Villiger oxidation of an α,α' -difluoroketone in which one fluorine is axial and one is equatorial occurs with 90% selectivity for migration of the equatorial CHF substituent. These data lead to the likely conclusion that in the systems we have examined, the primary stereoelectronic effect is of more importance than intrinsic migratory aptitudes, supporting the hypothesis advanced by Chandrasekhar and Roy.^[6, 23]

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- [17] The importance of the secondary stereoelectronic effect must also be considered. There is ample evidence that the hydrogen atom in systems such as 1-hydroxy-2-fluoro-4-*tert*-butyl-cyclohexanone is oriented toward the fluorine, although there is considerable controversy over whether or not a true hydrogen bond is involved: a) J. M. Bakke, L. H. Bjerkeseth, T. E. C. L. Ronnow, K. Steinsvoll, *J. Mol. Struc.* **1994**, *321*, 205; b) R. J. Abraham, E. J. Chambers, W. A. Thomas, *J. Chem. Soc. Perkin Trans. 2* **1994**, 949; c) M. Graupe, M. Takenaga, T. Koini, R. Colorado, Jr., T. R. Lee, *J. Am. Chem. Soc.* **1999**, *121*, 3222. Considering the orientation of the OH bond, the preferred orientations in Criegee intermediates **8_{eq}** and **8_{ax}** should be **I** for **8_{eq}** and **II** for **8_{ax}**. For **8_{ax}**, the preferred conformer (**II**) has both
- bonds properly aligned for migration according to the secondary stereoelectronic effect. However, for **8_{eq}**, only the fluorinated carbon is aligned for migration in the preferred conformation **I**. Thus, the secondary stereoelectronic effect could be considered to be the sole determinant of regiochemistry in this case. Experiments are currently underway to determine the relative importance of the secondary electronic effect in this system.
- [18] Although 2-fluorocyclohexanone would seem to be a more appropriate model, it has conformational biases, existing as a mixture of equatorial and axial isomers interconverting by a ring flip, the proportion of which is dependent on the polarity of the solvent. The equatorial isomer, which has a higher dipole moment, is preferred in more polar solvents, and the axial in less polar solvents. As this equilibrium will unnecessarily complicate our results, acyclic ketone **9** was chosen as the model compound. a) Y. Pan, J. B. Stothers, *Can. J. Chem.* **1967**, *45*, 2943; b) J. Allinger, N. L. Allinger, *Tetrahedron* **1958**, *2*, 64; c) N. L. Allinger, J. Allinger, *J. Am. Chem. Soc.* **1958**, *80*, 5476; d) R. J. Abraham, L. Griffiths, *Tetrahedron* **1981**, *37*, 575.
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- [21] In the isomeric **8_{ax}** series, the situation is different since equatorial attack puts the C–F and C–OO substituents at a dihedral angle of 60° , while axial attack puts these two at an angle of 180° . However, the conformer which activates the CH_2 substituent is not affected by any serious dipole/dipole interactions. See Supporting Information for a graphical representation.^[23]
- [22] Compounds **13** and **14** were separated by column chromatography and their structures determined by analysis of ^1H , ^{13}C , ^{19}F , HMQC, HMBC, COSY, and TOCSY experiments. NOE difference experiments carried out on both **13** and **14** demonstrated that the major compound had a *cis* relationship between the fluorine of the oxidized substituent and the *tert*-butyl group, indicating that the migration had occurred, as expected, from the carbon with the equatorially disposed fluorine atom. Compound **13** was more sensitive to hydrolysis than its isomer **14** and we were thus unable to obtain spectra of **13** unencumbered by the side product of this reaction. As compound **13** was still present in substantial amounts, and as the resonances of interest are clearly separated, we were able to assign the structure from these spectra.
- [23] Experimental details, stereoelectronic analysis of Criegee intermediate resulting from axial attack of mCPBA on ketone **5_{ax}**, and spectroscopic characterization of compounds **6_{eq}**, **6_{ax}**, **7_{eq}**, **7_{ax}**, **10**, **11**, **13**, and **14** are available as supporting information.



Dendritic Growth of a Supramolecular Complex**

Emilio Alvarez-Parrilla, Pedro Ramos Cabrer, Wajih Al-Soufi, Francisco Meijide, Eugenio Rodríguez Núñez, and José Vázquez Tato*

Dendrimers are branched polymers with star- or tree-type structures in which all the segments between branching points are of the same length. They can be used as spacers, building blocks for other structures, and as carriers of drugs, fragrances, cosmetics, herbicides, and other molecules.^[1] Although dendrimers are generally covalent polymers, dendrimerlike structures can also be formed by means of supramolecular polymer chemistry, by self-assembly of complementary monomeric components, by molecular interactions, and by recognition processes.^[2]

Cyclodextrins (CD) and their derivatives form supramolecular structures with a variety of organic molecules in water by means of molecular recognition processes (formation of inclusion complexes).^[3] Recently, we reported that β -cyclodextrin (β -CD) and two mono-amino β -CD derivatives form inclusion complexes in water with the naturally occurring bile salts sodium cholate (NaC) and sodium deoxycholate (NaDC) with 1/1 and 2/1 (CD/bile salt) stoichiometries, respectively.^[4, 5] An interesting supramolecular structure was obtained by the complexation of the head-to-head cyclodextrin dimer *N,N'*-bis(6-deoxy- β -cyclodextrin)pyromellitic acid diamide with NaDC.^[5] This structure resulted from the self-assembly of complementary monomeric units, as proposed by Lehn.^[2]

In view of these results, it seemed feasible to form a new dendrimerlike supramolecular polymer, reminiscent of a Cayley tree (Figure 1), by complexation of ditopic guests with β -CD trimers. To check this possibility the new cyclodextrin trimer **T** (Figure 2a) was synthesized, and its complexation behavior with the ditopic guest NaDC (Figure 2b) was studied.

First, the complexation behavior of **T** with the small molecule 2-naphthalenesulfonate (2NS) was studied by ¹³C NMR spectroscopy. This guest was chosen as a reference model because it forms a 1/1 complex with β -CD ($K \approx 8 \times 10^3 \text{ M}^{-1}$, a value close to that found for the NaC/ β -CD complex)^[5] The continuous variation plot^[6] for the **T**/2NS system (Figure 3) shows the formation of a 1/3 complex.

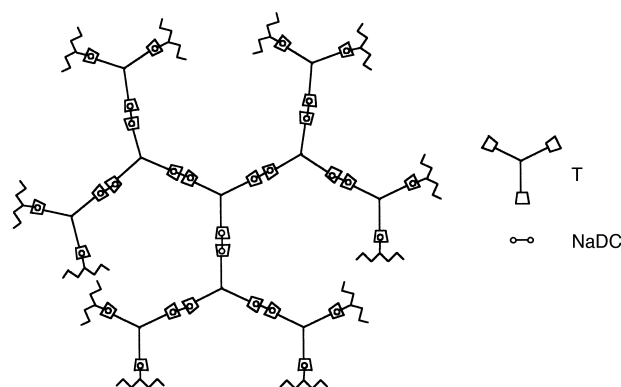


Figure 1. Schematic representation of the Cayley tree dendrimerlike supramolecular structure expected for the cyclodextrin complex **T**/NaDC.

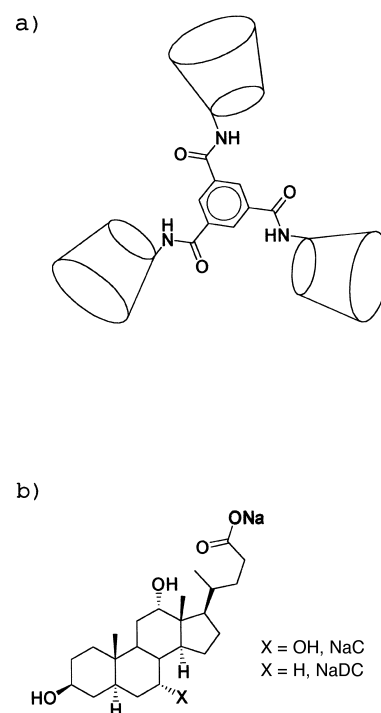


Figure 2. Structures of **T** (a) and the bile salts NaC and NaDC (b).

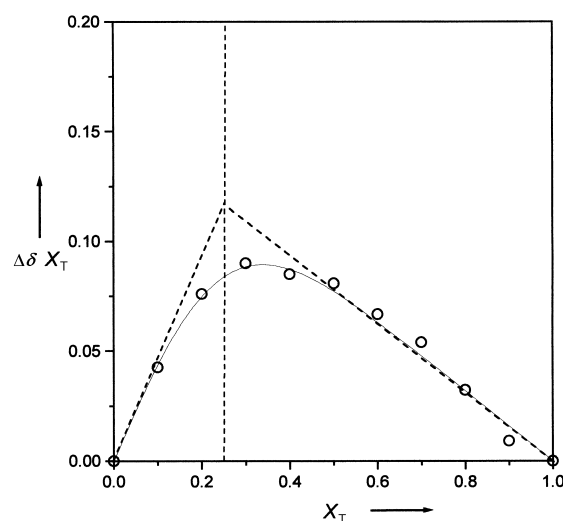


Figure 3. Job plot corresponding to the chemical shift displacement of C-1 of β -CD for the 1/3 **T**/2NS complex. Total concentration $[\mathbf{T}] + [2\text{NS}] = 5 \text{ mM}$.

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A time-dependent stoichiometry was observed by NMR studies with the continuous-variation plot technique^[6] for the complexation of NaC with **T**. Initially (1 h after preparation of the samples) the **T**/NaC system showed a mixture of 1/1 and 1/2 complexes. After one week a mixture of 1/2 and 1/3 complexes was observed, and only after 90 d was the final 1/3 stoichiometry reached. Although no further kinetic studies have been carried out so far, by comparison with former results with 2NS, it seems that steric hindrance plays an important role in the complexation of larger guests.

The complexation of NaDC by **T** was studied by mixing solutions in a 2/1 molar ratio, which corresponds to the stoichiometry of the building brick (two NaDC per **T**) that makes up the Cayley tree in Figure 1. After a few days, a white precipitate appeared on a stage placed at the bottom of the beaker. The mixture was allowed to stand for different periods of time. Figure 4 shows a scanning electron micrograph of the

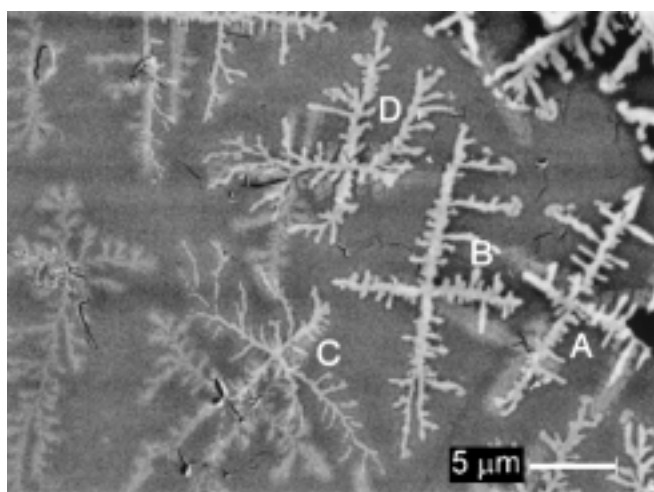


Figure 4. Scanning electron micrograph of the **T**/NaDC system after an aggregation period of 60 d.

surface obtained after two months. Visually, it is possible to distinguish two different types of structures: Structures A and B with a fractal dimension D_f of 1.58, and structure D with a D_f of 1.45. Structure C is a mixture of both. These different structures probably arise from different stoichiometries for each trimer unit. After three months a thin layer covered the stage. Figure 5a shows a general view of this surface obtained by confocal laser scanning microscopy. Structures with an average fractal dimension of 1.63 ± 0.02 can be observed at higher magnification (Figure 5b). This difference between the two groups of structures seems to depend on the aggregation time, which may be a consequence of the steric hindrance, which slows down the supramolecular growth kinetics. Depending on the number of cyclodextrins per trimer unit involved in the complexation process, the obtained fractal structure may present different shapes.

This is the first self-assembled supramolecular structure obtained from cyclodextrin derivatives that has a dendrimer-like structure. The synthesis of ditopic guests with less steric hindrance is in progress.

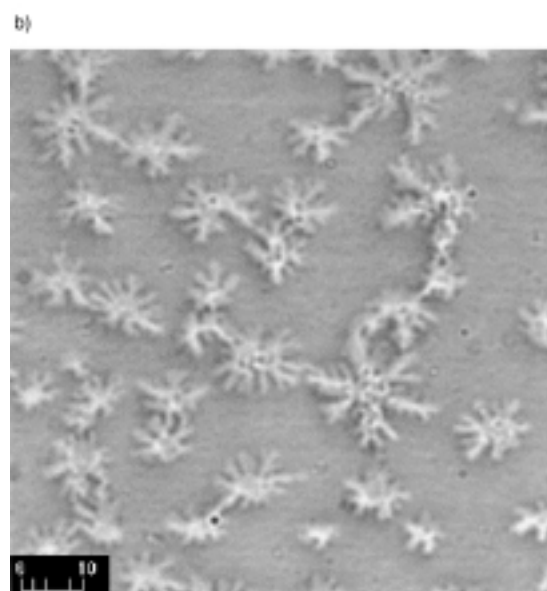
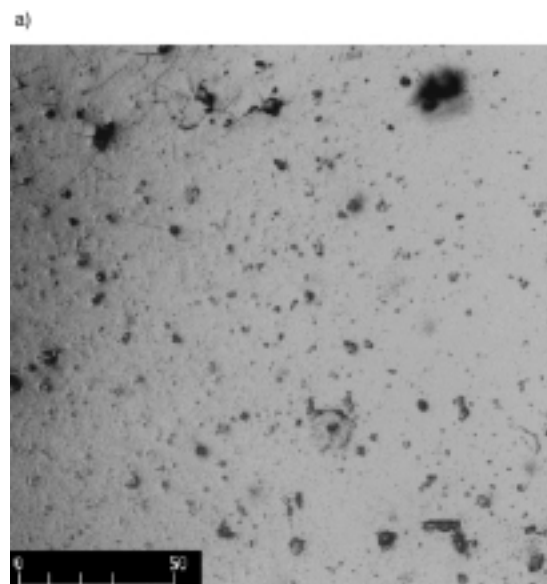


Figure 5. Confocal micrographs of the **T**/NaDC system after an aggregation period of 90 d. a) General view. b) Detail of some isolated dendrimers. The scale bars are in μm .

Experimental Section

Bile salts (Sigma-Aldrich) and β -cyclodextrin were purified as previously reported.^[4] Melting points and L-SIMS mass spectra were determined on a Gallenkamp apparatus and a Micromass Autospec spectrometer, respectively. The ^1H , ^{13}C , and DEPT 135 NMR spectra were recorded on a Bruker AC spectrometer at 300 and 75 MHz at 298.1 ± 0.1 K. All NMR experiments were carried out in D_2O . The stoichiometry of the inclusion complexes formed were determined using the continuous variation technique (Job plot).^[6] Laser scanning confocal micrographs were recorded on a Bio-Rad MRC 1024ES microscope in reflectance mode by using the 488-nm light from a 100-mW argon ion laser. Scanning electron micrographs were recorded on a LEO 435 VT variable-pressure electron microscope. The fractal dimension was determined from the digitized micrographs by using the box-counting method^[7] with a self-written program. This method counts the minimal number of boxes (pixels) that cover the fractal structure for decreasing box size.

T: 6-deoxy-6-amino- β -cyclodextrin^[5] (0.5 g, 0.44 mmol) and triethylamine (60 μ L, 0.44 mmol) were dissolved in dry DMF (10 mL) and cooled to 0 °C. 1,3,5-benzenetricarbonyl trichloride (0.0355 g, 0.134 mmol) was added, and the reaction mixture was kept at 0 °C for 1 h and then at RT for 12 h. The reaction product was precipitated with cold acetone (100 mL). The resulting solid was filtered and washed with cold acetone to remove the DMF. Finally the product was dissolved in water and purified through an Amicon 8050 ultrafiltration cell unit with a YM3 membrane ($M = 3000$) to give a white solid (0.279 g, 0.079 mmol, 59 %). $R_f = 0.12$ (ethyl acetate/isopropyl alcohol/water/concentrated NH_4OH 2/3/4/0.3); m.p. 218–225 °C (decomp); ^1H NMR (D_2O): $\delta = 8.23$ (s, 1H, Ar-H), 4.97–5.05 (brs, 7H, H-1), 3.48–3.96 (m, 42H, H-2, H-3, H-4, H-5, H-6); ^{13}C and DEPT 135: $\delta = 171.5$ (CONH), 137.38 (substituted C_{Ar}), 131.77, (CH_{Ar}), 104.49 (C-1), 85.90 (C-4'), 83.72 (C-4), 75.69 (C-5), 74.68 (C-3), 74.22 (C-2), 62.92 (C-6), 43.57 (C-6'); positive-ion FAB-MS: m/z : 3559.0485 [$M+1$].

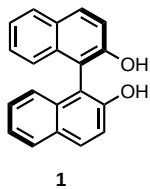
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Lithium Binaphtholates: Chiral Chains and Clusters**

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Alexander Steiner

Binaphthol (H_2binol) is chiral by virtue of restricted rotation about the C–C bond linking the two naphthyl units, and the absolute configuration of (*R*)-binaphthol (**1**) was first established unequivocally by X-ray diffraction in 1968.^[1] The flexibility of the dihedral angle between the two naphthyl rings of this C_2 -symmetric molecule allows coordination to a wide range of metal centers (examples are known from 45° to 110°), and H_2binol



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has been used as a chiral auxiliary in many metal-based enantioselective catalysts and reagents. Examples include the $\text{Yb}(\text{OTf})_3$ -catalyzed asymmetric Diels–Alder reaction,^[2] the TiCl_4 -derived catalyst for the asymmetric ene reaction,^[3] and the chiral modification of LiAlH_4 for use in asymmetric reductions.^[4] Multi-binaphthol species have attracted recent interest; particularly successful examples are Mikami's Ti–binaphtholate catalysts^[5] and the heterometallic alkali metal/lanthanide binaphtholates synthesised by Shibasaki et al.^[6] Examples of catalytically active multi-binaphthol Ti clusters have also been reported.^[7,8] In addition to these applications in asymmetric synthesis, binaphthol has also been of interest as a component of macrocycles for chiral recognition,^[9,10] and as a component of helical polymers with novel properties such as the emission of polarized light.^[11]

In view of this widespread interest in binaphthol-containing species, it is surprising that no structural studies of the lithiated derivatives have been reported (inclusion compounds of *rac*- H_2binol with NaOH and KOH have been structurally characterized^[12]). Lithiated H_2binol has been used in the preparation of many enantioselective catalysts and has also been investigated as a chiral auxiliary in its own right.^[13] Our interest in lithium binaphtholates arose from our use of LiHbinol in the synthesis of anhydrous analogues of Shibasaki's heterometallic catalysts,^[14] and herein we report on investigations of mono- and dilithiated derivatives of optically pure and racemic H_2binol . The previously overlooked structures of these compounds show unexpected diversity.

Lithiated H_2binol compounds [$\text{Li}(\text{rac-Hbinol})$] (**2**), [$\text{Li}((R)\text{-Hbinol})$] (**3**), [$\text{Li}_2(\text{rac-binol})$] (**4**), and [$\text{Li}_2((R)\text{-binol})$] (**5**) were prepared by reaction of H_2binol with stoichiometric quantities of $n\text{BuLi}$ in THF at 0 °C. All of these lithiated derivatives were precipitated as microcrystalline solids from THF solutions by the addition of petroleum ether, and we were able to grow X-ray-quality single crystals of **2**, **3**, and **4** by the slow diffusion of petroleum ether into the THF solutions at room temperature. Despite numerous attempts and using a range of precipitating solvents, good quality single crystals of **5** could not be obtained from THF solutions.

The monolithiated species **2** and **3** both crystallize as helical polymeric chains with Hbinol^- units linked by [$\text{Li}(\text{thf})_2$]⁺ bridges, as shown for **2** in Figures 1 and 2. The chain structure of **3** is very similar, but less symmetrical, containing two inequivalent Li atoms. Short fragments of the chains of **2** and **3** are shown in Figures 3 and 4, respectively. The structure of **3** is necessarily composed of chains of a single enantiomer whereas that of **2** consists of arrays of *R*-configured polymer and *S*-configured polymer. The remaining O–H group has not been

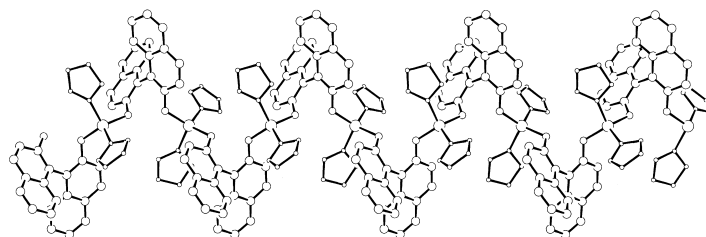


Figure 1. The [$\text{Li}((S)\text{-Hbinol})$] chain of [$\text{Li}(\text{rac-Hbinol})$] (**2**).

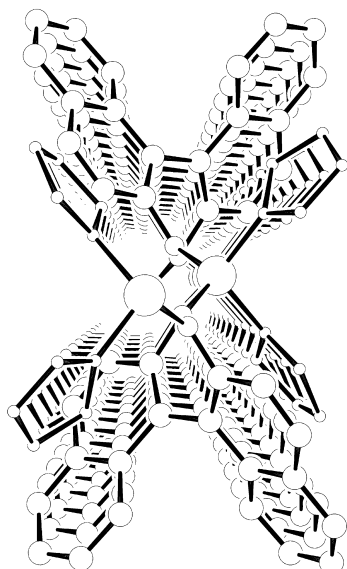


Figure 2. View along the $[\text{Li}\{(S)\text{-Hbinol}}]\text{ chain of } [\text{Li}(\text{rac-Hbinol})]$ (**2**) emphasizing the zigzag arrangement of Li atoms.

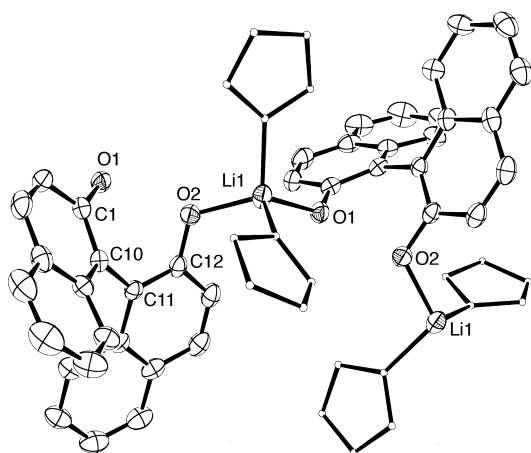


Figure 3. ORTEP plot of $[\text{Li}(\text{rac-Hbinol})]$ (**2**). Selected distances [$\text{\AA}$] and angles [$^\circ$]: Li1–O1 1.914(6), Li1–O2 1.942(6), O1...O2 (intramolecular) 2.4233(2), Li1...Li1 5.6755(5); O1–Li1–O2 124.2(3), C1–C10–C11–C12 50.1(4).

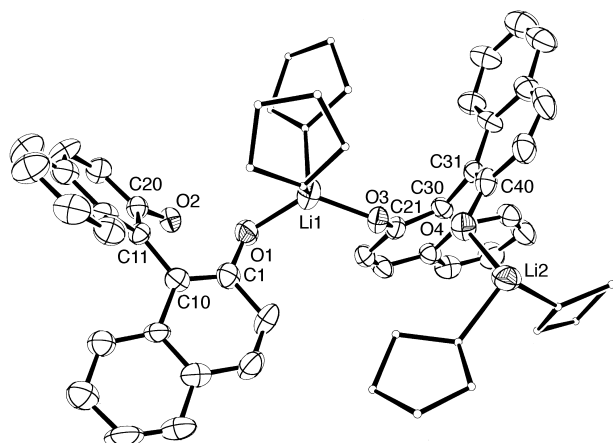


Figure 4. ORTEP plot of $[\text{Li}\{(R)\text{-Hbinol}}]\text{ (3)}$. Selected distances [$\text{\AA}$] and angles [$^\circ$]: Li1–O1 1.920(15), Li1–O3 1.881(13), Li2–O4 1.929(14), Li2–O2 1.921(15), Li1...Li2 5.6217(14), O1...O3 3.3708(6); O1–Li1–O3 124.9(9), O2–Li2–O4 123.7(8), C1–C10–C11–C20 48.2(10), C21–C30–C31–C40 50.2(9).

located in the X-ray structure but it is reasonable to suppose that it occupies a bridging position between the O atoms of a single binol unit. This is clearly the case in THF where the ^1H NMR spectra of both **2** and **3** show a broad resonance arising from the phenolic OH which is shifted to very low field (11.1 ppm), a shift that is consistent with strong hydrogen bonding. The ^1H NMR spectra of **2** and **3** were indistinguishable; although the spectra were well-resolved, little useful structural information could be extracted. Between room temperature and $+50^\circ\text{C}$, there was essentially no change in the well-resolved spectra apart from an upfield shift of the OH resonance to 10 ppm; however, on lowering the temperature to -50°C , considerable broadening was observed which would be consistent with the formation of an oligomeric structure similar to that observed in the solid state. The ^{13}C NMR spectra were also indistinguishable, and the ^7Li spectra showed a single resonance at $\delta = +2.70$ at temperatures between $+40$ and -50°C .

It is useful to compare the crystal structures of **2** and **3** with those of $(R)\text{-H}_2\text{binol}$ and $\text{rac-H}_2\text{binol}$, both of which crystallize as infinite H-bonded chains.^[15, 16] $(R)\text{-H}_2\text{binol}$ is made up of left-handed helical chains arranged along a 3_2 screw axis whereas the racemic compound crystallizes as arrays of 2_1 -helical all- R chains and all- S chains. The H_2binol units are linked by hydrogen bonds which bridge intermolecular O–O distances of 2.961(4) $\text{\AA}$ for $(R)\text{-H}_2\text{binol}$ and 2.852(3) $\text{\AA}$ for $\text{rac-H}_2\text{binol}$. The dihedral angles between the naphthyl groups of $(R)\text{-}$ and $\text{rac-H}_2\text{binol}$ are $101.65(5)^\circ$ and $90.58(4)^\circ$, respectively, these are very much larger than those of **2** and **3**.

The dilithiated species $[\text{Li}_2(\text{rac-binol})]$ (**4**) exists in the solid state as the C_2 -symmetric chiral cluster $[\text{Li}_6\{(\text{R})\text{-binol}\}_2\{(\text{S})\text{-binol}\}(\text{thf})_8]$ shown in Figures 5 and 6 and its SSR enantiomer (the unit cell contains two RRS clusters and two SSR clusters). Rings, stacked rings, and ladders are well known in lithium chemistry,^[17] but the chiral central Li_4O_4 fragment of **4** is a novel structural motif which is best likened to a section of a spiral staircase. Li1, Li2, and Li4 have distorted tetrahedral geometry whereas Li3 is planar, as are the three-coordinate

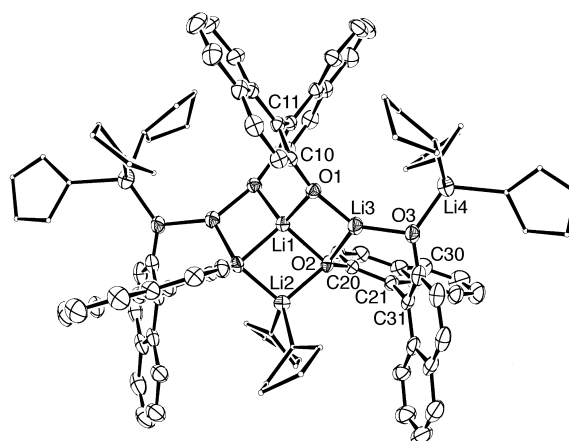


Figure 5. ORTEP plot of $(RRS)\text{-4}$. There is a C_2 axis along Li1–Li2. Selected distances [$\text{\AA}$] and angles [$^\circ$]: Li1–O1 1.902(9), Li1–O2 1.967(9), Li2–O2 1.945(10), Li3–O1 1.779(11), Li3–O2 1.923(10), Li3–O3 1.841(10), Li4–O3 1.842(12); O1–Li1–O1' 105.2(7), O1–Li1–O2 94.83(16), O2–Li1–O2' 97.7(6), O2–Li2–O2' 99.3(7), O2–Li3–O1 100.6(4), O1–Li3–O3 143.4(6), O2–Li3–O3 115.6(6), C20–C21–C31–C30 $-72.0(8)$, C10–C11–C11'–C10' $64.1(7)$.

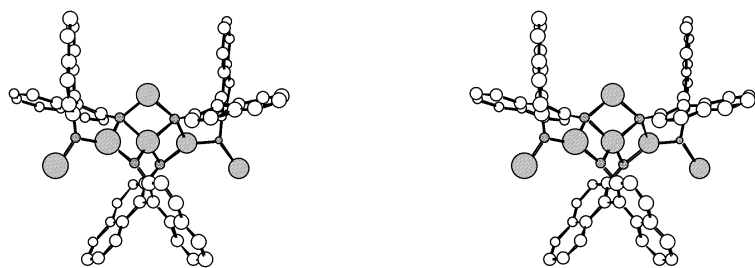


Figure 6. Stereoview of (RRS)-4. The thf ligands are omitted for clarity.

O1 and O3 atoms. The remarkable self-assembly of the chiral cluster 4 cannot be explained by steric arguments. However, dissecting 4, (Figure 7), into a) an *RR* fragment (a short section of an $[\text{Li}_2\{(R)\text{-binol}\}]$ chain), and b) an *S* fragment,

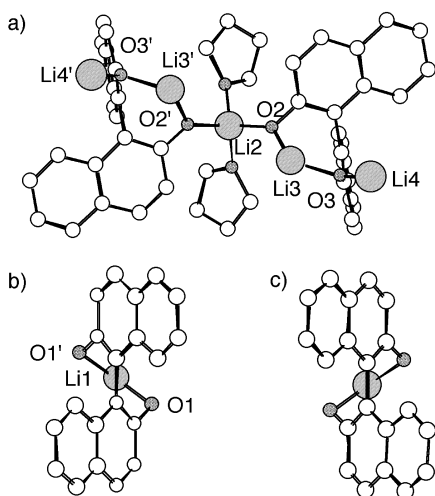


Figure 7. (RRS)-4 separated into its component fragments: a) (*RR*)- $[\text{Li}_5(\text{binol}_2)]^+$ fragment, b) complementary $[\text{Li}\{(S)\text{-binol}\}]^-$ fragment, c) $[\text{Li}\{(R)\text{-binol}\}]^-$ fragment which cannot interact with fragment a).

illustrates clearly how the two $[\text{Li}_2\{(R)\text{-binol}\}]$ units of fragment a) cooperate to form a chiral template for recognition of fragment b). This fragment is perfectly aligned for interactions between O1' and Li3' and between O2' and Li1, whereas these interactions are geometrically impossible for the enantiomeric fragment c). The low temperature solution structure of 4 is analogous to that found in the solid state: at 223 K the ^7Li NMR spectrum shows four resonances in the relative ratio 1:2:2:1, and 13 resonances are resolved in the aromatic region of the ^{13}C NMR spectrum. At 298 K the ^7Li spectrum shows only a single resonance although there is no apparent change in the ^{13}C NMR spectrum.

The previously overlooked structures of lithium binaphtholates have shown unexpected diversity. The well-known helical motif has manifested itself again in the monolithiated binaphthol derivatives, in this case with $[\text{Li}(\text{thf})_2]^+$ acting as the intermolecular link, rather than hydrogen-bonding or covalent linkages. The novel chiral cluster, which self-assembles from $[\text{Li}_2(\text{rac-binol})]$, has provided a new structural motif in lithium alkoxide chemistry, and has also demonstrated that aggregates must always be considered when Li binaphtholates are used as reagents in solution.

Experimental Section

All the preparations described below were performed under strictly anaerobic conditions using standard Schlenk techniques. Solvents were distilled from sodium/benzophenone ketyl (non-deuterated) or CaH_2 (deuterated) and stored under N_2 over 4 Å molecular sieves prior to use. Samples for NMR spectroscopy were sealed under vacuum. ^1H and ^{13}C NMR spectra were recorded on a Gemini300 spectrometer. ^7Li NMR spectra were recorded on a Bruker WM250 spectrometer. Elemental analyses were performed in duplicate by Mr S. Apter of this Department.

Preparation of LiHbinol: A solution of *n*BuLi in hexanes (2.69 M, 1.22 cm³, 3.297 mmol) was added to a solution of H₂binol (*rac* or *R*, 0.944 g, 3.297 mmol) in THF (10 cm³) at 0 °C. The very pale yellow solution was allowed to warm to room temperature. Removal of the solvent in vacuo resulted in a white solid which was recrystallized from THF/petroleum ether. Li₂binol was prepared in an analogous manner using two equivalents of *n*BuLi.

Elemental analysis $[\text{Li}\{(R)\text{-binol}\}(\text{thf})_2] \cdot 1/4\text{THF}$ calcd (%) for $\text{C}_{29}\text{H}_{30}\text{LiO}_{4.25}$: C 76.64, H 6.87; found: C 73.04, H 6.53; $[\text{Li}(\text{rac-Hbinol})(\text{thf})_2]$ calcd (%) for $\text{C}_{28}\text{H}_{29}\text{LiO}_4$: C 77.05, H 6.70; found: C 75.60, H 6.72; $[\text{Li}_2\{(R)\text{-binol}\}(\text{thf})_n]$ calcd (%) for $\text{C}_{24}\text{H}_{20}\text{Li}_2\text{O}_3$: C 77.85, H 5.44; found: C 76.52, H 5.72; $[\text{Li}_6(\text{rac-binol})_3(\text{thf})_8] \cdot 2\text{THF}$ calcd (%) for $\text{C}_{100}\text{H}_{116}\text{Li}_6\text{O}_{16}$: C 74.34, H 7.24; found: C 72.57, H 7.07.

^{13}C NMR (75 MHz, $[\text{D}_8]\text{THF}$, 25 °C, TMS), $[\text{Li}\{(R)\text{-binol}\}]$: δ = 118.9, 121.3, 123.7, 125.2, 127.1, 128.3, 128.4, 128.8, 136.1, 160.4; $[\text{Li}(\text{rac-Hbinol})]$: δ = 121.0, 123.9, 125.0, 127.1, 128.3, 128.6, 136.2; $[\text{Li}_2\{(R)\text{-binol}\}]$: δ = 108.3, 113.4, 118.9, 119.4, 123.7, 124.9, 126.1, 127.4, 135.8, 162–164(br); $[\text{Li}_2(\text{rac-binol})]$: δ = 108.8, 118.6, 119.6, 123.8, 124.2, 126.3, 126.7, 127.4, 127.6, 127.8, 128.1, 137.1, 165.0. ^7Li NMR (217 MHz, $[\text{D}_8]\text{THF}$), $[\text{Li}_2\{(R)\text{-binol}\}]$ (298 K): δ = −2.4; (223 K): δ = −2.35, −3.01; $[\text{Li}_2(\text{rac-binol})]$ (298 K): δ = −2.4; (223 K): δ = −0.92 (1 Li), −2.34 (2 Li), −2.62 (2 Li), −3.36 (1 Li).

X-ray crystallography intensity data were collected using a STOE-IPDS image plate diffractometer (MoK_α , graphite monochromator) in the φ rotation scan mode. Data were collected at 213(2) K. The structures were solved by direct methods with the SHELXS97 package and refined using full-matrix least-squares on F^2 (SHELXL97).^[18]

Crystal data for 2: $\text{C}_{28}\text{H}_{28}\text{LiO}_4$, M_r = 435.50, monoclinic, space group $P2_1/c$, a = 11.9330(17), b = 10.8035(10), c = 18.755(3) Å, β = 100.093(17), V = 2380.5(5) Å³, Z = 4, ρ_{calcd} = 1.215 g cm^{−3}, μ (MoK_α) = 0.044 mm^{−1}, F_{000} = 924. A total of 3110 reflections were measured and used in the refinement. Refinement converged to R = 0.0545 for the 1719 reflections with $F > 2\sigma(F)$.

Crystal data for 3: $\text{C}_{29}\text{H}_{30}\text{LiO}_{4.25}$, M_r = 453.53, monoclinic, space group $C2/c$, a = 40.252(8), b = 12.084(2), c = 10.737(2) Å, β = 105.47(3), V = 5033.3(16) Å³, Z = 4, ρ_{calcd} = 1.197 g cm^{−3}, μ (MoK_α) = 0.044 mm^{−1}, F_{000} = 1928. A total of 6332 reflections were measured and used in the refinement. Refinement converged to R = 0.0958 for the 4698 reflections with $F > 2\sigma(F)$.

Crystal data for 4: $\text{C}_{100}\text{H}_{116}\text{Li}_6\text{O}_{16}$, M_r = 1615.80, monoclinic, space group $C2/c$, a = 22.083(4), b = 25.685(5), c = 17.446(3) Å, β = 113.76(3), V = 9057(3) Å³, Z = 4, ρ_{calcd} = 1.185 g cm^{−3}, μ (MoK_α) = 0.043 mm^{−1}, F_{000} = 3448. A total of 5471 reflections were measured and used in the refinement. Refinement converged to R = 0.0758 for the 2621 reflections with $F > 2\sigma(F)$.

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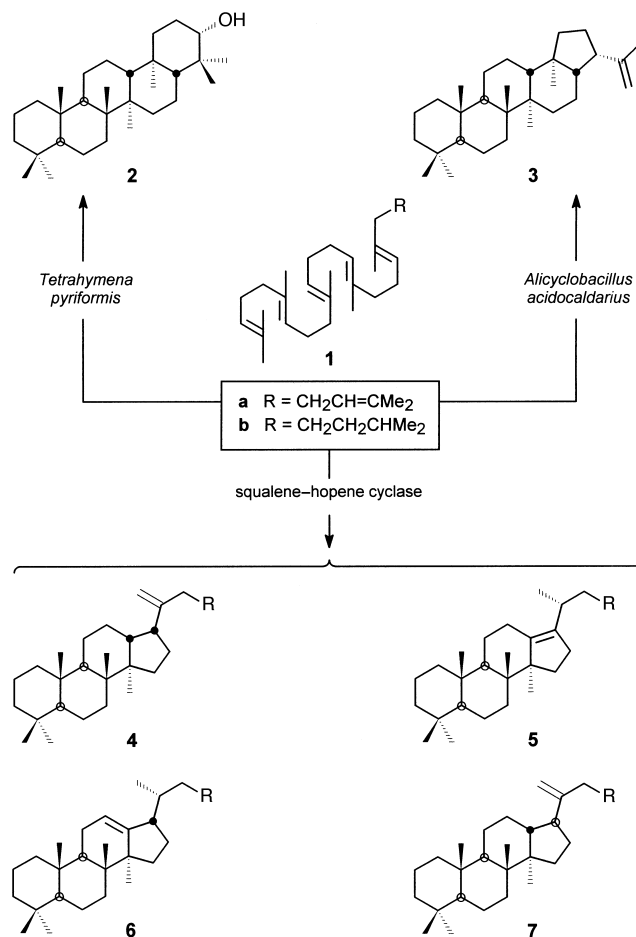
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A Model for the Nonenzymatic BCD Cyclization of Squalene**

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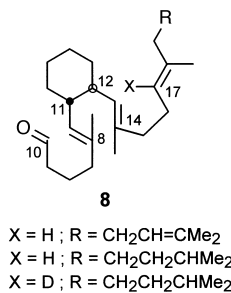
The enzymatic polycyclization of squalene (**1a**) and 2,3-oxidosqualene (epoxysqualene) is a fascinating biogenetic process.^[1] Whereas oxidosqualene cyclases produce sterols and plant triterpenes, the squalene cyclases (SC) of the protozoan *Tetrahymena pyriformis* and of the thermoacidophilic bacterium *Alicyclobacillus acidocaldarius* convert squalene (**1a**) into tetrahymanol (**2**) and hop-22(29)-ene (**3**,

diploptene), respectively.^[2, 3] Interestingly, a few years ago it was reported that squalene–hopene cyclase converts non-natural 2,3-dihydrosqualene (**1b**) in high yield into a mixture of the tetracyclic triterpenes (20*R*)-dammar-13(17)-ene (**5b**) and (20*R*)-dammar-12-ene (**6b**) (Scheme 1).^[4] More surprising even is the recent report about the isolation, next to the



Scheme 1. Triterpenoids originating from cyclization of squalene (**1a**) and 2,3-dihydrosqualene (**1b**) catalyzed by bacterial squalene cyclases.

classical hopanoids, of the tetracyclic derivatives **4a**, **5a**, **6a**, and **7a**, each representing less than 2 % relative to **3**, when the natural substrate squalene (**1a**) is treated with purified squalene–hopene cyclase.^[5] This result raises the intriguing possibility that this enzyme has been “prepared” for performing the seemingly more complex transformation that bears analogy with the formation of lanosterol.^[6] In this evolutionary context, however, one fundamental question remains unanswered: what is the nonenzymatic course of the poly-(tetra, penta)cyclization of squalene (and epoxysqualene)? Here we report on the results of the Lewis acid catalyzed cyclization of aldehydes **8a–c** that can provide an answer to the above question.



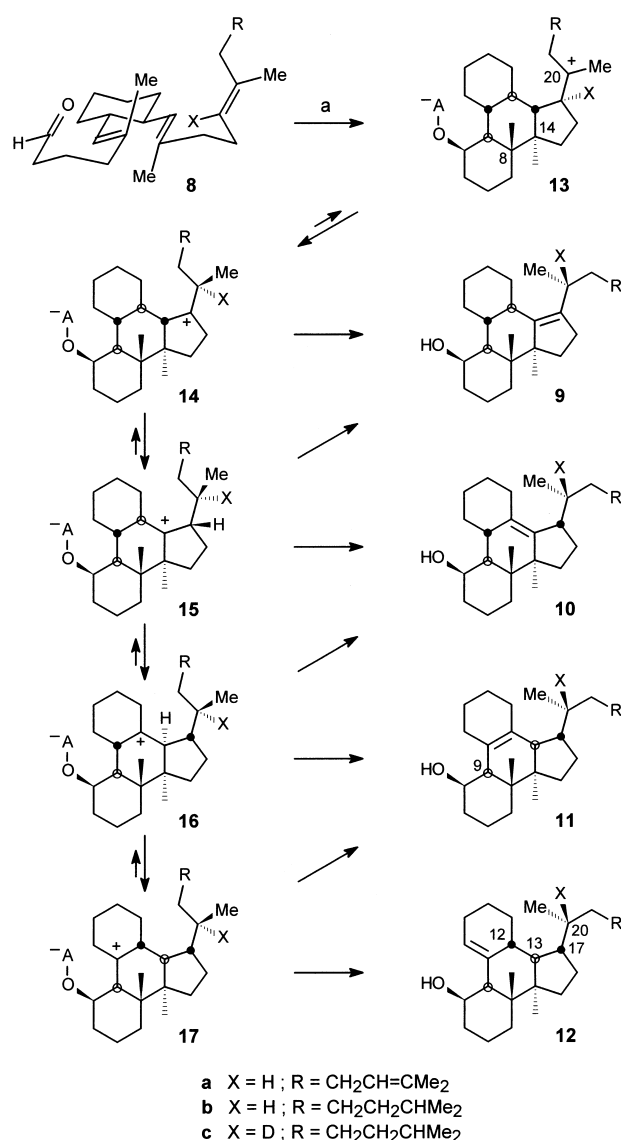
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The enzymatic conversion of both squalene and epoxy-squalene produces a variety of cyclization products depending on the cyclase used. This diversity arises through different folding patterns that are adopted by the polyene in either the early (AB-ring formation) or the late (D- and E-ring formation) stages of the process.^[7] On the other hand, all tetra- and pentacyclizations have in common an anti-Markovnikov like closure of the six-membered C-ring. From the mere chemical point of view this third ring closure constitutes a formidable challenge. Not surprisingly, the (Lewis) acid-catalyzed cyclizations of squalene and epoxysqualene afford tricyclic products whose structures, and in particular the resulting five-membered C-ring, are determined by Markovnikov's rule.^[8, 9] Considering the C-ring region as the crucial one in the polycyclization, substrates **8**^[10] were designed to 1) initiate the cyclization at pro-C10, hence skipping the early stage of the cyclization, and to 2) enforce the anti-Markovnikov six-membered C-ring formation through the incorporation of an extraneous cyclohexane ring at pro-C(11),C(12).^[11] The role of the latter consists not only in inducing the prechair geometry of the chain in the 11,12-region through the *trans*-diequatorial substitution and in enforcing the proximity of C8 and C14, which should ease the entropic burden of the polycyclization, but also and foremost in preventing the Markovnikov five-membered C-ring formation; indeed, concerted cyclization should lead to a C-ring that is connected to two six-membered rings by *trans*-fusions at 11,12 and at 8,9—a situation that is better accommodated by a six- than by a smaller five-membered ring.^[12] Thus, surmounting the activation energy that is involved with the C-ring formation should enable us to take a view on the nonenzymatic course of the polycyclization beyond the C-ring.

Treatment of aldehyde **8a** under standard conditions (see Scheme 2; saturated NaHCO₃ workup) led to an apolar fraction (19%), to a second fraction (48%) consisting of a complex mixture of pentacyclic derivatives (as judged from the absence of the characteristic resonances of the olefinic protons of the polyene chain), and to the two tetracyclic alcohols **9a** (13%) and **12a** (4%).^[13] The structure of the latter cyclization product was solved by X-ray diffraction,^[14] which revealed, next to a genuine BCD-skeleton containing the two quaternary centers at C8 and C14, a most interesting stereochemical pattern at C12, C13, C17, and C20. Indeed, the latter pattern indicates that **12a** is formed in two steps (Scheme 2): a) the cyclization of **8a** involving a polyene chain folding with least steric interactions, which affords the dammarane-type cation **13**, followed by b) a series of four consecutive antiparallel 1,2-hydride migrations which led to carbocation **17**. The sequence is then terminated by proton elimination. At this point it is evident that any of the intermediate cations encountered en route to **17**, such as **14**–**16**, could be subject to proton elimination leading to the cyclization derivatives **9**–**11**, whose isolation would further confirm the above pathway. Indeed, the spectroscopic data of the second isolated product were in full accord with structure **9a**, in particular the ¹H NMR (i.e., low-field resonance δ = 3.06 for H20) and ¹³C NMR/DEPT data.^[13] The full configurational assignment was established by X-ray diffraction on the related compound **9c** (vide infra).



Scheme 2. Stereochemical course of the Lewis acid catalyzed cyclization of **8a**–**c**. a) 0.03 M in dichloromethane, 0.5 equiv tin(IV) chloride (A), room temperature, 15 min.

In order to avoid the formation of the major pentacyclic fraction, the cyclization of aldehydes **8b** and **8c**, in which the double bond at pro-C(24),C(25) is missing, was studied. Furthermore, substrate **8c** was provided with a deuterium label at pro-C17 in order to ascertain that the cyclization products possessing a stereocenter at C20 originate from a direct 1,2-shift (and not from deprotonation–reprotonation sequences). Treatment of aldehyde **8b** under the standard conditions afforded, next to a major apolar fraction (78%), two tetracyclic alcohols, the expected **9b** (6%) and a novel cyclization product (7%) to which structure **10b** was tentatively assigned. We assume that the apolar fraction originates from the acid-catalyzed E1 elimination of the sterically congested axial hydroxy group. By careful exclusion of traces of water the formation of apolar derivatives could be reduced; under these improved conditions the cyclization of **8c** led to an apolar fraction (39%), and to the expected **9c** (20%) and **10c** (17%).

That **9a**, **9b**, and **9c** belong to the same series was proven by partial hydrogenation of **9a** (Wilkinson's catalyst, toluene)^[13] to **9b** (58% yield next to starting material), and by comparison of the relevant ¹H NMR spectral data of **9b** and **9c** which were identical except for the low-field resonance at $\delta = 3.03$ of H20 (absent in **9c**) and the doublet at $\delta = 0.93$ of the protons at C21 (singlet in **9c**). The final unambiguous identification of the structures in the **9**-series was established by the X-ray diffraction analysis of crystalline **9c**.^[14]

Although full understanding of the process will necessitate further experiments to minimize the apolar fraction, the described cyclization of **8** is remarkable in that it constitutes a rare example of a nonenzymatic polycyclization that leads to anti-Markovnikov like cyclization products with a six-membered C-ring (**9**, **10** and **12**). The reactions are furthermore characterized by bond formation at vicinal quaternary centers.^[15, 16] The "natural" pathway, which is now believed to involve a classical Markovnikov cyclization to a five-membered C-ring followed by ring expansion,^[9] is considered in our case to be less likely,^[12] although this alternative cannot be excluded at this stage.^[17] The observed 20*R* configuration further confirms what one may have expected on the basis of chemical intuition: 1) the chain folding in the D-region is one that minimizes the steric interaction between C20 and the methyl group at C14, and 2) the C17 to C20 hydride shift which establishes the absolute configuration at C20 occurs faster than rotation at the C17–C20 bond. The analogy between structures **5b/6b** and **9b/10b**, respectively, is also striking. It may indicate that the cyclohexane ring at the 11,12 bond operates as a mimic of a rather tight binding of the central part of the natural substrate in the hydrophobic cleft of the enzyme.^[18]

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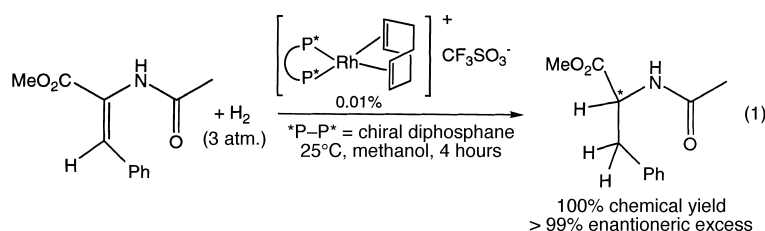
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- [14] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-140162 (**9c**) and -140169 (**12a**). Copies 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 Simple Model for the Origin of Enantioselection and the Anti "Lock-and-Key" Motif in Asymmetric Hydrogenation of Enamides as Catalyzed by Chiral Diphosphine Complexes of Rh(η)²

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The historical and commercial significance of catalytic, enantiospecific enamide hydrogenation^[1–4] has generated intense interest in the reaction mechanism [Eq. (1)].



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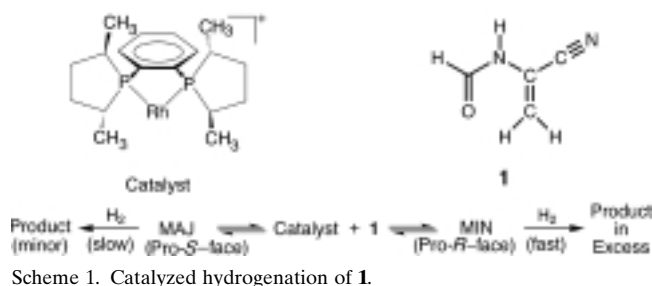
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Investigations led by Halpern and Brown elucidated a surprising anti “lock-and-key” mechanistic motif, in which the major product enantiomer arises from hydrogenation of the C=C enantioface that binds less favorably to the catalyst.^[5–8] Why these catalysts exhibit such anti “lock-and-key” behavior and how widely this mode of stereochemical control applies to other catalysts are unanswered questions. We present results of computer modeling studies, which suggest a simple model for enantioselectivity in enamide hydrogenation and, in the process, demonstrate the power of modern computational methods in catalysis research.

The kinetic studies of Landis and Halpern established the following mechanism for the catalytic hydrogenation of enamides: Reversible binding of the substrate to the catalyst followed by rate-determining activation of H₂ and the subsequent rapid elimination of the hydrogenated product.^[8] For a chiral catalyst and a prochiral enamide, the catalytic cycle consists of two diastereomeric manifolds, designated Major (MAJ) and Minor (MIN), in correspondence with the relative stabilities of the Rh-substrate adducts.^[8] The product, which can be produced in an enantiomeric excess (*ee*) as high as 99.9 %, is generated almost exclusively by the small fraction (< 10 %) of the catalyst in the minor diastereomeric manifold.

We have computed free-energy surfaces for the hydrogenation reaction of a model enamide, α -formamidoacrylonitrile (**1**), as catalyzed by the highly selective, chiral catalyst, [Rh{(R,R)-Me-DuPHOS}]⁺ (Me-DuPHOS = 2',5',2'',5''-tetramethyl 1,2-bis(phospholanyl)benzene; Scheme 1).^[9, 10] As in



a previous investigation of the achiral catalyst, [Rh(PH₃)₂]⁺,^[11] we chose the amidoacrylonitrile, rather than the more common amidoacrylate species,^[12] because hydrogenation of acetamidocinnamitrile has been shown to yield a high *ee*, comparable to methyl acetamidocinnamate, and because nitrile groups span a smaller conformational space.^[2] We present results of gas-phase three-layer hybrid ONIOM computations.^[13] The enantiospecificities of enamide hydrogenations are commonly insensitive to the nature of the solvent, which justifies a gas-phase analysis.^[14]

The computed properties of the diastereomeric catalyst–substrate adducts, pro-*S* MAJ and pro-*R* MIN,

agree with experiment (Figure 1).^[8, 15–17] The enamide substrate binds to the (R,R)-Me-DuPHOS-ligated catalyst in a bidentate fashion, with coordination of both the amide oxygen and the C=C double bond. Metal binding to the

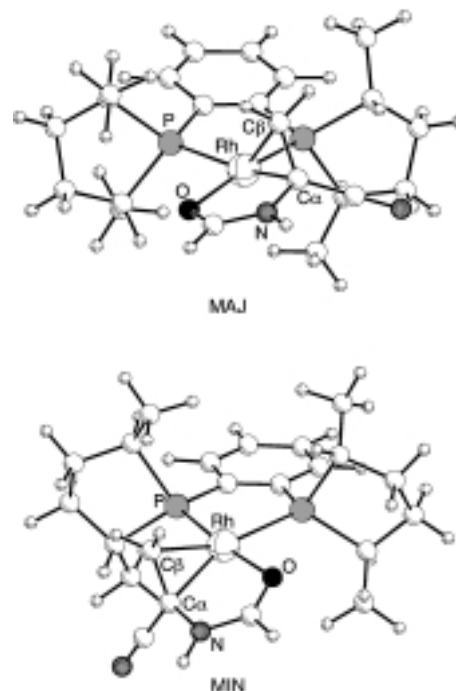
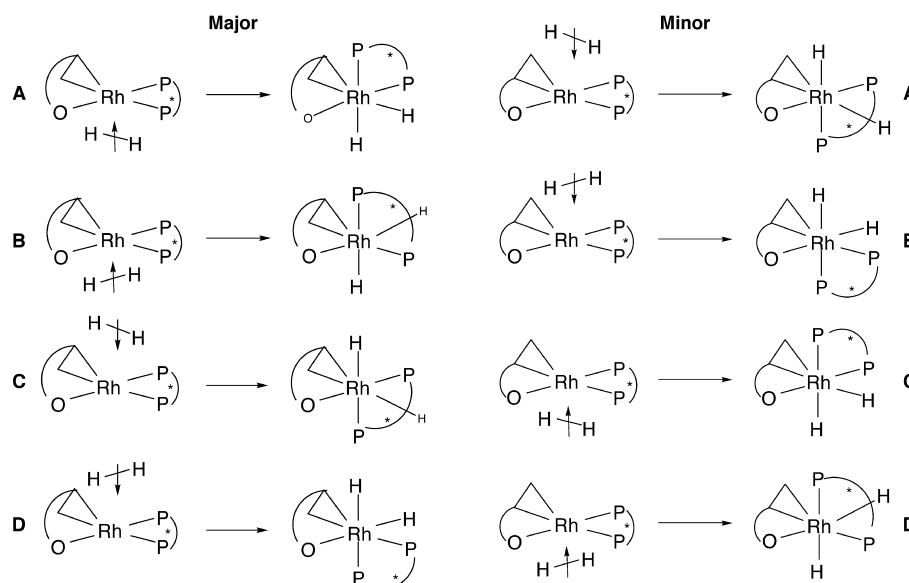


Figure 1. Computed structures of the catalyst–substrate diastereomers.

substrate's pro-*S* face (MAJ) is stabilized with a free energy of 3.6 kcal mol^{–1} at 298 K relative to the pro-*R* face (MIN), which corresponds to a 500:1 ratio of [MAJ]:[MIN].

In principle, reaction of MAJ and MIN with H₂ leads to eight diastereomeric reaction pathways, as illustrated in Scheme 2.^[5, 18] We have followed all eight pathways computa-



Scheme 2. Eight possible pathways arising from *cis* addition of H₂, either along the C-Rh-P axis (A and C) or along the O-Rh-P axis (B and D).

tionally. Each pathway proceeds along the same sequence of elementary steps: First, an ion-induced dipole complex (IID) of hydrogen and the catalyst–enamide cation is formed. Addition of H_2 yields a five-coordinate, trigonal bipyramidal molecular H_2 complex ($MOLH_2$). Oxidative addition of H_2 generates the six-coordinate dihydride intermediate ($DIHY$). Migratory insertion of $C=C$ into the $Rh-H$ bond forms the alkyl hydride intermediate ($ALHY$). Finally, reductive elimination generates the hydrogenation product, still coordinated to the catalyst. Direct experimental evidence for the intermediacy of alkyl hydride complexes has previously been published^[19] and the overall sequence of elementary steps agrees with previous proposals.^[8, 20] As we previously found for the achiral model system, $[Rh(PH_3)_2(1)]^+$,^[11] only the **A** pathways are kinetically active. The free energy surfaces for these pathways are shown in Figure 2. Significant participation of the **B**, **C**, and **D** pathways is precluded by >20 kcal mol⁻¹ barriers in at least one of the elementary steps; the origins of these barriers are electronic as they are found both in the simple model system and the sterically bulky DuPHOS system.

Taking into account the initial difference in stability of MAJ and MIN, we find the free energy of activation for the minor diastereomer to be 4.4 kcal mol⁻¹ lower than the major diastereomer. Thus, the anti “lock-and-key” motif, in which the less stable diastereomer produces the most product, is reproduced by computation.^[21]

Why is MAJ more stable than MIN? The primary difference between the two structures, as seen in Figure 1, is that the α -carbon lies closest to the coordination plane for MAJ; for MIN, the β -carbon lies in the coordination plane. We approximate a 7 kcal mol⁻¹ energetic penalty for placing the β -carbon, rather than α -carbon, in the coordination plane as follows: Mutation of the DuPHOS methyl groups of MAJ and MIN into H atoms followed by geometric optimization of only the phospholane rings creates sterically relaxed diastereomers with the α -carbon and β -carbon, respectively, in the coordination plane. The structure derived from MIN is 7 kcal mol⁻¹ higher in energy than that derived from MAJ. Subsequent optimization of the full structures produces a pair of energetically identical enantiomers with the α -carbon lying in the coordination plane. For the fully methylated DuPHOS, steric interaction between a DuPHOS methyl group and the

substrate β -methylene forces the α -carbon out of the coordination plane and destabilizes MIN relative to MAJ.

Why is MAJ so much less reactive toward H_2 than MIN? Quadrant maps^[1] qualitatively illustrate the primary steric interactions that occur when H_2 adds to either diastereomer (Figure 3). Addition of H_2 to MAJ is disfavored by motion of

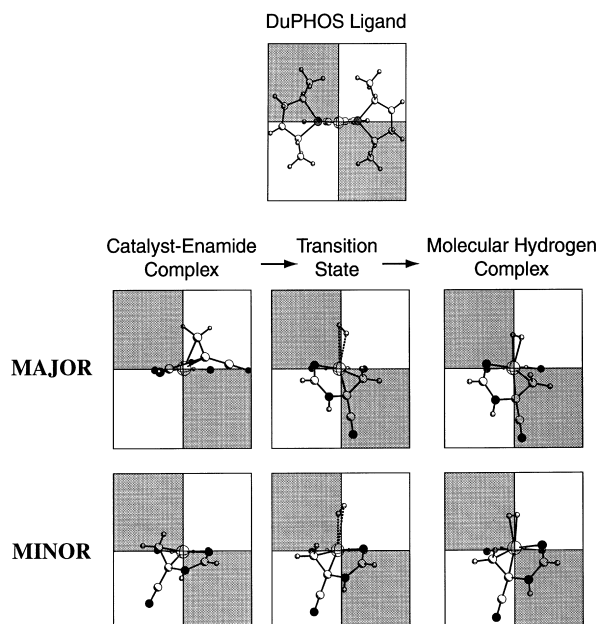


Figure 3. Formation of the molecular hydrogen complexes as viewed from a quadrant diagram perspective. For both pathways, hydrogen adds parallel to the P–Rh–C axis from the side of the β -carbon.

the nitrile group across a hindered quadrant. This motion yields both a high energy molecular hydrogen intermediate and a high energy transition state for formation of the dihydride. In contrast, the sterically induced distortion of MIN, which results in the β -carbon of the alkene lying in the coordination plane, *promotes* reaction with H_2 . Adoption of the trigonal bipyramidal structure of the molecular hydrogen complex is unencumbered as the nitrile group swings across an unhindered quadrant and requires little net motion. After the molecular hydrogen complex is formed, both manifolds have similar barriers for the subsequent steps (oxidative addition, migratory insertion, and reductive elimination).

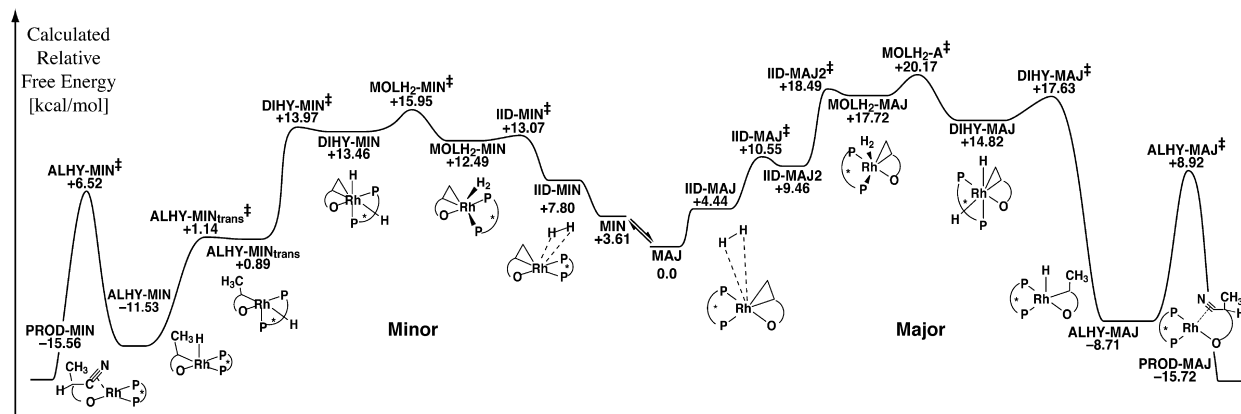


Figure 2. Free energy diagrams for pathway **A** on both diastereomeric manifolds at 298 K. Energies are in kcal mol⁻¹ and are referenced to the energy of MAJ.

The computational results agree well with all relevant empirical results. Computation predicts the following. 1) A 99.8% *ee* at 298 K in favor of the *R* product for hydrogenation of enamides with the [Rh{(*R,R*)-Me-DuPHOS}]⁺ catalyst, similar to the >99% *ee* of *R* products routinely observed experimentally for closely related substrates.^[9, 22] 2) The equilibrium concentration of MIN should be less than 0.25%, in accord with the failure of NMR experiments to detect the MIN diastereomer of [Rh{(*S,S*)-DuPHOS}-(enamide)]⁺ adducts.^[23] 3) Dihydrides are intermediates in the catalytic cycle^[24] although not at levels detectable by common NMR methods due to their high free energies and the low barriers for migratory insertion. Recently, Bargon and co-workers have reported direct observation of dihydrides by the very sensitive *para*-hydrogen induced polarization (PHIP) method during the hydrogenation of dimethyl itaconate.^[25] 4) Similar activation free energies for H₂ activation and reductive elimination steps. Previous studies involving different diphosphines^[19] have shown that lowering the temperature can result in a switch from rate-determining H₂ activation to rate-determining reductive elimination.

It is intriguing that enantiospecificities of enamide hydrogenation are quite sensitive to the nature of the enamide α -substituent.^[26] We anticipate that modulation of the electronic characteristics of the α -substituent will significantly affect both the orientation of the enamide to the coordination plane in the square planar catalyst–substrate adducts and the relative stabilities of the α - and β -alkyl hydrides. Computational evaluations of such effects are in progress.

Computational Methods

The three ONIOM^[13] layers (B3LYP:HF:UFF) are defined as follows: The core layer (B3LYP) consists of the enamide, H₂, rhodium, and an ethylene bridged bis-phosphine ligand (hydrogen atoms replace the phospholane rings); the intermediate layer (HF) includes the core plus the phospholane rings without the methyl groups; and the outer layer (MM) consists of the entire molecule (B3LYP = Becke's 3-parameter hybrid functional with Lee, Yang, and Parr correlation energies; HF = Hartree–Fock; MM = Universal Force Field molecular mechanics). All intermediates and transition states were fully optimized at the ONIOM (B3LYP/LANL2DZ:HF/LANL2MB:UFF) level of theory in a modified version of Gaussian 98^[27] (these modifications can be found in the Supporting Information). Intermediates were verified by the absence of negative frequencies and transition states were verified both by the presence of a single negative eigenvalue and by optimization in both directions along the negative eigenvector coordinate. All structures reported in this paper were fully reoptimized using ONIOM (B3LYP/II:HF/LANL2MB:UFF), where II refers to an expanded (311111/22111/411) basis set for rhodium^[28] and a 31/31 basis set for phosphorus.^[29] An additional d function with an exponent of 0.34^[30] was added to the P atoms. All other atoms used a 6-31G(d,p) basis set. Free energies were taken unscaled from the thermochemical section of the vibrational frequency analysis. No attempt was made to correct for hindered rotation vibrational entropy.

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Stepwise Ethene and/or Methyl Acrylate/CO Insertions into the Pd–C Bond of Cationic Palladium(II) Complexes Stabilized by a (P,O) Chelate**

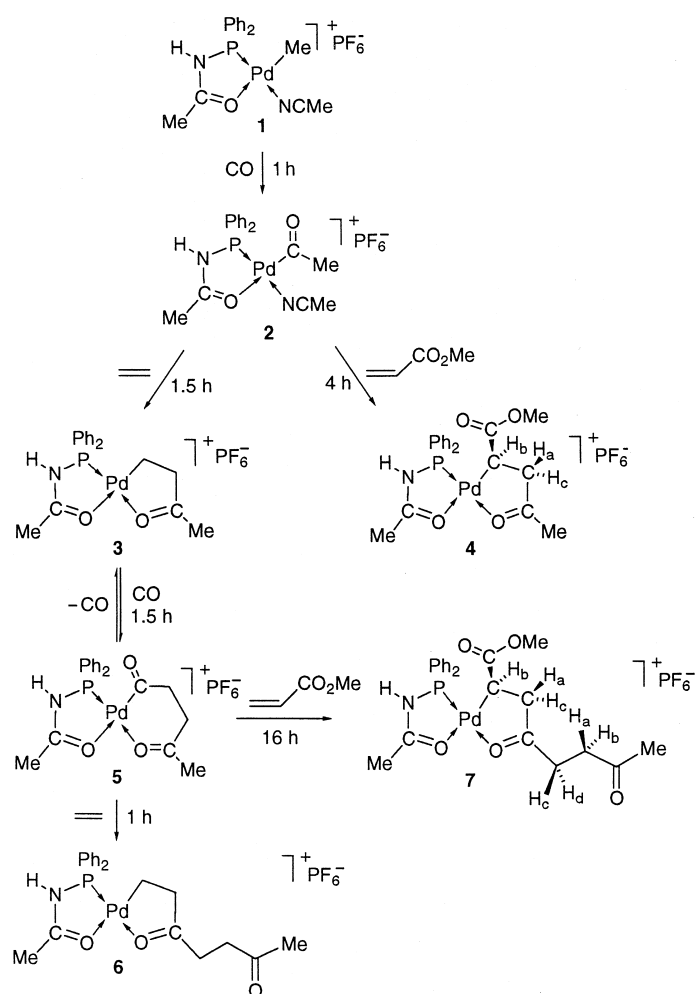
Pierre Braunstein,* Céline Frison,* and Xavier Morise*

Dedicated to Professor R. Guilard
on the occasion of his 60th birthday

The palladium-catalyzed alternating copolymerization of olefins and CO has become a major field of research in academic and industrial laboratories.^[1–4] This is because of the possibility of fine-tuning the reaction at the molecular level, the low cost of the monomers, and the properties, applications, and possible subsequent functionalization of the resulting polyketones.^[5–8] Theoretical and experimental studies have established the kinetic and thermodynamic factors controlling the chain-growth mechanism, which consists of alternating CO and olefin migratory insertion into the metal–alkyl and the metal–acyl bond, respectively.^[1–3, 9–13] However, owing to difficulties encountered in the isolation of intermediates, detailed investigations on the early stages of the chain-growth process have been carried out mainly with strained alkenes and to date only one structural report on ethene–CO coupling has been published.^[14] Furthermore, there is considerable interest in the copolymerization of olefins with polar monomers, such as methyl acrylate. However, despite recent achievements,^[15] the need to increase the percentage of functional groups in the final copolymers remains. To do this requires methods that enhance the relative rates of insertion of the polar monomer into the growing chain.

Here we present results obtained with Pd^{II} complexes in which the acetamido-derived ligand Ph₂PNHC(O)Me acts as a dissymmetric P,O chelate. These complexes have enabled us to 1) isolate and structurally characterize the metallacyclic complex resulting from the first CO–ethene coupling step, 2) isolate a complex obtained after insertions of CO and methyl acrylate into a Pd–Me bond, and 3) prepare complexes resulting from successive CO, ethene, CO, and then either incorporation of an ethene or methyl acrylate molecule.

When a dichloromethane solution of complex **1** (P,O = Ph₂PNHC(O)Me) was exposed to 1 atm of CO, the acyl complex **2** was formed quantitatively (Scheme 1) as evidenced



Scheme 1. All reactions performed at RT in CH₂Cl₂.

by a $\nu(\text{CO})$ vibration at 1716 cm^{−1} in the IR spectrum and a resonance at $\delta = 217.0$ in the ¹³C NMR spectrum. This resonance has a small ²J(P,C) coupling of 4 Hz which indicates that the acyl group in **2** resides in a *cis* position relative to the phosphorus atom just like the methyl ligand in **1**.^[10, 16, 17] Further corroboration comes from the large high-field shift ($\Delta\delta = -20.4$) of the ³¹P NMR resonance of **2** ($\delta = 58.9$), compared to **1** ($\delta = 79.3$), formation of a *trans* isomer would cause a smaller shift (ca. −10 ppm).^[18, 19] Owing to the *cis* nature of the migratory insertion of CO into palladium–carbon bonds,^[3, 13, 18, 20–22] an isomerization process, either before or after formation of the acyl ligand, must occur during the formation of **2** which appears to be the thermodynamic isomer. The preference for a soft carbon ligand (either alkyl or acyl) to avoid a position *trans* to a phosphorus atom is consistent with Pearson's antisymbiotic effect.^[23] Complex **2** shows a remarkable stability towards decarbonylation and/or decomposition, both in solution and in the solid state. This result contrasts with the majority of known cationic acylpalladium complexes, which rapidly decompose, even in the solid state, and are therefore rarely isolated.^[2, 9, 11, 12, 17, 21, 24]

Insertion of ethene into the palladium–acyl bond of **2** occurred at ambient temperature under atmospheric pressure. The reaction was completed after approximately 90 minutes (³¹P{¹H} NMR monitoring) and afforded complex **3**

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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). Coordination of the ketonic oxygen atom to the Pd center ($\nu_{\text{CO}} = 1635 \text{ cm}^{-1}$) forms a stabilizing chelate which makes β -hydrogen elimination less likely.^[25, 26] In fact **3** is stable at ambient temperature for several hours in solution and for days in the solid state, in contrast to diphosphane (P,P) complexes of the type $[\text{Pd}(\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{Me})(\text{P,P})]$.^[9] This observation illustrates the beneficial role of our P,O ligand. The $^{31}\text{P}\{^1\text{H}\}$ NMR signal of **3** ($\delta = 77.4$) is shifted to low field relative to that of **2**. In the ^1H NMR spectrum of **3** the methylene protons give rise to two triplets at $\delta = 2.11$ (Pd–CH₂) and 3.24 (CH₂C=O). Related metallacycles have been isolated upon insertion of propene,^[2, 10] styrene,^[2, 27, 28] and cyclic mono-^[11, 24, 29–33] and diolefins^[2, 11, 12, 18, 31–35] into palladium–acyl bonds. For ethene, however, there are few precedents,^[3, 9, 14, 35] and **3** represents the first complex of this type containing a P,O ligand. Many such heterobidentate P,O ligands play key roles in homogeneous catalysis, including the nickel-catalyzed ethene oligomerization to linear α -olefins in which minor changes are known to have considerable chemical consequences.^[36, 37] The structure of **3** was unambiguously determined by X-ray diffraction (Figure 1) and is only the second ethene–acyl coupling product to be so characterized.^[14] Even with other olefins, very little structural data is

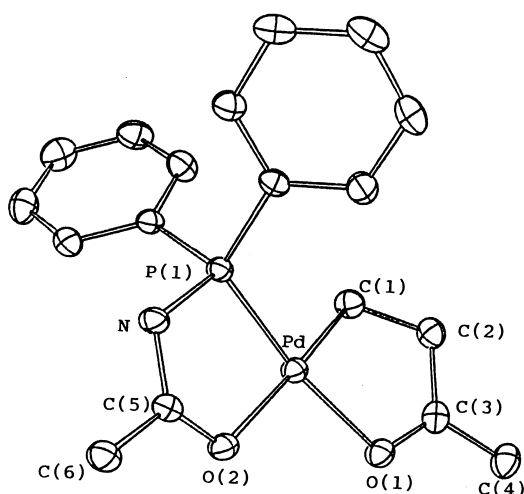


Figure 1. Molecular structure of the cation $[\text{Pd}\{\text{C}(\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{OCH}_3)(\text{P,O})\}]^+$ in **3** (H atoms not shown). Selected bond lengths [Å] and angles [°]: Pd–C(1) 2.002(3), Pd–O(1) 2.141(2), Pd–O(2) 2.176 (2), Pd–P 2.1719(8), C(1)–C(2) 1.530 (5), C(3)–O(1) 1.237(4), O(2)–C(5) 1.233(4), C(5)–N 1.359(4), N–P 1.709(3); O(1)–Pd–C(1) 80.8(1), C(1)–Pd–O(2) 177.4(1), O(1)–Pd–P(1) 170.95(7), O(1)–Pd–O(2) 101.66(8), P–N–C(5) 119.5(2), N–C(5)–O(2) 121.9(3).

available.^[11, 38] Our structure confirms the presence of the C,O and P,O chelates, with a *cis* arrangement of the two carbonyl moieties (O(1)–Pd–O(2) 101.66°). The Pd–O(1) bond (2.141(2) Å) is longer than in related norbornene–CO coupling products (2.016(5)–2.033(5) Å).^[11] The Pd–C(1) bond length in the five-membered ring (2.002(3) Å) is comparable to those of Pd-bound sp^3 -carbon atoms *trans* to a carbonyl oxygen atom.^[21, 38–40]

When complex **2** was treated with methyl acrylate, $^{31}\text{P}\{^1\text{H}\}$ NMR monitoring revealed the complete disappearance of **2** after approximately 4 h at ambient temperature. A new

resonance appeared at $\delta = 81.9$, the same region as that of **3**. The ^1H NMR spectrum of the new complex **4** (Scheme 1) in $[\text{D}_6]$ acetone contains three methyl signals at $\delta = 2.45$, 2.61 (Me–C(O)), and 2.91 (OMe), whereas the CH and CH₂ protons H_a, H_b, and H_c resonate at $\delta = 3.16$, 3.22, and 3.44, respectively. The values for the vicinal and geminal $J(\text{H,H})$ as well as the $J(\text{P,H})$ couplings (see Experimental Section) are consistent with a five-membered C,O chelate in **4**. The $\nu(\text{CO})$ vibration, assigned to the ester group at 1692 cm^{-1} (compared to 1744 cm^{-1} in EtC(O)OMe and 1731 cm^{-1} in methyl acrylate), reflects the presence of the palladium in the position α to the C(O)OMe group.^[41]

Interestingly, the stability of the C,O chelate in **3** did not prevent the facile CO insertion into the Pd–C bond to give the α -keto chelate **5** (Scheme 1), as evidenced by a new $\nu(\text{CO})$ vibration at 1708 cm^{-1} and a $^{31}\text{P}\{^1\text{H}\}$ NMR resonance ($\delta = 63.0$) in the same region as that of the acyl complex **2**. This carbonylation reaction is, however, easily reversible. The signals assigned to the methylene protons have drawn closer together ($\delta = 2.66$ and 2.84) compared with **3**. Whereas Brookhart et al. have described the formation of such a six-membered chelate in a 1,10-phenanthroline palladium complex,^[3] CO insertion into other $\text{Pd}(\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{Me})$ systems either did not occur^[35] or led to a nonchelating C(O)CH₂CH₂–C(O)Me chain.^[9, 14, 17]

The six-membered metallacycle in **5** inserted ethene under ambient conditions to afford complex **6** (Scheme 1). Its spectroscopic data resemble those for **3**, but with additional characteristic signals for a pendant ketone chain. Here again, the ketonic oxygen atom of the chelate is *trans* to the phosphorus atom. Complex **5** was also treated with methyl acrylate (under a CO atmosphere to avoid decarbonylation to **3**) and insertion into the Pd–C(O) bond occurred after 16 h at ambient temperature ($^{31}\text{P}\{^1\text{H}\}$ NMR monitoring). This afforded complex **7** as the sole product in 75 % yield (Scheme 1). This yellow complex was fully characterized by spectroscopic methods (see Supporting Information). The $\nu(\text{CO})$ vibrations at 1631 and 1601 cm^{-1} are assigned to the coordinated C=O groups of the five-membered chelates C,O and P,O, respectively. Two other absorptions at 1714 and 1700 cm^{-1} are assigned to the pendant keto group of the growing chain and to the ester function of the acrylate, respectively. Like **4**, complex **7** does not react with CO under ambient conditions, which may be the result of a stronger Pd–C bond than in **3**.

The use of a dissymmetric P,O ligand in this chemistry offers the advantage over symmetrical P,P or N,N ligands of orientating the *trans* ligands in a more selective manner. Catalytic investigations are currently being performed to see if terpolymerization is achievable.

Experimental Section

Reactions were carried out under inert atmosphere in purified solvents. All compounds gave satisfactory elemental analyses. Full details are given in the Supporting Information.

Selected data: **1**: Solid $[\text{PdCl}(\text{Me})(\text{cod})]$ (cod = 1,5-cyclooctadiene)^[42] (1.086 g, 4.098 mmol) was added to a solution of $\text{Ph}_2\text{PNHC}(\text{O})\text{Me}$ (1.004 g, 4.098 mmol) in MeCN (300 mL) followed by addition of TIPF_6 (1.432 g, 4.098 mmol). The solution was stirred for 30 min, filtered and the solvent evaporated. The residue was washed with diethylether (25 mL) and

dried in vacuo to afford **1** as a white solid (2.030 g, 90 % yield). IR (CH₂Cl₂): $\bar{\nu}_{\text{CO}} = 1614 \text{ s cm}^{-1}$; ¹H NMR (200 MHz, CD₂Cl₂): $\delta = 0.76$ (d, ³J(P,H) = 1.8 Hz, 3H; Pd-CH₃), 2.22 (s, 3H; CH₃), 2.25 (s, 3H; CH₃), 8.40 (brs, 1H; NH); ¹³C{¹H} NMR (50 MHz, CD₂Cl₂): $\delta = -4.0$ (s; Pd-CH₃), 3.0 (s, CH₃CN), 22.4 (s; NC(O)CH₃), 119.4 (s; C≡N), 182.9 (d, ²⁺³J(P,C) = 5 Hz; CO); ³¹P{¹H} NMR (121.5 MHz, CD₂Cl₂): $\delta = 79.3$.

2: CO was bubbled through a solution of **1** (0.500 g, 0.908 mmol) in CH₂Cl₂ (70 mL) at room temperature for 1 h. After filtration and removal of the volatiles under vacuum, the residue was washed with pentane (20 mL) and dried in vacuo, to afford **2** as a yellow powder (0.464 g, 88 % yield). This complex can be stored for weeks in a Schlenk flask under an atmosphere of N₂ and in CH₂Cl₂ no decomposition was observed after 1 week. IR (CH₂Cl₂): $\bar{\nu}_{\text{CO}} = 1716 \text{ s cm}^{-1}$, 1618 s cm^{-1} ; ¹H NMR (300 MHz, [D₆]acetone): $\delta = 2.30$ (d, ⁴J(P,H) = 1.5 Hz, 3H; Pd-C(O)CH₃), 2.32 (s, 3H; CH₃CN), 2.35 (d, ⁴J(P,H) = 1.2 Hz, 3H; NC(O)CH₃), 10.00 (brs, 1H; NH); ¹³C{¹H} NMR (50 MHz, CD₃CN): $\delta = 23.3$ (s; NC(O)CH₃), 37.0 (d, ³J(P,C) = 26 Hz; PdC(O)CH₃), 182.5 (d, ²⁺³J(P,C) = 4 Hz; NC(O)CH₃), 217.0 (d, ²J(P,C) = 4 Hz; PdC(O)CH₃); ³¹P{¹H} NMR (121.5 MHz, [D₆]acetone): $\delta = 58.9$.

3: C₂H₄ was bubbled through a solution of **2** (0.245 g, 0.422 mmol) in CH₂Cl₂ (30 mL) at room temperature for 1.5 h. The workup as described for **2**, afforded **3** as an orange powder (0.180 g, 75 % yield). Yellow crystals suitable for X-ray diffraction were obtained by recrystallization from CH₂Cl₂/pentane. IR (CH₂Cl₂): $\bar{\nu}_{\text{CO}} = 1635 \text{ m}$, 1611 m cm^{-1} ; ¹H NMR (300 MHz, [D₆]acetone): $\delta = 2.11$ (t, ³J(H,H) = 6.1 Hz, 2H; PdCH₂), 2.44 (s, 3H; CH₃), 2.52 (s, 3H; CH₃), 3.24 (t, ³J(H,H) = 6.1 Hz, 2H; PdCH₂CH₂), 10.37 (brs, 1H; NH); ¹³C{¹H} NMR (125 MHz, CD₂Cl₂): $\delta = 18.1$ (s, PdCH₂), 22.3 (d, ³J(P,C) = 4.2 Hz; NC(O)CH₃), 28.2 (d, ⁴J(P,C) = 2.2 Hz; CH₂C(O)CH₃), 51.03 (s; PdCH₂CH₂), 183.8 (d, ²⁺³J(P,C) = 4.9 Hz; NC(O)CH₃), 234.4 (d, ³J(P,C) = 1.9 Hz; CH₂C(O)CH₃); ³¹P{¹H} NMR (121.5 MHz, [D₆]acetone): $\delta = 77.4$.

4: Methyl acrylate (1.64 equiv, 139.0 μL , 1.542 mmol) was added to a solution of **2** (0.545 g, 0.940 mmol) in CH₂Cl₂ (70 mL). The solution was stirred for 4 h at ambient temperature to give a yellow solution. After filtration and removal of the volatiles under vacuum, the yellow residue was washed with pentane (2 $\times$ 20 mL) and dried in vacuo to afford **4** (0.490 g, 83 % yield). IR (CH₂Cl₂): $\bar{\nu}_{\text{CO}} = 1692 \text{ m}$, 1632 m , 1601 m cm^{-1} ; ¹H NMR (500 MHz, [D₆]acetone): 2D NMR experiments (COSY, HMBC, HSQC) and simulation with the program gNMR were used to determine the chemical shifts and coupling constant of the complex spin systems formed by the CHCH₂ protons and to distinguish the different methylene groups; $\delta = 2.45$ (d, ⁴J(P,H) = 0.6 Hz, 3H; NC(O)CH₃), 2.61 (s, 3H; CH₂C(O)CH₃), 2.91 (s, 3H; OCH₃), ABCX (X = P) system for Pd-CH_b-CH_aH_c: 3.16 (ddd, ²J(H_a,H_b) = 19.3, ³J(H_a,H_b) = 2.4, ⁴J(P,H) = 0.6 Hz, 1H; H_a), 3.22 (ddd, ³J(H_c,H_b) = 6.1, ³J(H_a,H_b) = 2.4, ³J(P,H) = 2.3 Hz, 1H; H_b), 3.44 (dd, ²J(H_c,H_a) = 19.3, ³J(H_a,H_b) = 6.1 Hz, 1H; H_c), 9.20 (brs, 1H; NH); ¹³C{¹H} NMR (125 MHz, [D₆]acetone): $\delta = 22.0$ (d, ³J(P,C) = 4.1 Hz, NC(O)CH₃), 28.3 (s, CH₂C(O)CH₃), 33.2 (s, PdCH), 51.3 (s, C(O)OCH₃), 51.8 (s, PdCHCH₂), 176.5 (s, C(O)OCH₃), 185.8 (d, ²J(P,C) = 4.2 Hz, NC(O)CH₃), 235.8 (d, ³J(P,C) = 2.5 Hz, CH₂C(O)CH₃); ³¹P{¹H} NMR (121.5 MHz, [D₆]acetone): $\delta = 81.9$.

5: Complex **5** was obtained in a similar manner to **2** from a CO purged solution of **3** (1.800 g, 3.186 mmol) in CH₂Cl₂ (100 mL) as a yellow powder (1.509 g, 79 % yield). IR (CH₂Cl₂): $\bar{\nu}_{\text{CO}} = 1708 \text{ s}$, 1657 m , 1616 m cm^{-1} ; ¹H NMR (500 MHz, CD₂Cl₂): $\delta = 2.30$ (d, ⁴J(P,H) = 1.0 Hz, 3H; NHC(O)CH₃), 2.48 (s, 3H; CH₂C(O)CH₃), 2.66 (t, ³J(H,H) = 6.0 Hz, 2H; PdC(O)CH₂), 2.84 (t, ³J(H,H) = 6.0 Hz, 2H; CH₂C(O)CH₃), 8.65 (brs, 1H; NH); ¹³C{¹H} NMR (125 MHz, CD₂Cl₂): $\delta = 23.1$ (d, ³J(P,C) = 4.2 Hz, NC(O)CH₃), 31.1 (d, ⁴J(P,C) = 1.9 Hz, CH₂C(O)CH₃), 38.0 (s, CH₂C(O)CH₃), 39.2 (d, ³J(P,C) = 24.6 Hz, PdC(O)CH₂), 183.5 (d, ²⁺³J(P,C) = 5.4 Hz, NC(O)CH₃), 214.3 (d, ²J(P,C) = 8.8 Hz, PdC(O)), 219.6 (s, Pd(O)CCH₂); ³¹P{¹H} NMR (121.5 MHz, [D₆]acetone): $\delta = 63.0$.

6: Complex **6** was obtained in a similar manner to **3** from a C₂H₄-purged solution of **5** (0.050 g, 0.084 mmol) in CH₂Cl₂ (15 mL) after 1 h as a yellow powder (0.036 g, 69 % yield). IR (CH₂Cl₂): $\bar{\nu}_{\text{CO}} = 1715 \text{ m}$, 1630 m , 1613 s cm^{-1} ; ¹H NMR (500 MHz, CD₂Cl₂): $\delta = 1.76$ (dt, ³J(H,H) = 6.0, ³J(P,H) = 2.0 Hz, 2H; PdCH₂), 1.92 (s, 3H; CH₂C(O)CH₃), 2.08 (d, ⁴J(P,H) = 0.5 Hz, 3H; NHC(O)CH₃), 2.65 (m, 4H; CH₂CH₂C(O)CH₃), 2.84 (dt, ³J(H,H) = 6.0, ⁴J(P,H) = 0.5 Hz, 2H; PdCH₂CH₂), 8.81 (brs, 1H; NH); ¹³C{¹H} NMR (125 MHz, CD₂Cl₂): $\delta = 18.4$ (s; PdCH₂CH₂), 22.3 (d, ³J(P,C) = 4.2 Hz; NC(O)CH₃), 29.5 (s; CH₂C(O)CH₃), 34.5 and 38.3 (2 $\times$ s;

C(O)CH₂CH₂C(O)), 50.4 (s; PdCH₂CH₂), 183.8 (d, ²J(P,C) = 4.8 Hz; NC(O)CH₃), 206.6 (s; CH₂C(O)CH₃), 235.0 (s; CH₂C(O)CH₂); ³¹P{¹H} NMR (121.5 MHz, [D₆]acetone): $\delta = 77.5$.

7: Complex **7** was prepared from **5** (0.780 g, 1.311 mmol) and 1.2 equiv of methyl acrylate (141 μL , 1.573 mmol) in CH₂Cl₂ (100 mL). The reaction mixture was stirred at ambient temperature for 16 h under an atmosphere of CO. The solution was then filtered, and the volatiles were removed under vacuum to leave a yellow powder (0.670 g, 75 % yield). IR (CH₂Cl₂): $\bar{\nu}_{\text{CO}} = 1714 \text{ s}$, 1700 s , 1631 m , 1601 s cm^{-1} ; ¹H NMR (500 MHz, [D₆]acetone): 2D NMR experiments (COSY, HMBC, HSQC) and simulation with the program gNMR were used to determine the chemical shifts and coupling constants of the complex spin systems formed by the CHCH₂ and CH₂CH₂ protons and to distinguish the different methylene groups; $\delta = 2.19$ (s, 3H; CH₂C(O)CH₃), 2.44 (s, 3H; NC(O)CH₃), 2.92 (s, 3H; OMe), ABCD spin system for C(O)CH₂H_dCH_aH_bC(O)CH₃: 2.96 (ddd, ²J(H_a,H_b) = 20.3, ³J(H_a,H_c) = 7.0, ³J(H_a,H_d) = 5.2 Hz, 1H; H_a), 3.00 (ddd, ²J(H_a,H_b) = 20.3, ³J(H_b,H_c) = 4.7, ³J(H_b,H_d) = 7.7 Hz, 1H; H_b), 3.10 (ddd, ³J(H_c,H_a) = 7.0, ³J(H_c,H_b) = 4.7, ²J(H_c,H_d) = 18.5 Hz, 1H; H_c), 3.20 (ddd, ²J(H_d,H_c) = 18.5, ³J(H_d,H_b) = 7.7, ³J(H_d,H_a) = 5.2 Hz, 1H; H_d), ABCX (X = P) spin system for Pd-CH_b-CH_aH_c: 3.20 (dd, ²J(H_c,H_a) = 19.0, ³J(H_a,H_b) = 2.6 Hz, 1H; H_a), 3.24 (ddd, ²J(H_c,H_b) = 6.0, ³J(H_a,H_b) = 2.6, ³J(P,H) = 2.0 Hz, 1H; H_b), 3.42 (dd, ³J(H_c,H_b) = 6.0, ²J(H_c,H_a) = 19.0 Hz, 1H; H_c), 10.54 (brs, 1H; NH); ¹³C{¹H} NMR (125 MHz, [D₆]acetone): $\delta = 21.7$ (d, ³J(P,C) = 4.8 Hz, NC(O)CH₃), 29.6 (s, CH₂C(O)CH₃), 33.0 (s, PdCH), 35.4 (d, ⁴J(P,C) = 1.9 Hz, CH₂CH₂C(O)CH₃), 37.7 (s, CH₂C(O)CH₃), 50.9 (s, PdCHCH₂), 51.2 (s, C(O)OCH₃), 176.2 (s, C(O)OCH₃), 185.6 (d, ²⁺³J(P,C) = 3.8 Hz, NC(O)CH₃), 210.0 (s, CH₂C(O)CH₃), 236.3 (d, ³J(P,C) = 1.9 Hz, CH₂C(O)CH₂); ³¹P{¹H} NMR (121.5 MHz, [D₆]acetone): $\delta = 81.6$; FAB-MS: *m/z*: 534 (27 %) [*M*⁺].

Crystallographic data for **3**: C₁₈H₂₁F₆NO₂P₂Pd; *M*_r = 565.71, monoclinic, space group *P*2₁/*n*, *a* = 8.6887(2), *b* = 18.822(1), *c* = 13.0904(6) Å, β = 100.983(3)°, *V* = 2101.6(3) Å³, *Z* = 4, ρ_{calc} = 1.79 g cm⁻³, μ = 1.103 mm⁻¹, *F*(000) = 1128, $\lambda(\text{MoK}\alpha)$ = 0.71073 Å, *T* = 173 K. Red crystal, dimensions 0.20 $\times$ 0.15 $\times$ 0.15 mm³. A total of 12017 reflections were collected on a Kappa CCD diffractometer (ϕ scans, 2 < θ < 29°). The structure was solved using direct methods and refined against $|F|$. Absorption corrections were computed from the psi scans of four reflections. For all computations the Nonius MoLEN package^[43] was used: *R* = 0.041, *R*_w = 0.059, GOF = 1.049, maximum residual electron density 0.801 e Å⁻³ for 4413 reflections having *I* > 3σ(*I*). All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were introduced as fixed contributors (*d*_{C-H} = 0.95 Å, *B*(H) = 1.3 *B*_{eq}(C) Å²). 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-136484. 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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An Efficient, Modular Synthetic Route to Oligomers Based on Zirconocene Coupling: Properties for Phenylene–Thiophene-1-Oxide and Phenylene–Thiophene-1,1-Dioxide Chains**

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The increasing interest in π -conjugated polymers has resulted in a very large research effort, focused on the development of new conducting materials for electronic devices.^[1] Associated with this effort has been the synthesis and study of conjugated oligomers with well-defined structures and properties.^[2] Oligomeric species allow detailed investigations of the fundamental properties of conjugated chains, and provide insight into the behavior of the analogous polymers. In addition, the discrete properties of the oligomers make them attractive materials in their own right, since they may exhibit properties that are not associated with the related polymers.

We have developed a new route to conjugated polymers based on the zirconocene coupling of diynes.^[3,4] This approach provides synthetic intermediate polymers containing zirconacyclopentadiene units, which are readily converted into a variety of new structures. These chemical transformations provide a modular approach to control the electronic and optical properties of the polymer, through substitution at a particular site in the conjugated chain by various structural units (for example dienylene, phenylene, thiophene, selenophene, germole, and phosphole). Related strategies which complement this approach involve the zirconocene coupling of functionalized diynes, to provide new monomers for polymerization^[3f,4] and the incorporation of zirconacyclopentadiene units by the coupling of diyne segments in a pre-existing polymer.^[3b,c,g]

A recent discovery in our laboratories^[4] allows the efficient incorporation of thiophene-1-oxide (T_O) and thiophene-1,1-dioxide (T_{O_2}) rings into the backbone of a conjugated polymer by the reaction of zirconacyclopentadiene derivatives with SO_2 . Thus, we have been able to prepare the first polymers containing T_O groups and develop general routes to alternating arylene– T_O and arylene– T_{O_2} copolymers.^[3g] Interestingly, these polymers have more narrow band gaps, and have higher electron affinities than the corresponding thiophene-based polymers. In related work based on a different synthetic strategy, Barbarella et al. have shown that T_{O_2} groups may be incorporated into oligomers which exhibit decreased electronic band gaps and higher electron affinities than the

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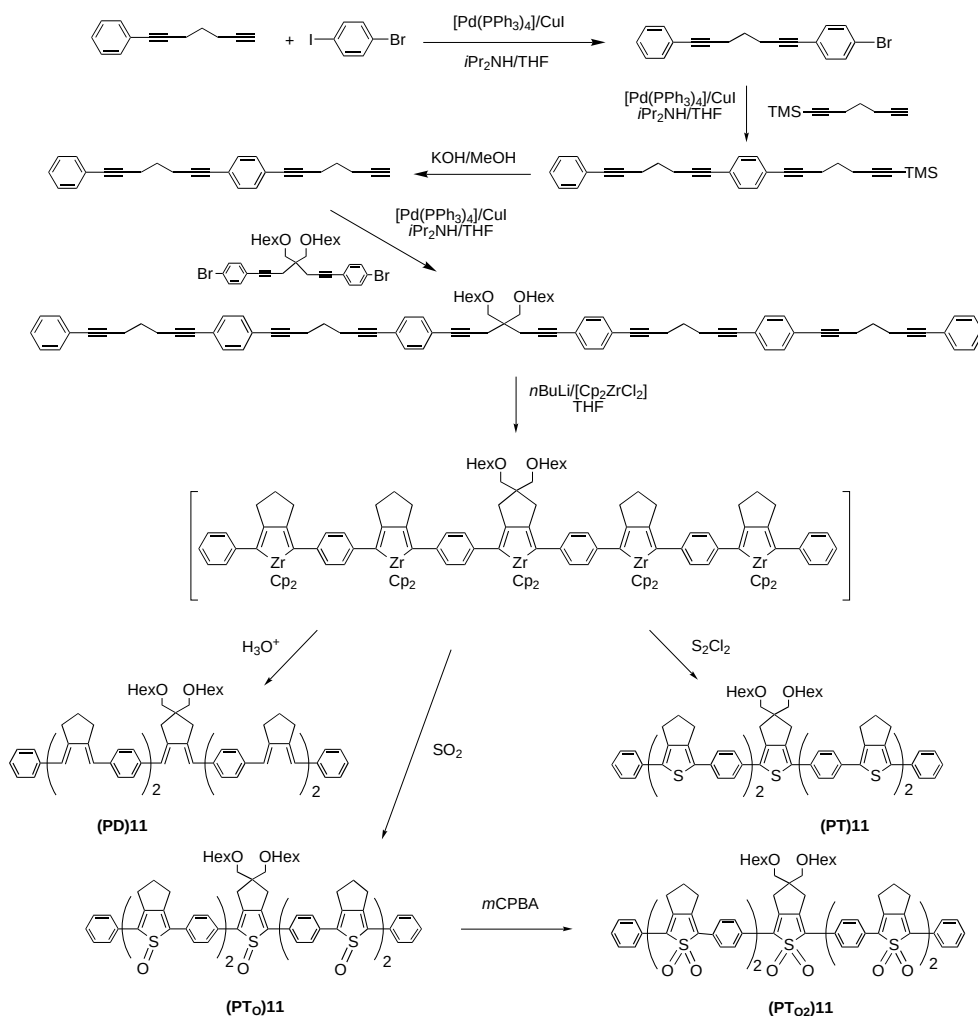
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analogous thiophene oligomers.^[5] Thus, T_{O^-} and $T_{O_2^-}$ -based polymers have emerged as good candidates for electron-transporting materials in polymer-based devices.

To further investigate the fundamental electronic properties of T_{O^-} and $T_{O_2^-}$ -based conjugated systems we have prepared a series of well-defined oligomers which allows systematic comparison of T_{O^-} , $T_{O_2^-}$, thiophene (T), and dienyne (D) groups. Our synthetic route to these oligomers takes advantage of versatile and efficient methods based on zirconocene couplings. Traditional synthetic approaches to oligomers principally rely on series of coupling steps to produce a particular structure. Such step by step procedures are tedious and provide only one type of oligomer.^[2] The method described here makes use of a high-yielding coupling reaction (the palladium coupling of alkynes and aryl halides) to produce oligomers which, by zirconocene coupling methods, may be converted into a family of new conjugated structures with fundamentally different properties.

The general strategies for conversions of linked diyne units to diene, thiophene, thiophene-1-oxide, and thiophene-1,1-dioxide groups by zirconocene coupling have been described.^[4] A high-yielding route to the required precursor diyne oligomers (of arbitrary length) is described in Scheme 1. This procedure relies on the availability of unsymmetrical diynes, such as 1-phenyl-1,6-heptadiyne and 1-trimethylsilyl-1,6-heptadiyne, which are obtained in high yields by a literature method.^[6] By repeated sequences of coupling reactions of alkyne and aryl halides (with the high-yielding Hagihara procedure) and deprotection chain lengths may be built up rapidly. The precursor diyne oligomers were converted into the corresponding zirconacyclopentadiene derivatives by the Negishi method,^[7] and these compounds were then converted in situ into four different types of oligomers (Scheme 1). The zirconocene coupling and subsequent transformations are very efficient as indicated by the high yields for the latter conversions (92–99%). This procedure provided oligomers with phenylene–dienylene, phenylene–thiophene, phenylene–thiophene-1-oxide, and phenylene–thiophene-1,1-dioxide, structures (Table 1; $(PD)_n$, $(PT)_n$, $(PT_O)_n$, and $(PT_{O_2})_n$, respectively, where P = phenylene



Scheme 1. Modular synthesis of various oligomers by zirconocene coupling methods.

and n indicates the total number of rings and/or diene units).

Figure 1 provides views of the molecular structure of $(PT)5$.^[8] This oligomer crystallized (with 0.5 equivalents of chloroform solvent) as pairs of loosely associated molecules related by inversion. The inner phenylene and thiophene rings are coplanar (with angles between the least-squares planes of $<6.5^\circ$), while the terminal phenyl rings are rotated by 31.4 and 27.6° with respect to the central phenylene ring. The association of the oligomers is such that a symmetry related pair of carbon atoms in the central phenylene group (C19) are separated by $3.24 \text{ \AA}$.

To investigate the electronic properties of each type of oligomer structure, and to determine the conjugation lengths for the corresponding polymers, we examined the UV/Vis spectra for each oligomer. The $(PT_O)_n$ oligomers exhibit a distinct bathochromic shift which is associated with elongation of the oligomer chain (Figure 2a). The λ_{max} values for these oligomers asymptotically approach the observed value for poly(phenylene–thiophene-1-oxide) (PPT_O , 497 nm). The corresponding optical band gaps, determined from the band edges, are plotted as a function of oligomer length (Figure 2b). This smooth convergence curve indicates that the oligomers and polymers obtained by zirconocene coupling are

Table 1. Optical properties of oligomers.

Oligomer structure	Oligomers	$\lambda_{\max}$ [nm]	ϵ [$10^5 \text{ cm}^{-1} \text{ M}^{-1}$]	$E_{\text{g,opt}}$ [eV] ^[a]
	X = 2H, (PD)3	338	0.33	3.23
	X = S, (PT)3	330	0.32	3.31
	X = SO, (PT _O)3	392	0.14	2.71
	X = SO ₂ , (PT _{O2})3	378	0.20	2.86
	X = 2H, (PD)5	392	0.66 (0.39) ^[b]	2.75
	X = S, (PT)5	384	0.66 (0.39)	2.86
	X = SO, (PT _O)5	440	0.39 (0.23)	2.44
	X = SO ₂ , (PT _{O2})5	430	0.27 (0.16)	2.51
	X = 2H, (PD)7	412	0.81 (0.35)	2.55
	X = S, (PT)7	408	1.10 (0.47)	2.66
	X = SO, (PT _O)7	462	0.44 (0.19)	2.29
	X = SO ₂ , (PT _{O2})7	452	0.56 (0.24)	2.35
	X = 2H, (PD)11	436	1.20 (0.33)	2.40
	X = S, (PT)11	430	1.25 (0.34)	2.49
	X = SO, (PT _O)11	484	0.56 (0.15)	2.11
	X = SO ₂ , (PT _{O2})11	476	1.01 (0.28)	2.24

[a] Optical band gap from absorption edge. [b] Molar absorption values in parantheses are based on the fundamental repeating unit of the oligomer.

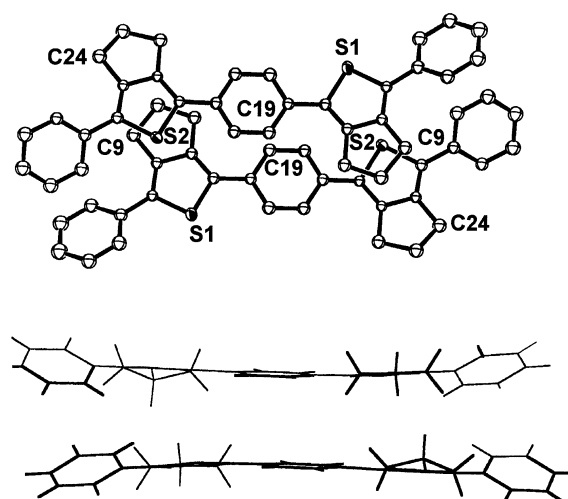


Figure 1. Views of the molecular structure of (PT)5.

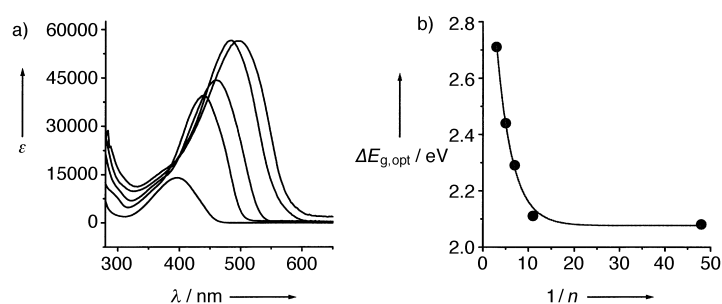


Figure 2. a) UV/Vis spectra and b) optical band gap convergence curve, for the (PT_O)_n series.

well defined and largely defect free, and allows the conjugation length in PPT_O to be estimated at 15 rings (7–8 monomer units). Note that similar convergence curves were observed for the (PD)_n, (PT)_n, and (PT_{O2})_n systems, from which conjugation lengths of 15–20 rings may be estimated (see Supporting Information). This estimate is consistent with

results for polythiophenes which exhibit conjugation lengths of approximately 12 rings,^[9] whereas polyphenylenes have been estimated to have a conjugation length of only 4–5 rings.^[10]

A comparison of the convergence curves for all the oligomeric systems described above reveals that the phenylene–thiophene-1-oxide structure has the lowest energy optical band gap: PPT_O (2.08 eV) < PPT_{O2} (2.18 eV) < PPD (2.35 eV) < PPT (2.44 eV).^[11] This trend in band gap energy reflects the generally greater conjugation along the chain for diene (compared to aromatic) structures, and also reflects the more planar diene units present in thiophene-1-oxide and thiophene-1,1-dioxide (compared to the acyclic diene).^[12] Barbarella and co-workers have previously shown that incorporation of thiophene-1,1-dioxides into thiophene oligomers results in a reduction of the band-gap energy, and their data indicates that this narrowing of the band gap principally occurs by a stabilization of the LUMO level.^[5a]

Cyclic voltammetry data for the oligomers and polymers give the same ordering of the band gaps, with values that are slightly greater: PPT_O (2.12 eV) < PPT_{O2} (2.31 eV) < PPD (2.50 eV) < PPT (2.69 eV). These data also allow estimates for the HOMO and LUMO energy levels (Figure 3).^[13] The calculated energy levels reveal that the more narrow band gaps for the thiophene-1-oxide and thiophene-1,1-dioxide systems result mainly from a stabilization of the LUMO levels, as has been observed for thiophene–thiophene-1,1-dioxide oligomers.^[5] However, this LUMO stabilization is similar for PPT_O and PPT_{O2}, and the narrower band gap for the thiophene-1-oxide chains results because the more electronegative sulfonyl group stabilizes both the HOMO and LUMO, whereas the sulfoxide does not stabilize the HOMO significantly relative to the thiophene polymer. An alternative explanation for the difference in band gaps for (PT_O)_n and (PT_{O2})_n oligomers is based on differences in dihedral angles between moieties in the chains. This is suggested by the structural data for 2,5-diphenylthiophene and its sulfoxide and its sulfone derivatives. In these

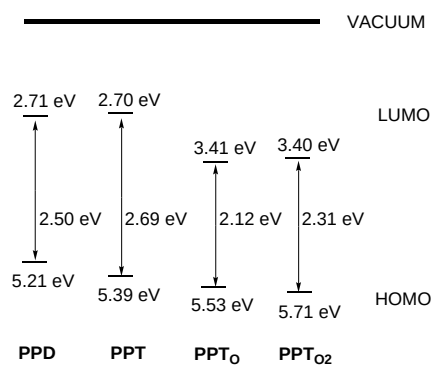


Figure 3. Estimated HOMO and LUMO energy levels of **PPD**, **PPT**, **PPT_O**, and **PPT_{O2}**.

structures, the dihedral angles between the central ring and those of the phenyl groups vary from an average of 8.5° for the thiophene to 12.5° for the sulfoxide and 7.4(2) and 39.1(2)° for the sulfone.^[14] Thus, conjugation in the thiophene-1-oxide derivative may be more effective because it is more planar than the corresponding sulfone; this planarity may result from a lower barrier to rotation about the ring–ring bonds.^[15]

The fluorescence properties of the oligomers and polymers were investigated for dilute samples dissolved in chloroform. The chains containing T_O and T_{O2} units exhibit very low luminescence efficiencies, and for the polymers the ordering of the quantum efficiencies is: PPT (0.12) > PPT_O (0.016) > PPD (0.012) > PPT_{O2} (0.003).^[3b] The Stokes shifts associated with these emissions range from 50 to 80 nm. For the highly luminescent (PT)*n* oligomers, the Stokes shift is approximately 50 nm and the quantum efficiency decreases with molecular weight from the very high value for (PT)5 ($\Phi = 0.77$) to a value of 0.36 for (PT)11 (for (PT)7, $\Phi = 0.50$).^[16]

In conclusion, we have described a versatile new approach to the synthesis of a series of conjugated oligomers, based on the zirconocene coupling of diynes. This has allowed us to prepare the first arylene–T_O and arylene–T_{O2} co-oligomers, and investigate fundamental electronic properties for conjugated chains involving thiophene-1-oxide and thiophene-1,1-dioxide. Of particular note are the narrow band gaps and high electron affinities associated with such monomers, and that the presence of the less electron-withdrawing sulfoxide group results in the lowest band gap.

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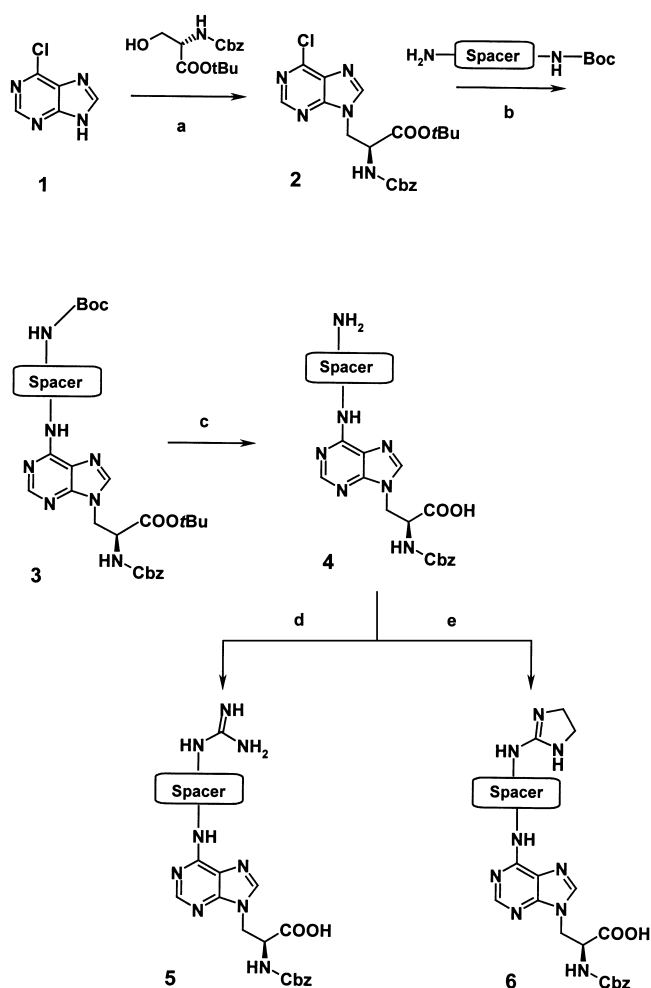
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Vitronectin Receptor Antagonists: Purine-Based Peptidomimetics

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Integrins are a widely expressed family of α/β heterodimeric cell surface receptors which bind to extracellular matrix adhesive proteins such as fibrinogen, fibronectin, vitronectin, laminin, and osteopontin.^[1–3] They are composed of dimers of at least fifteen α subunits and eight β subunits. The β_3 class of the integrin family, $\alpha_{IIb}\beta_3$ (GPIIb/IIIa or fibrinogen receptor) and $\alpha_v\beta_3$ (vitronectin receptor), has received special attention in recent drug research.^[4, 5] $\alpha_{IIb}\beta_3$ is prevalent on platelets and plays a role in thromboembolic disorders,^[5] while $\alpha_v\beta_3$ is the dominant receptor for mediating the attachment of osteoclasts to bone during bone resorption^[6, 7] and has been implicated in tumor progression, angiogenesis,^[5, 8, 9] and restenosis.^[10] Many integrins, including $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$, interact with a common Arg-Gly-Asp (RGD) binding motif in their target proteins.^[11–13] Kessler et al. were able to identify the structural properties required for the selective inhibition of either $\alpha_{IIb}\beta_3$ or $\alpha_v\beta_3$ using stereoisomeric cyclic peptide libraries.^[14–16] In drug development peptidomimetic compounds are often preferred over peptides^[17], and several selective peptidomimetic antagonists of $\alpha_v\beta_3$ have been reported recently.^[18–25] This has led to an increased interest in finding new building blocks for integrin antagonists that allow the correct spatial arrangement of pharmacophorically important groups. Although the purine scaffold has been used in the synthesis of a variety of chemical libraries, especially in the design of kinase inhibitors,^[26, 27] there are to our knowledge no reports on its use as a central scaffold in peptidomimetics. Here we introduce the purine scaffold in the design and synthesis of selective antagonists of $\alpha_v\beta_3$ and present the first structure–activity relationship within this series.

The synthesis of the $\alpha_v\beta_3$ antagonists **5** and **6** is outlined in Scheme 1. Alkylation of 6-chloropurine **1** with *N*-Cbz-L-serine *tert*-butyl ester under Mitsunobu conditions afforded **2** in 50% yield. Nucleophilic substitution with building blocks containing one *tert*-butoxycarbonyl (Boc) protected and one unprotected amino group gave aminopurines **3**. Only (4-piperidylmethyl)amine was used without Boc-protection, reacting selectively (>95%) with the secondary amine. (No trace of the corresponding product of the primary amine was found.) The Boc group and the *tert*-butyl ester were cleaved simultaneously by treatment with 95% TFA/5% water and



Scheme 1. Synthesis of the $\alpha_v\beta_3$ antagonists **5** and **6**. a) PPh_3 , DEAD, THF, 0 °C; 50%; b) DMF, DIPEA, 6 h 50 °C; 60–80%; c) 95% TFA, 2 h, RT; 100%; d) 1*H*-pyrazole-1-carboxamide, H_2O , DIPEA, 48 h, RT; 50–85%; e) 2-methylsulfanyl-2-imidazoline, DIPEA, 24 h, 50 °C; 50–70%. DEAD = diethyl azodicarboxylate, TFA = trifluoroacetic acid, DIPEA = diisopropylethylamine.

the resulting molecules **4** were converted into the guanidines **5** by treatment with 1*H*-pyrazole-1-carboxamide^[28] and DIPEA in water with yields ranging from 50 to 85%. Alternatively **4** was converted to the (1*H*-imidazolin-2-yl)amines **6** using 2-methylsulfanyl-2-imidazoline and DIPEA in DMF with yields of 50 to 70%. The synthesis of antagonist **7**, which contains a 2-aminobenzimidazole group as an arginine mimetic, is depicted in Scheme 2. *N*-(1*H*-benzimidazol-2-yl)butane-1,4-diamine, prepared by standard methods, was treated with **2** to give antagonist **7** after cleavage of the *tert*-butyl ester. A second series of $\alpha_v\beta_3$ antagonists (**10** and **11**) is characterized by a “reversed” orientation of the central purine scaffold (amino acid substituent not on the imidazole but on the pyrimidine ring), and its synthesis is shown in Scheme 3. Compound **1** was first alkylated with *N*-Boc-protected aminoalkyl tosylates to give the chloropurines **8** in high yields. These compounds reacted with the amino group of *tert*-butyl (*S*)-*N*²-Cbz-2,3-diaminopropionate and its homologues, and after treatment with TFA, compounds **9** are obtained. Subsequent conversion to the guanidines **10** and the

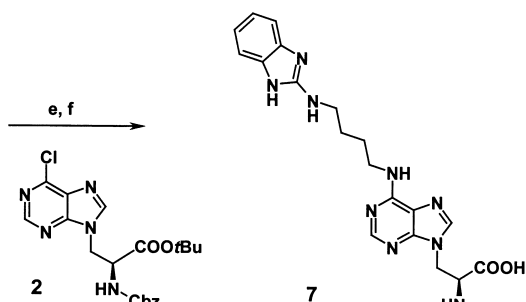
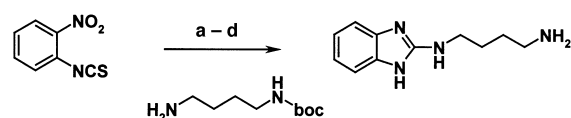
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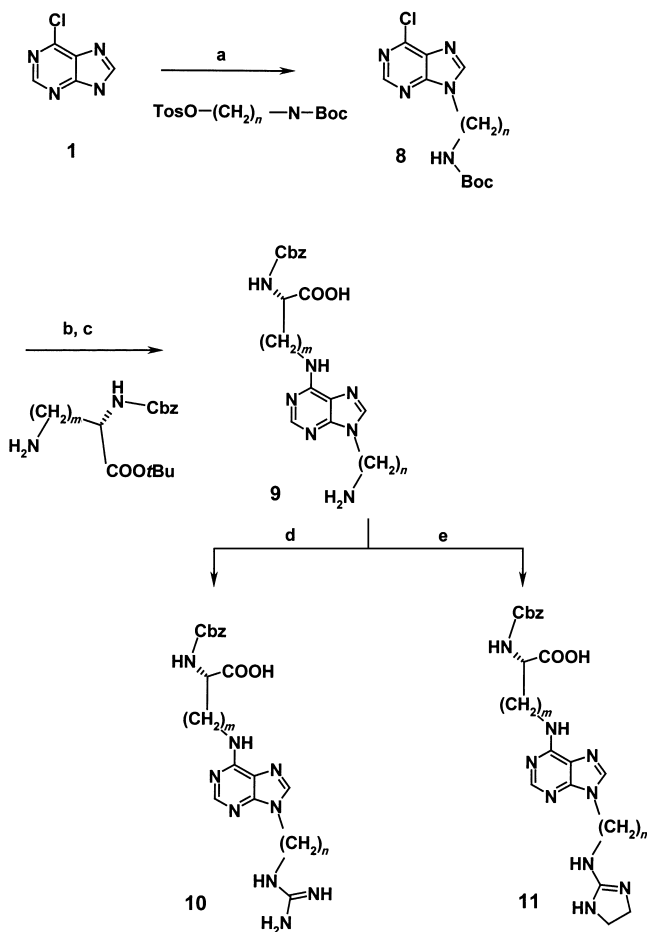
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Scheme 2. Synthesis of the $\alpha_v\beta_3$ antagonist **7**. a) DMF, 0 °C, 1 h; 95 %; b) H_2 /Pd-C; 100 %; c) HgO, sulfur, 50 °C; 43 %; d) 95 % TFA, 2 h, 0 °C; 100 %; e) DMF, DIPEA, 32 h, 50 °C; 32 %; f) 95 % TFA, 2 h, 0 °C; 100 %.

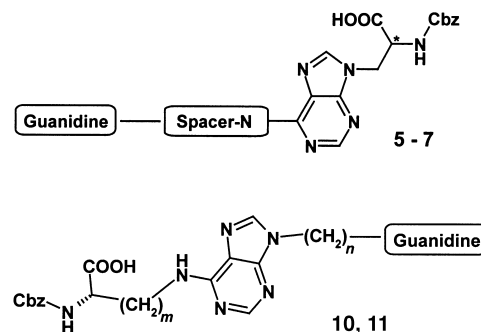


Scheme 3. Synthesis of the $\alpha_v\beta_3$ antagonists **10** and **11**. a) DMF, K_2CO_3 , RT; 70–80 %; b) DMF/DIPEA (2/1), 70 °C, 7 d; 40–50 %; c) 95 % TFA, 2 h, 0 °C; 100 %; d) 1*H*-pyrazol-1-carboxamidine, H_2O , DIPEA, 48 h, RT; 50–60 %; e) 2-methylsulfanyl-2-imidazoline, DIPEA, 24 h, 50 °C; 50–60 %.

amino-2-imidazolines **11** was accomplished with the same methods as for the synthesis of **5** and **6**.

One of the most interesting challenges in this area is the design of selective antagonists since both $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ bind

to the same recognition motif RGD and there seem to be only subtle differences in the ligand structure that determine selectivity. The IC_{50} values of compounds **5–7**, and **10**, **11** (Scheme 4) for the inhibition of binding of fibrinogen to $\alpha_{IIb}\beta_3$ and of Kistrin (a disintegrin with high affinity to $\alpha_v\beta_3$ ^[7, 29]) to $\alpha_v\beta_3$ are summarized in Tables 1 (“normal” orientation) and 2 (“reversed” orientation).



Scheme 4. General formulas for the antagonists **5–7**, and **10**, **11**.

The most striking criterion for selectivity is the distance between the carboxy group and the N-terminal guanidine group, as can be seen from a comparison of **5a**, **5b**, and **5c** or in the “reversed” series by comparing **10a**, **10b**, and **10c**. The optimum distance for $\alpha_v\beta_3$ binding within these two series is 12 bonds (**5b**, **10b**), while $\alpha_{IIb}\beta_3$ prefers a substrate where the two groups are further apart, as in **5a** or **10a** with a distance of

Table 1. Activity and selectivity of $\alpha_v\beta_3$ antagonists **5–7** (Scheme 4). The IC_{50} values [μM] denote the concentration required to reduce binding of fibrinogen (Fg) to $\alpha_{IIb}\beta_3$ or of Kistrin (K) to $\alpha_v\beta_3$ by 50 %.^[30]

	Configura- tion (*)	Guanidine	Spacer	IC_{50} K/ $\alpha_v\beta_3$	IC_{50} Fg/ $\alpha_{IIb}\beta_3$
5a	S		$-(\text{CH}_2)_5\text{-NH-}$	1.1	1.9
5b	S		$-(\text{CH}_2)_4\text{-NH-}$	0.7	> 10
5c	S		$-(\text{CH}_2)_3\text{-NH-}$	2.2	> 10
6a	S		$-(\text{CH}_2)_4\text{-NH-}$	0.15	> 10
7	S		$-(\text{CH}_2)_4\text{-NH-}$	0.16	> 10
6b	S			0.05	4.0
6c	R			0.34	> 10
6d	R		$-(\text{CH}_2)_4\text{-NH-}$	1.0	> 10

Table 2. Activity and selectivity of $\alpha_v\beta_3$ antagonists **10** and **11** (Scheme 4). The IC_{50} values [μM] denote the concentration required to reduce binding of fibrinogen (Fg) to $\alpha_{IIb}\beta_3$ or of Kistrin (K) to $\alpha_v\beta_3$ by 50%.^[30]

	Guanidine	<i>m</i>	<i>n</i>	IC_{50} K/ V_{nR}	IC_{50} Fg/ $IIbIIIa$
10a		1	5	0.65	0.6
10b		1	4	0.17	5.0
10c		1	3	0.54	3.5
10d		2	3	0.58	> 10
11a		1	4	0.075	> 10
11b		1	5	0.21	> 10
11c		2	3	0.19	> 10

13 bonds. This observation is in good agreement with the results obtained for other inhibitors^[18, 19] and with Kessler's observation that in cyclopeptides $\alpha_v\beta_3$ -selective molecules are strongly bent, while $\alpha_{IIb}\beta_3$ -selective molecules bind in a more extended conformation.^[14, 16]

A comparison of **5b**, **6a**, and **7** illustrates that cyclic guanidines have a higher affinity for $\alpha_v\beta_3$ than noncyclic ones. The cyclic guanidino antagonist **6a** (IC_{50} = 150 nM) exhibits a fivefold affinity over the open guanidino compound **5b** (IC_{50} = 700 nM). The 2-aminobenzimidazole antagonist **7** shows approximately the same potency as **6a**. Interestingly this increase in affinity is not observed for $\alpha_{IIb}\beta_3$ binding. A similar trend can be observed in the "reversed" series; however, the increase in affinity from **10b** (IC_{50} = 170 nM) to the cyclic guanidino compound **11a** (IC_{50} = 75 nM) is only by a factor of 2, but at the same time there is a decrease in affinity to $\alpha_{IIb}\beta_3$. This decreased affinity to $\alpha_{IIb}\beta_3$ for cyclic guanidines can also be observed in a comparison of the extended antagonists **10a** and **11b**. One could speculate that the antagonists bind to $\alpha_{IIb}\beta_3$ through an "end-on" interaction, while $\alpha_v\beta_3$ -binding is achieved through "side-on" binding on the guanidine.

Another enhancement in $\alpha_v\beta_3$ binding is obtained through the rigidification of the spacer from aminobutane in **6a** (IC_{50} = 150 nM) to methylpiperidine in **6b** (IC_{50} = 50 nM), keeping the length of the spacer constant. The piperidine spacer seems to bring the guanidino group into the correct spatial orientation and, in addition, it has the advantage that no additional stereocenter is introduced.

The *S* configuration is clearly preferred for the only stereocenter that is connected with the lipophilic side chain, the benzyloxycarbonylamino group, as can be seen from the IC_{50} values for **6c** (340 nM) and **6d** (1000 nM) which both have

the *R* configuration and which are approximately sevenfold less active than the *S* enantiomers **6b** and **6a**, respectively.

Unexpectedly, the binding affinity is quite insensitive to the orientation of the central purine scaffold—a comparison of, for example, **6a** (IC_{50} = 150 nM) and **11a** (IC_{50} = 75 nM) reveals only a small preference for the "reversed" orientation. The difference is of the same order of magnitude as the one induced by changing the position of the purine scaffold within a given orientation ("slidomers"); compare, for example, **11a** (IC_{50} = 75 nM) and **11c** (IC_{50} = 190 nM).

In conclusion, we have been able to show that the purine scaffold is a highly versatile building block in the synthesis of peptidomimetics. To mimic the RGD peptide recognition sequence of the vitronectin receptor, we could easily vary spacer length, rigidity, and pharmacophoric groups. This led to a very consistent structure–activity relationship and resulted in highly active and selective new antagonists of $\alpha_v\beta_3$. The ease of derivatization will certainly encourage many future uses of this scaffold in peptidomimetic chemistry.

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Highly Efficient and Versatile Acylation of Alcohols with Bi(OTf)₃ as Catalyst

Akihiro Orita, Chiaki Tanahashi, Atsushi Kakuda, and Junzo Otera*

The acylation of alcohols is an important transformation in organic synthesis.^[1] Despite a number of precedents, new efficient methods are still in strong demand. Acid anhydrides are the most commonly used reagents in the presence of an acid or base catalyst^[2] and the utility of this protocol was boosted by the discovery of the dimethylaminopyridine (DMAP) catalyst.^[2a] More recently, metal triflates, such as scandium triflate,^[2d] trimethylsilyl triflate,^[2e] and indium triflate,^[2f] were found to be effective as well. These catalysts are very useful, but also suffer from some drawbacks. Scandium triflate is rather expensive and must be used under anhydrous conditions. Trimethylsilyl triflate is labile towards moisture, and its acidity is too strong for acid-sensitive alcohols as reagents.^[2e] In the development of organotin

acylation catalysts,^[2g,h,3] we have established that they are so mild that various selective acylation reactions are feasible but their acidity is not strong enough to perform acylation of sterically hindered alcohols. In this context, we were intrigued to employ bismuth triflate Bi(OTf)₃^[4] since it had proved to be easy to handle due to its stability in the air and to be acidic enough to catalyze Friedel–Crafts,^[5] Diels–Alder,^[6] and ene reactions.^[7]

As shown in Table 1, Bi(OTf)₃ can promote the acetylation of primary, secondary, and tertiary alcohols in acetic anhydride at 25 °C [Eq. (1)]. For the acetylation of 2-phenylethanol a catalyst loading as low as 0.01 mol % was sufficient to afford the acetate quantitatively within 10 min (entry 1).

Table 1. Bi(OTf)₃-catalyzed acetylation of alcohols [Eq. (1)].^[a]

$\text{R-OH} + \text{Ac}_2\text{O} \xrightarrow{\text{Bi(OTf)}_3} \text{R-OAc} \quad (1)$				
Entry	Alcohol	Mol % of the catalyst	Time [h]	Yield [%] ^[b]
1	2-phenylethanol	0.01	0.167	98
2	2-phenylethanol	0.005	2	99
3 ^[c]	2-phenylethanol	0.5	4	92
4	1-phenylethanol	0.005	2	95
5	2-octanol	0.005	17	98
6 ^[d]	2-octanol	0.005	3	98
7	1-adamantanol	0.01	6	98

[a] Reaction conditions: alcohol (1.0 mmol), acetic anhydride (10 equiv), 25 °C. [b] Determined by GC. [c] Acetic anhydride (1.5 equiv), CH₂Cl₂ (wet, 1.0 mL). [d] At 40 °C.

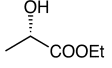
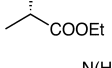
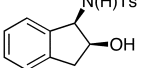
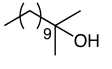
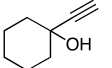
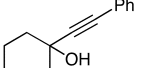
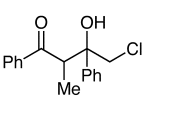
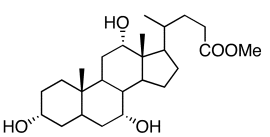
Even with 0.005 mol % of Bi(OTf)₃, this acetylation proceeded quantitatively, but took longer to complete (entry 2). It is possible to employ a solvent different from the anhydride as well. For example, 2-phenylethanol in CH₂Cl₂ was acetylated by 1.5 equivalents of Ac₂O, where CH₂Cl₂ was used without any purification (vide infra and entry 3). Secondary alcohols such as 1-phenylethanol and 2-octanol were also smoothly converted into the corresponding acetates in the presence of 0.005 mol % of Bi(OTf)₃ (entries 4 and 5). Although 2-octanol is less reactive than 1-phenylethanol, quantitative acetylation was possible by using longer reaction times or higher reaction temperatures (entries 5 and 6). Surprisingly, even for the acetylation of 1-adamantanol a catalyst loading of 0.01 mol % was enough (entry 7).

After these preliminary examinations, acetylation of functionalized alcohols was scrutinized at 25 °C (Table 2). Although acid-sensitive geraniol and furfuryl alcohol were acetylated in 81 % and 80 % yields, respectively (entries 1 and 3), further improvements of the yields were attained by adding a donor solvent, such as acetonitrile or THF (entries 2 and 4). Not only primary but also secondary and tertiary alcohols with functional groups or phenol units underwent smooth acetylation (entries 5–15). Alcohols having an ester or amine function did not react more slowly or show decomposition of the functional groups. More importantly, neither racemization nor epimerization was detected (entries 7–9).

Notably, tertiary alcohols can be acetylated at ambient temperatures (entries 10–14). For example, the Bi(OTf)₃-catalyzed acetylation of an α,α -dimethyl-substituted tertiary

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Table 2. Acetylation of functionalized alcohols [Eq. (1)].^[a]

$\text{R-OH} + \text{Ac}_2\text{O} \xrightarrow{\text{Bi(OTf)}_3} \text{R-OAc} \quad (1)$					
Entry	Alcohol	mol % of the catalyst	Conditions		
			cosolvent/vol. [mL]	time [h]	yield [%] ^[b]
1	geraniol	0.01	none	3.5	81
2	geraniol	1.0	MeCN/1	8.5	95
3	furfuryl alcohol	0.05	none	5.5	80
4	furfuryl alcohol	0.05	THF/0.5	4	93
5	borneol	0.5	THF/3	7	99
6	borneol	0.5	toluene/1 ^[c]	3	95
7		0.5	THF/5	2	91
8		0.1	CH ₂ Cl ₂ /5 ^[c]	5	92
9		1.0	none	5	98 ^[d]
10		0.5	THF/5	7	95
11		0.5	MeCN/2	4	94
12		0.5	THF/2	2	81
13		3.0	MeCN/3	1	96 ^[d]
14	linalool	0.5	THF/3	12	80
15	2,4,6-trimethylphenol	0.5	CH ₂ Cl ₂ /1 ^[c]	2.5	92
16		5.0	THF/1	7	100 ^[e]

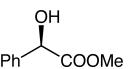
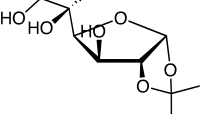
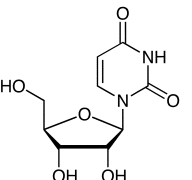
[a] Reaction conditions: alcohol (1.0 mmol), acetic anhydride (10 equiv), 25 °C. [b] Determined by GC. [c] Wet solvent was used. [d] Isolated product. [e] The triacetate was obtained as the sole product.

alcohol proceeded smoothly at 25 °C (entry 10), while the use of Sc(OTf)₃ at room temperature for a similar substrate was reported to lead to the elimination product in 39 % yield.^[2d] Exposure of tertiary propargyl alcohols to Bi(OTf)₃/Ac₂O at 25 °C furnished the desired acetates in 94 % and 81 % yields (entries 11 and 12). On the other hand, acetylation of these substrates under Sc(OTf)₃ or Me₃SiOTf catalysis should be run at lower temperatures (0 to –20 °C) for obtaining good yields.^[2d,e] Furthermore, the present protocol allows the acetylation of a tertiary aldol with a β-chlorine substituent at 25 °C without significant elimination (entry 13). This aldol substrate is sensitive toward acids as well as bases, so the conventional DMAP/Ac₂O method is not applicable. Linalool underwent acetylation at 25 °C to provide linalyl acetate quantitatively without any concomitant formation of the migration product geranyl acetate (entry 14). On the other hand, acetylation of linalool under Sc(OTf)₃ catalysis at –20 °C gave rise to a mixture of the desired acetate (68 %) and geranyl acetate (8 %), and Me₃SiOTf at –10 °C led to a

mixture of the desired acetate and the starting alcohol.^[2d,e] As a further example, a steroidal alcohol was triacetylated efficiently despite the sterically hindered 12α-hydroxy group (entry 16).^[8] Of synthetic use is also the successful employment of wet cosolvents. In essence, the existence of a small amount of water in the solvents (possibly less than ca. 2 %) has no influence on the reactions, yet the increase of the water content retards the reaction. Thus, CH₂Cl₂ directly from the bottle that contains only a small amount of water always gave satisfactory results (entries 8 and 15). THF and CH₃CN can also be used without purification, but reproducible yields were not easily obtained, probably due to a varying amount of water contained.

The Bi(OTf)₃–acid anhydride system was further extended to benzoylation and pivalation [Eq. (2), Table 3]. A primary and an aromatic alcohol could be benzoylated smoothly (entries 1 and 6), but benzoylation of both secondary and tertiary alcohols failed (entries 3 and 5). On the other hand, a series of primary to tertiary alcohols was smoothly transformed into pivalates when exposed to pivalic anhydride in the presence of Bi(OTf)₃ in CH₂Cl₂ (entries 2, 4 and 7–9).

Table 3. Benzoylation^[a] and pivalation^[b] of alcohols with the Bi(OTf)₃–acid anhydride system [Eq (2)].

$\text{R}^1\text{-OH} + (\text{R}^2\text{CO})_2\text{O} \xrightarrow{\text{Bi(OTf)}_3 (3.0 \text{ mol}\%)} \text{R}^1\text{-OCOR}^2 \quad (2)$					
Entry	Alcohol	anhydride/equiv	Conditions		
			time [h]	yield [%] ^[c]	
1 ^[d]	Ph-CH ₂ -CH ₂ -OH	(PhCO) ₂ O/1.5	24	90 ^[e]	
2	Ph-CH ₂ -CH ₂ -OH	(<i>t</i> BuCO) ₂ O/1.5	4	96	
3 ^[f]		(PhCO) ₂ O/1.5	8	2 ^[e]	
4		(<i>t</i> BuCO) ₂ O/3.0	4	99	
5 ^[f]	Ph-CH ₂ -CH(OH)-CH ₃	(PhCO) ₂ O/5.0	48	0	
6 ^[d]	2-naphthol	(PhCO) ₂ O/3.0	1	100 ^[e]	
7	borneol	(<i>t</i> BuCO) ₂ O/1.5	4	97	
8		(<i>t</i> BuCO) ₂ O/1.5	4	97	
9	1-adamantanol	(<i>t</i> BuCO) ₂ O/1.5	4	93	
10		(<i>t</i> BuCO) ₂ O/4.5	7	91 ^[g]	
11		(<i>t</i> BuCO) ₂ O/4.5	23	99 ^[g]	

[a] Benzoylation of the alcohol (1.0 mmol) was done in CH₂Cl₂ (3 mL, wet) at 25 °C. [b] Pivalation of the alcohol (1.0 mmol) was done in CH₂Cl₂ (3 mL, wet) at 25 °C. After the pivalation, methanol (10 mL) was added and the mixture was stirred at 50 °C for 7 h in order to destroy the remaining anhydride. [c] Isolated product. [d] Catalyst concentration: 1.0 mol %. [e] Determined by GC. [f] Catalyst concentration: 5.0 mol %. [g] Isolated tripivalate.

After pivalation, it is often difficult to separate the pivalate esters from the remaining pivalic anhydride by column chromatography because of their similar polarity. In our protocol, methanol was added after consumption of the alcohol in order to convert the remaining anhydride to methyl pivalate. Bi(OTf)₃ effected the methanolysis of the pivalic anhydride quantitatively at 50 °C where methanol as well as CH₂Cl₂ was used without purification. The product pivalate esters could be readily isolated from the crude product mixture by simple filtration through a thin pad of silica gel with hexane as the solvent. The pivalate esters thus obtained were of sufficient purity. Methanolysis of the excess anhydride in the presence of Sc(OTf)₃ or Me₃SiOTf^[9] under the same reaction conditions was not complete. Apparently, Bi(OTf)₃ is superior to the other metal triflates.

The pivalation of a mandelic ester proceeded with complete retention of the configuration (entry 8). Furthermore, this pivalation method was applicable to a sugar having an acetal as protecting group, which can not survive in the Me₃SiOTf protocol (entry 10).^[2e] The acylation of a nucleoside with Bi(OTf)₃/(*t*BuCO)₂O led quantitatively to the triply pivalated nucleoside despite the existence of an amide function (entry 11). In all the trials for pivalation, CH₂Cl₂ could be used directly from the bottle without purification.

In conclusion, a powerful and versatile acylation method has been developed based on Bi(OTf)₃ and acid anhydride. This method has several advantages: the catalyst is cheap and easy to handle, a variety of primary, secondary, and tertiary alcohols can be transformed, and the operations are simple because neither dry reaction conditions nor lowering of the reaction temperature for tertiary alcohols is required. The reactivity of Bi(OTf)₃ can be modified, if necessary, by changing the coordinating character of the cosolvent.

Experimental Section

Representative procedure for pivalation (entry 8, Table 3): A CH₂Cl₂ solution (3 mL, unpurified and wet) of methyl (*R*)-mandelate (166.2 mg, 1.0 mmol) and (*t*BuCO)₂O (299.4 mg, 1.5 mmol) was stirred at 25 °C in the presence of Bi(OTf)₃ (21.8 mg, 3.0 mol %, calculated as the tetrahydrate) for 4 h. MeOH (10 mL, unpurified and wet) was added and the mixture was stirred at 50 °C for 7 h. The mixture was passed through a pad of silica gel with hexane as the solvent and the filtrate was evaporated. Ethyl acetate (30 mL) was added to the crude product, and the organic layer was washed three times with aqueous NaHCO₃ and dried (MgSO₄). Evaporation furnished the pure pivalate ester (97 % yield, 242.8 mg).

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Remarkable *In/out* Inversions at Bridgehead Phosphorus Atoms**

Roger W. Alder* and David Read

Phosphanes are more pyramidal and invert much less readily than amines. The C-P-C angles for trialkylphosphanes are typically about 100° and they have inversion barriers around 150 kJ mol⁻¹,^[1] hence temperatures of well over 100 °C are required for the racemization of chiral phosphanes. In medium-sized bicyclic ring systems^[2] *in/out* isomerism^[3] is possible, and bridgehead nitrogen atoms are known to adopt whichever arrangement is more stable.^[4] Thus 1,4-diazabicyclo[2.2.2]octane is *out,out*, 1,6-diazabicyclo[4.4.4]tetradecane is *in,in*,^[5] 1,5-diazabicyclo[3.3.3]undecane has nearly planar nitrogen atoms,^[6] and 1,9-diazabicyclo[7.3.1]tridecane is *in, out*.^[7] We expected that in related phosphorus compounds *in,out* and *in,in* isomers would become preferred to *out,out* isomers as the ring systems became larger, and also that unstable isomers might be isolated as a result of the high inversion barriers for phosphanes. We find however that these barriers are much lower than expected. We focus here on a

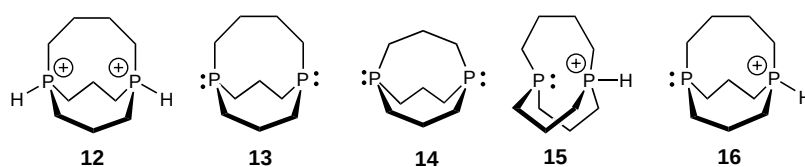
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case where phosphorus atoms not only invert readily, but do so under the control of an unexpected kinetic effect (inversion at the leaving group in a nucleophilic substitution), in which they go from *in,in* to *in,out* via a less-stable *out,out* structure.

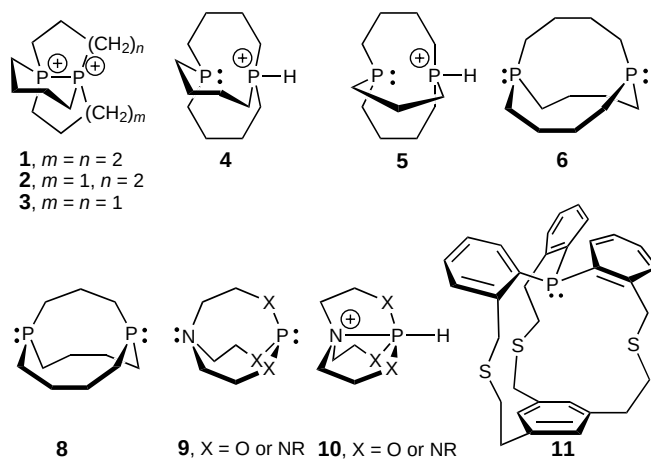
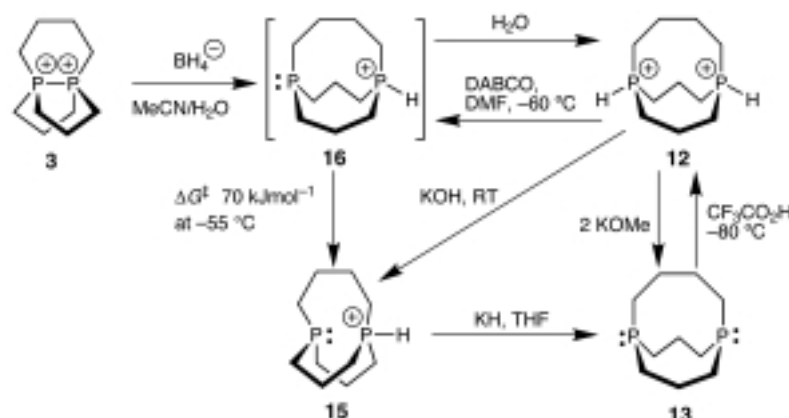
The preparations of salts of dication **1**, **2**, and **3** have been described.^[2] Reactions of **1** and **2** with borohydride lead to **4** and **5**, respectively, which are related to diphosphanes **6** and **8**, but clearly have *in,out* structures with significant P–P bonding. Deprotonation of **4** leads to **7** through an unexpected rearrangement, but deprotonation of **5** with strong base gives *out,out* diphosphane **8**, which is easily re-protonated to **5**,^[2] with inversion at the nonprotonated phosphorus atom at ambient temperature. The protonation of **8** to give **5**, and its strong basicity, are reminiscent of prophosphatranes **9**, which are strong bases that lead to **10**.^[8] Pascal and co-workers have shown that the reaction of **11** with sulfur to give the *out* sulfide involves a rate-limiting inversion of the phosphorus atom, but here the energy barrier (146 kJ mol^{−1}) is relatively normal.^[9]

When **3**(OTf)₂ is treated with less than one equivalent of KBH₄ in wet CH₃CN, the product is **12**(OTf)₂, namely *out,out*-diprotonated **13**—both phosphorus atoms have inverted! The [3.3.3] diphosphane **14**^[2] only forms *out*-mono- and *out,out*-diprotonated ions, and so the formation of **12** might simply reflect a preference for *out,out* structures in the [4.3.3] series too. However, deprotonation of **12**(OTf)₂ with one equivalent of base at room temperature immediately gives the *in,out* monoprotonated salt **15**(OTf), with a coupling constant $J_{\text{PP}} = 253$ Hz observed in the ³¹P NMR spectrum. This salt can be further deprotonated by strong base to **13**, which can also be prepared directly from **12** by treatment with two equivalents of KOMe in CH₃CN. Addition of more than two equivalents of trifluoroacetic acid at −80 °C to **13** leads back to **12** (Scheme 1). Addition of acid to **15**, however, does not give **12**, but gives dication **3** and hydrogen, with the P–H acting as a hydride source.



Scheme 1.

It seems clear that **12** is formed in the borohydride reduction by protonation of *out,out* monoprotonated ion **16** by water (or borate-derived species). Compound **16** must be the kinetic product of the addition of hydride to **3**. When **12** is treated with one equivalent of 1,4-diazabicyclooctane (DABCO) in DMF at −55 °C and the solution monitored by ³¹P NMR spectroscopy, a new species is observed with broad ³¹P



lines at $\delta = -40$ and $+10$ (no P–P coupling in the spectrum, the broadening may be a consequence of slow exchange via **12** or **13**). This new species has a half-life of 2.5 h at −55 °C for conversion into **15**. If this new species is **16**, then $\Delta G^\ddagger$ for inversion to **15** is 70 kJ mol^{−1} at −55 °C, which we believe to be the lowest measured barrier for a trialkylphosphane and is less than half the typical value.

We made strenuous efforts to obtain structural data on **12**, **13**, and **15** without success. B3LYP/6-311G(d,p)//B3LYP/6-31G(d) density functional calculations^[10] predict that **15** is more stable than **16** by 24 kJ mol^{−1}, which is in agreement with our observations. The calculated structures for **12**, **13**, **15**, and **16** are interesting (Table 1). Diphosphane **13** has C–P–C angles which are approximately 7° larger than normal, and the C–P–C angles increase substantially on protonation of **12** (about 5° above a tetrahedral angle), which allows some reduction in the C–C–C angles. The *out,out* isomer **16** appears to be a normal protonated phosphane with a Natural Bond Orbital (NBO)^[11] charge of +0.02 on the hydrogen atom, but has strongly flattened phosphorus atoms. The P(H) atom in the *in,out* isomer **15** has essentially trigonal pyramidal geometry, with the other phosphorus atom and the hydrogen atom as apical substituents. The apical

Table 1. Calculated structural data for **12**–**16**.

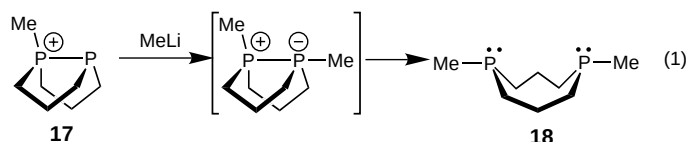
	Energy	P...P [Å]	Average P–H [Å]	Average C–P–C [°]	Average C–P(H)–C [°]
13	–1075.777927	4.39	–	107.1	–
15	–1076.184252	2.55	1.44	113.1	119.4
16	–1076.173587	4.06	1.41	107.9	115.3
12	–1076.442301	3.88	1.40	114.5	–

hydrogen atom shows an NBO charge of -0.10 , which suggests it has hydridic character, and is in agreement with the chemical behavior of these species; thus **12** is also formed by reaction of **3** with **4** in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$.

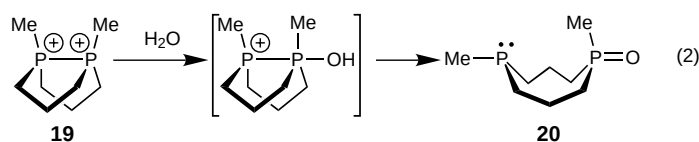
Two aspects of the chemistry involved in these apparently unnecessary journeys from *in,in* to *in,out* via *out,out* (Scheme 1) invite further comment: 1) Why are inversion barriers at these bridgehead phosphanes much lower than normal? 2) Why does the phosphorus atom distal from the borohydride invert in the reaction of **3**?

These bridgehead phosphanes are clearly much flatter than normal (Table 1), and so ground-state destabilization surely contributes to the lowered barriers. We only have actual X-ray structural data for **14**, which shows an average C–P–C angle of 105.7° ,^[2] but the larger angles in the calculated structure for **13** continue the expected trend.

The second question raised above is much more puzzling. Formation of **16** demands kinetically controlled inversion at the phosphorus atom, which is the leaving group in the $\text{S}_\text{N}2(\text{P})$ reaction. There is little evidence concerning inversion or retention at the leaving group in $\text{S}_\text{N}2$ reactions, since stereochemical information can only be preserved if the leaving atom is tricoordinate, and nitrogen atoms invert too easily. A phosphorus leaving group may provide the best possibility of observing such a process, but the only examples we know of come from our own work. Reaction of monocations such as **17** with nucleophiles leads stereoselectively to *cis* products such as **18**,^[12] presumably by a least-motion process [Eq. (1)] that

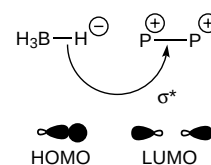


corresponds to retention at the leaving group. Also, attack of water on **19** gives *trans*-monooxide **20** by single inversion [Eq. (2)]. The formation of **4** and **5** from **1** and **2** involves



apparent retention at the leaving group, but we cannot rule out the fleeting formation of *out,out* ions such as **16**, which might invert too rapidly to be trapped by protonation. Even if the reaction of **3** to give **12** is an exceptional case, where the balance of strain on the *in,out* and *out,out* isomers is just right

for observation of inversion at the leaving group, the cause of the non-least motion behavior must still be sought. A tentative suggestion can be based on frontier MO arguments. If electron transfer from the HOMO of the hydride donor to the $\text{P-P } \sigma^*$



orbital (LUMO) is extensive at the transition state, this could be enough to drive the phosphorus atoms apart, and lead to double inversion. This question could benefit from computational studies.

Experimental Section

All operations involving phosphanes were carried out under dry N_2 .

12(OTf)₂: KBH_4 (45 mg; 0.83 mmol) was added to a stirred solution of **3**(OTf)₂ (832 mg; 1.66 mmol) in CH_3CN (15 mL). After one hour, the cloudy solution was filtered and the filtrate evaporated to give a sticky white solid, which was recrystallized from EtOH to give colorless needles (701 mg, 84%), m.p. $163\text{--}165^\circ\text{C}$. ^1H NMR (400 MHz, CD_3NO_2 , 25°C): $\delta = 2.25\text{--}2.44$ (4H, m), $2.50\text{--}2.73$ (4H, m), $2.75\text{--}3.02$ (12H, m), 6.58 (d, $^1J(\text{P,H}) = 519$ Hz, 2H); ^{13}C NMR (100 MHz): $\delta = 11.71$ (t, $^2J(\text{P,C}) = 7$ Hz, C-8, C-11), 12.21 (d, $^1J(\text{P,C}) = 41$ Hz, C-7, C-9, C-10, C-12), 14.70 (d, $^1J(\text{P,C}) = 40$ Hz, C-2, C-5), 15.52 (d, $^2J(\text{P,C}) = 6$ Hz, C-3, C-4); ^{31}P NMR (162 MHz) $\delta = 7.71$ (d, $^1J(\text{P,H}) = 519$ Hz); electrospray-MS: m/z : 501.0154 [$M^- - \text{H}$] ($\text{C}_{12}\text{H}_{22}\text{P}_2\text{S}_2\text{F}_6\text{O}_6$ requires 501.0159). The salt **12**(PF₆)₂ precipitated on addition of NH_4PF_6 (179 mg, 1.11 mmol) to a solution of **12**(OTf)₂ (251 mg; 0.50 mmol) in a minimum volume of water, and was recrystallized from EtOH as colorless plates (143 mg, 58%), m.p. $136\text{--}137^\circ\text{C}$. Elemental analysis calcd for $\text{C}_{10}\text{H}_{22}\text{P}_4\text{F}_{12}$ · $\text{C}_2\text{H}_5\text{OH}$ C 26.68, H 5.22; found: C 26.16, H 4.89.

15(OTf): Methanolic KOH (55 μL , 1.04 M, 0.06 mmol) was added to a stirred solution of **12**(OTf)₂ (29 mg; 0.06 mmol) in CH_3CN (10 mL). After stirring the mixture for 10 min, the solvent was removed, the residue extracted into CH_2Cl_2 (3×10 mL), filtered and evaporated to give a colorless waxy solid (16 mg; 82%). ^1H NMR (400 MHz, CD_2Cl_2 , 25°C): $\delta = 1.65\text{--}2.40$ (18H, m), $2.62\text{--}2.70$ (2H, m), 6.15 (dd, $^1J(\text{P,H}) = 279$ Hz, $^2J(\text{P,H}) = 83$ Hz, 1H); ^{13}C NMR (100 MHz): $\delta = 19.07$ (t, $^2J(\text{P,C}) = 8$ Hz, 2C), 22.58 (d, $^2J(\text{P,C}) = 5$ Hz, 1C), 22.78 (d, $^2J(\text{P,C}) = 6$ Hz, 1C), 25.35 (d, $^1J(\text{P,C}) = 12$ Hz, 1C), 28.34 (dd, $^1J(\text{P,C}) = 82$ Hz, $^2J(\text{P,C}) = 66$ Hz, 2C), 33.81 (d, $^1J(\text{P,C}) = 93$ Hz, 1C), 35.19 (dd, $^1J(\text{P,C}) = 47$ Hz, $^2J(\text{P,C}) = 41$ Hz, 2C); ^{31}P NMR (162 MHz): $\delta = -91.49$ (dd, $^1J(\text{P,P}) = 253$ Hz, $^1J(\text{P,H}) = 279$ Hz), -52.17 (dd, $^1J(\text{P,P}) = 253$ Hz, $^2J(\text{P,H}) = 83$ Hz); electrospray-MS: m/z : 203.1119 [$M^+ - \text{CF}_3\text{SO}_3$] ($\text{C}_{10}\text{H}_{21}\text{P}_2$ requires 203.1119). This compound is stable in the crystalline state, but decomposes slowly in solution to give a complex mixture of products (^{31}P NMR).

13: Methanolic KOCH₃ (0.33 M, 3.38 mL, 1.12 mmol) was added to stirred solution of **12**(OTf)₂ (280 mg, 0.56 mmol) in CH_3CN (5 mL). After stirring the mixture for 1 h, the volatiles were removed. The solid residue was then extracted with pentane (2×10 mL) and filtered through a glass sinter. Removal of the solvent furnished a waxy white solid (92 mg, 81%). ^{13}C NMR (100 MHz; C_6D_6): $\delta = 21.69$ (t, $^2J(\text{P,C}) = 5$ Hz, C-8, C-11), 22.30 (d, $^1J(\text{P,C}) = 28$ Hz, C-2, C-5), 22.59 (d, $^1J(\text{P,C}) = 25$ Hz, C-7, C-9, C-10, C-12), 24.46 (d, $^2J(\text{P,C}) = 5$ Hz, C-3, C-4); ^{31}P NMR (162 MHz): $\delta = -25.24$; CI-MS: m/z : 203.1119 [$M^+ + \text{H}$] ($\text{C}_{10}\text{H}_{21}\text{P}_2$ requires 203.1119). Diphosphane **13** can also be prepared from **15**(OTf) by treatment with KH in THF.

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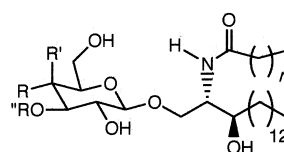
Gp120 Binds Cooperatively to Several Biologically Relevant Glycosphingolipids: Quantitative Measurements at Equilibrium by Total Internal Reflection Fluorescence Microscopy**

John C. Conboy, Katherine D. McReynolds, Jacquelyn Gervay-Hague, and S. Scott Saavedra*

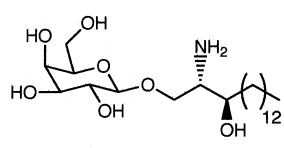
Understanding the molecular events responsible for HIV-1 viral recognition and binding to receptors expressed on the host cell is critical to developing treatment strategies based on HIV-1 inhibition. The virus is encapsulated by a lipid bilayer which supports the envelope glycoprotein gp160. This protein is comprised of two noncovalently linked subunits: 1) a 41-kD

transmembrane protein (gp41) which anchors the assembly in the viral membrane and 2) a 120-kD protein (gp120) which coats the outer surface of the viral particle and is responsible for initial recognition and attachment to the host cell.^[1]

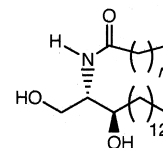
HIV-1 infection of a host cell can be initiated by gp120 binding to galactosyl ceramide (GalCer), a glycosphingolipid (GSL) expressed in human vaginal epithelial, colonic epithelial, and sperm cells.^[2–7] The literature on gp120–GSL binding activity is surprisingly inconsistent. Harouse et al.^[3, 4] and Bhat et al.^[8] used high-performance thin-layer chromatography (HPTLC) and enzyme-linked immunosorbent assays (ELISA) to study the binding of several glycolipids to rgp120 including GalCer (**1**), galactosyl sulfatide (GalS, **2**), glucosyl ceramide (GlcCer, **3**), lactosyl ceramide (LacCer, **4**), psychosine (**5**), and ceramide (Cer, **6**), and found that **1**, **2**, and



R=H, R'=OH, R''=H: GalCer **1**
 R=H, R'=OH, R''=SO₃⁻: GalS **2**
 R=OH, R'=H, R''=H: GlcCer **3**
 R=O-β (1-4)-D-Gal, R'=H, R''=H: LacCer **4**



Psychosine **5**



Ceramide **6**

5 bound. Latov and co-workers used immunospot assays on nitrocellulose and TLC plates as well as ELISA to test the binding affinity of **1** and **2** for recombinant gp120 (rgp120).^[5] They reported that in both the nitrocellulose immunospot assay and ELISA **2** bound to rgp120 but **1** did not. However, in the immunospot TLC assay, both **1** and **2** bound. Long et al.^[9] studied interactions of rgp120 and liposomes doped with various glycolipids (**1–5** and sphingomyelin). They reported that **1** bound to rgp120 strongly, **2–4** were less efficient binders, and **5** was inactive. More recently, McReynolds et al.^[10] found that rgp120 binds to **1**, **3**, and **4**, with **4** being the preferred receptor. In contrast, Hammache et al. have reported minimal binding to **3** and **4**.^[11]

It is evident that considerable confusion exists regarding the relative affinities of gp120 for GalCer and similar lipids. Furthermore, quantitative studies of gp120–glycolipid binding have not been performed, and thus the structural variations among these GSLs that are responsible for the apparent differences in affinity for gp120 are not known. The confusion may be in part a result of the inherent limitations of the binding assays used to study gp120–GSL interactions to date. Employing a heterogeneous solid-phase assay (for example, ELISA) to quantitatively characterize protein ligand–receptor binding processes that occur in vivo at the extracellular surface of a plasma membrane is fraught with difficulties. Although such methods are useful in screening for such interactions, proper geometric presentation of a mem-

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brane-bound receptor at a defined surface coverage in a bioactive conformation is not possible. Furthermore, none of these methods can provide a quantitative measurement of the binding affinity at equilibria, which makes comparisons of binding data obtained in different laboratories problematic.

Total internal reflection fluorescence (TIRF) microscopy is a well-established technique for the quantitative studies of the behavior of proteins at interfaces.^[12–15] Total internal reflection at a planar fused-silica–water interface spatially confines the excitation energy to the near surface region, which minimizes the contribution from dissolved fluorophores (that is, unbound ligands) to the measured fluorescence signal. In combination with the availability of methods to deposit supported phospholipid bilayers on planar fused silica substrates, a diverse array of behaviors at model membrane surfaces can be examined under equilibrium conditions.^[15–19]

In an effort to elucidate the chemical and structural properties important in gp120–GSL interactions, we have measured rgp120 binding to **1**, **3**, **4**, and **6** when incorporated into the upper leaflet of a supported planar bilayer of 2-oleoyl-1-palmitoylphosphatidylcholine (POPC). Representative binding curves are plotted in Figure 1. Glycosylation of ceramide is clearly required for gp120 recognition. There is minimal adsorption of rgp120 to **6**, which lacks the terminal carbohydrate group, and virtually no binding to a pure POPC

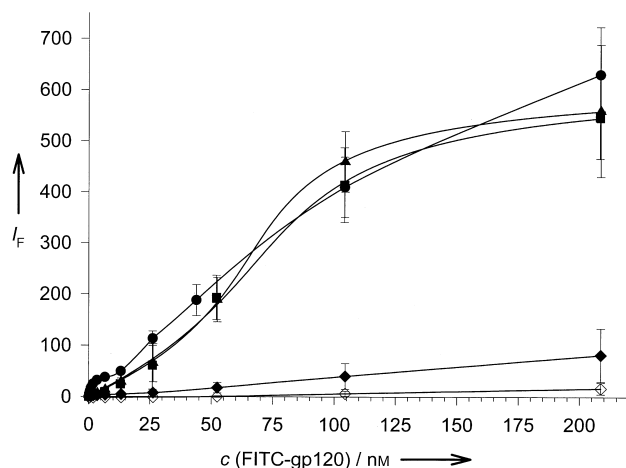


Figure 1. TIRF isotherms of FITC–rgp120 adsorption to POPC membranes, either pure ($\diamond$) or doped with 5% ($\text{mol}\cdot\text{mol}^{-1}$) of GalCer ($\bullet$), GlcCer ($\blacksquare$), LacCer ($\blacktriangle$), or Cer ($\blacklozenge$). The data plotted represent the mean and standard deviation of three independent measurements for each sample. The solid lines through the data of GlcCer and LacCer represent fits obtained with a cooperative Langmuir isotherm of the form $\omega F_{\text{in}} K_a [\text{gp120}] = \frac{F}{1-F}$, where $F = \frac{F}{F_{\text{max}}}$ is the normalized fluorescence intensity, K_a is the association constant, and ω is an additional term to account for cooperativity in the binding process ($\omega > 1$ is cooperative, $\omega = 1$ is noncooperative, $\omega < 1$ is negative cooperativity).^[21, 22] An average number of four protein-bound neighbor receptors was assumed ($n=4$). For GlcCer and LacCer, the fitted parameters are $K_a = 3.6 \pm 0.1 \times 10^6$, $\omega = 2.0 \pm 0.3$, and $K_a = 3.8 \pm 0.3 \times 10^6$, $\omega = 1.8 \pm 0.2$, respectively. For GalCer the sum of two independent Langmuir isotherms of the form $K_a [\text{gp120}] = \frac{F}{1-F}$, where $F = \frac{F}{F_{\text{max}}}$, were used to fit the data. The isotherms were considered independent as a result of a difference of three orders of magnitude in the recovered binding constants. The K_a obtained at high concentrations of gp120 was $7.5 \pm 1.8 \times 10^6$. The solid lines through the data for Cer and POPC are shown only as a guide to the eye.

bilayer. More significantly, the structure of the carbohydrate linked to ceramide apparently has little influence on the binding activity in the high rgp120 concentration range (25–200 nM). The respective affinity constants (K_a) for rgp120 at POPC membranes bearing **3**, **4**, and **1** are 3.8×10^6 , 3.6×10^6 , and 7.5×10^6 , respectively.^[20] These results are contrary to studies that report strong binding to **1** and minimal interaction with **3** and **4**,^[11] but are consistent with the ELISA results obtained by McReynolds et al.^[10]

Considerably different behavior is observed at low concentrations of rgp120 (0.2–20 nM). As shown in Figure 2 **1** is clearly the stronger receptor, with a K_a of 1.6×10^9 , whereas bilayers doped with **3** and **4** show only a small, linear increase in protein adsorption in this concentration regime. The strength of rgp120 binding to **1** at a POPC membrane is comparable to the binding of antibodies to surface-bound antigens ($K_a = 10^5$ – 10^7),^[13, 16] and of avidin and streptavidin to a biotin-bearing lipid bilayer ($K_a = 10^7$ – 10^8).^[21–23]

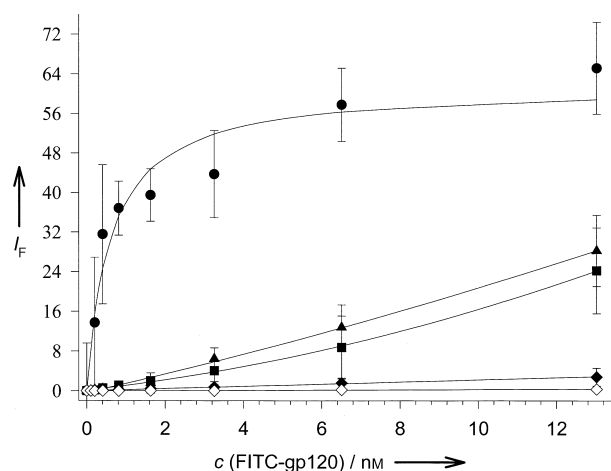


Figure 2. TIRF isotherms of FITC–rgp120 adsorption to POPC membranes, either pure ($\diamond$) or doped with 5% ($\text{mol}\cdot\text{mol}^{-1}$) of GalCer ($\bullet$), GlcCer ($\blacksquare$), LacCer ($\blacktriangle$), or Cer ($\blacklozenge$). The data plotted represent the mean and standard deviation of three independent measurements for each sample. The solid lines through the data of GlcCer and LacCer represent fits obtained with a cooperative Langmuir isotherm (see caption to Figure 1). For GalCer a single Langmuir isotherm of the form $K_a [\text{gp120}] = \frac{F}{1-F}$, where $F = \frac{F}{F_{\text{max}}}$, was used to fit the data. The association constant (K_a) at low concentrations of gp120 (0.2–20 nM) was determined to be $1.6 \pm 0.2 \times 10^9$. The solid lines through the data for Cer and POPC are shown only as a guide to the eye.

Another important difference is the mode of binding. Two models were used in fitting the isotherms plotted in Figures 1 and 2: 1) a Langmuir-like model that includes a cooperative binding process (that is, rgp120 binding promotes the binding of additional protein molecules);^[21, 22] and 2) a conventional Langmuir model (that is, noncooperative, in which all binding sites are equivalent). For **3** and **4**, the data are fitted satisfactorily only when the cooperative model is used, which explains the minimal protein adsorption at low concentrations and the subsequent sharp increase at higher concentrations.

The behavior of **1** is more complex. Strong, Langmuir-like binding occurs in the low concentration regime, whereas a weaker (but also Langmuir-like) binding process is observed

at rgp120 concentrations greater than 20 nM. Comparison of these data with those for **3** and **4** suggests that the initial strong binding of gp120 to GalCer triggers the further adsorption of rgp120 at higher concentrations (namely, the binding of **1** to rgp120 at higher concentrations is cooperative). This proposal would explain the similar behavior observed for all three GSLs at high concentrations of rgp120. In other words, the strong binding event at low protein concentrations differentiates the activity of **3** and **4** from **1**.^[24]

The fact that this complex binding behavior has not been observed previously is likely a consequence of the limitations of the methods that have been used to study gp120–receptor interactions in the past. However, such complex behavior is not unexpected. Previous studies have suggested the occurrence of a conformational change in gp120 upon binding to a receptor, which results in the exposure of a hydrophobic region in the protein,^[25] and possibly stimulating the cooperative adsorption of additional protein. The occurrence of adsorption-induced conformational changes is a probable explanation for the similar binding observed for **1**, **3**, and **4** at high gp120 concentrations. The mechanism of cooperative binding is currently unclear. Specifically, we do not know if, in the high concentration range, additional gp120 molecules bind to glycosphingolipids, or to membrane-bound gp120 molecules, or to a combination thereof. This issue will be the subject of future investigations.

In summary, the results presented here represent the first quantitative measurements of the complex binding process between rgp120 and glycosphingolipid receptors at a membrane surface. At low concentrations of rgp120, GalCer is the preferred receptor, with an affinity constant of about 10^9 , which is at the upper range of measured protein–receptor interactions at membrane surfaces. At higher concentrations, cooperative binding occurs, regardless of the chemical structure of the carbohydrate on Cer. Finally, we emphasize that the results presented here cannot be obtained from ELISA and other nonequilibrium based techniques, although they do complement and further substantiate the results reported by McReynolds et al.^[10]

Experimental Section

POPC and cerebroside were purchased from Matreya Lipids and Sigma and used as received. Rgp120 expressed in baculovirus and labeled with fluorescein isothiocyanate (FITC) was obtained from Intracel Corporation and used without further purification. The FITC:rgp120 labeling ratio was determined to be about 4:1 by UV/Vis and fluorescence spectroscopy.

Phospholipid bilayers composed of pure POPC in the inner leaflet and 19:1 POPC:cerebroside (mol mol^{-1}) in the outer leaflet were deposited on fused silica slides (Dynasil) at a surface pressure of 35 mN m^{-1} ($58 \text{ Å}^2 \text{ molecule}^{-1}$) using the Langmuir–Blodgett–Schaefer technique.^[26] Nano-Pure water with a resistivity of 18 Mohms cm was used as the subphase. All depositions were carried out at 25°C . Substrates were cleaned by sonication in 1:1 isopropanol:water, rinsing in water, soaking in 30% H_2O_2 :70% concentrated H_2SO_4 for 30 min, and rinsing in water.

Supported bilayers were maintained in an aqueous environment during mounting in a micro-TIRF flowcell. The micro cell (volume of approximately $50 \mu\text{L}$) was constructed to minimize the amount of gp120 needed to perform binding studies; details of its construction will be provided in a later publication. The flowcell was mounted on a Nikon Diaphot inverted microscope. FITC–rgp120 was injected into the flowcell and allowed to equilibrate for 30 min prior to each measurement, which was sufficient to

achieve a steady-state response. Fluorescence emission was back-collected through the quartz slide with a 10X objective, optically filtered, and detected with a photomultiplier tube.

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A Mixed-Valence Mixed-Spin Prussian-Blue-Like Heptanuclear Complex**

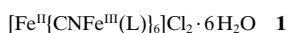
Guillaume Rogez, Arnaud Marvilliers, Eric Rivière, Jean-Paul Audière, Francesc Lloret, François Varret, Antoine Goujon, Nieves Mendenez, Jean-Jacques Girerd, and Talal Mallah*

Cyanide-bridged systems have been at the forefront in the field of molecular magnetism during the last eight years.^[1] Room-temperature molecule-based magnets as well as electro- and photo-induced magnetization in Prussian blue analogues have been reported.^[2–4] In addition, high-spin molecules were prepared by using $[M^{III}(\text{CN})_6]^{3-}$ ($M = \text{Cr}, \text{Fe}$) as building blocks to assemble heptanuclear and pentanuclear complexes with ground spin states up to 27/2.^[5, 6]

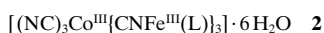
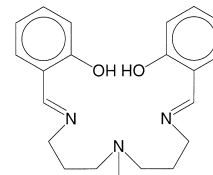
Prussian blue, a three-dimensional network with a cubic structure and the formula $\text{Fe}_4^{III}[\text{Fe}^{II}(\text{CN})_6]_3 \cdot 15 \text{H}_2\text{O}$, has been known for almost three hundred years.^[7, 8] It is well established that the Fe^{II} ions surrounded by the carbon ends of the cyanide bridges are in the low-spin $S = 0$ state while Fe^{III} ions surrounded by the nitrogen atoms of the cyanide groups and by water molecules are in the high-spin $S = 5/2$ state. This compound is famous because of its intense blue color, which arises from an intervalence band centered at 14080 cm^{-1} associated with a charge transfer from the diamagnetic low-spin Fe^{II} ion to the high-spin Fe^{III} ion.^[9] Another, maybe less well known, property of Prussian blue is the occurrence of ferromagnetic ordering below the Curie temperature (T_C) of 5.6 K .^[10] Mayoh and Day proposed a model for the ferromagnetic exchange interaction based on a partial delocalization of the electrons occupying the $\text{Fe}^{II} t_{2g}$ orbitals on to the neighboring high-spin Fe^{III} sites.^[11]

In order to gain more insights into the mechanism responsible for the ferromagnetic interaction we decided to use a molecular approach and prepare a discrete species that may reproduce the optical and the magnetic properties of Prussian blue. Thus, a new mixed-valence mixed-spin hepta-

nuclear complex **1** containing a central low-spin $S = 0$ Fe^{II} ion linked to six high-spin $S = 5/2$ $\text{Fe}^{III}(\text{L})$ species through cyanide bridges was prepared.



H_2L is the pentadentate ligand bis(3-salicylideneaminopropyl)methylamine. The magnetic and the optical properties of **1** are compared to those of the tetranuclear compound **2** having the diamagnetic low-spin $S = 0$ Co^{III} as a central ion surrounded by three $[\text{Fe}^{III}(\text{L})]$ complexes.



The Mössbauer spectrum of **1** at $T = 77 \text{ K}$ shows two absorption peaks with an isomer shift ν of -0.159 and 0.797 mm s^{-1} (Figure 1). The experimental spectrum can be fitted by two absorptions, one at $\nu = -0.195 \text{ mm s}^{-1}$, attributed

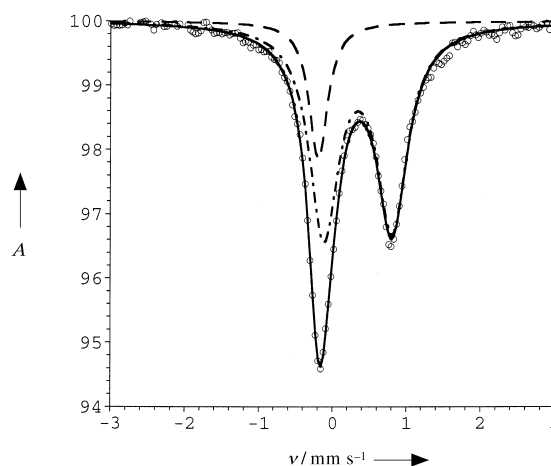


Figure 1. Absorption versus isomeric shift Mössbauer spectrum of **1**, experimental (○), best fit (—), calculated absorption for Fe^{II} (---), calculated absorption for Fe^{III} (— · —).

to low-spin $\text{Fe}^{II}(\text{CN})_6$, and the other centered at $\nu = 0.337 \text{ mm s}^{-1}$ with a quadrupole splitting ΔQ of 0.923 mm s^{-1} , as expected for high-spin Fe^{III} ions in a distorted environment. The analysis of the band intensities leads to a $_{\text{HS}}\text{Fe}^{III}/_{\text{LS}}\text{Fe}^{II}$ ratio of 5.5 which is compatible with the expected value of 6.

The first striking difference between **1** and **2** is their color. Complex **2** has the same color as the mononuclear complex $[\text{Fe}(\text{L})\text{Cl}]$ (dark red) while **1** is dark blue. The electronic spectrum of **2** and that of the mononuclear complex $[\text{Fe}(\text{L})\text{Cl}]$ in the $10000\text{--}32000 \text{ cm}^{-1}$ region (Figure 2) are almost identical. This is not surprising as the substitution of Cl^- ligands by the nitrogen end of $[\text{Co}(\text{CN})_6]^{3-}$ in the coordination sphere of the Fe^{III} ion should not have a dramatic influence on the spectrum. The band centered at 18700 cm^{-1} ($\epsilon(\text{per Fe}^{III}) = 2000 \text{ L mol}^{-1} \text{ cm}^{-1}$) is actually a result of the phenolate $\rightarrow \text{Fe}^{III}$ charge transfer.^[12] In contrast the spectrum of **1** shows the presence of an additional band in the $10000\text{--}20000 \text{ cm}^{-1}$

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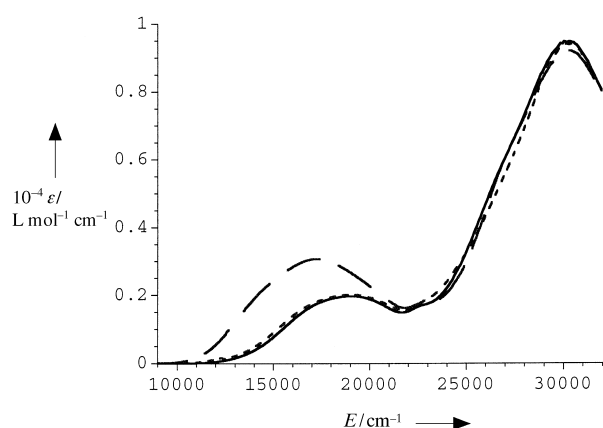


Figure 2. Absorption per Fe^{III} center versus energy in solution (CH_2Cl_2) electronic spectra for $[\text{Fe}(\text{L})\text{Cl}]$ (—), **2** (---), and **1** (- - -).

region (Figure 2). The deconvolution of the spectrum shows that the new band is centered at 14950 cm^{-1} ($\epsilon = 7800\text{ L mol}^{-1}\text{ cm}^{-1}$). This new optical transition can be assigned as the intervalence band associated with the mixed-valence compound **1**.

The investigation of the magnetic properties of compound **2** shows a Curie-law behavior between room temperature and 80 K with a $\chi_{\text{M}}T$ value ($13.62\text{ emu mol}^{-1}\text{ K}$; $1\text{ emu} = 10^{-6}\text{ m}^3$) corresponding to that expected for three uncoupled high-spin Fe^{III} ions. A decrease of $\chi_{\text{M}}T$ is observed below $T = 80\text{ K}$ (Figure 3), which indicates a weak antiferromagnetic ex-

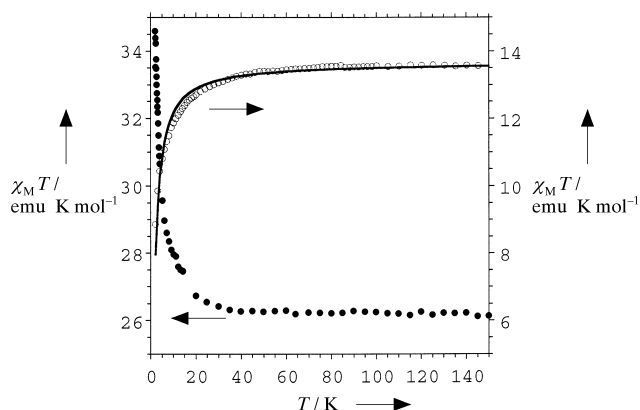


Figure 3. $\chi_{\text{M}}T = f(T)$ curves for **1** (●) and **2** (experimental (○), and best fit (—)).

change coupling between the localized $S = 5/2$ Fe^{III} ions through the diamagnetic Co^{III} central ion.^[13] The magnetic behavior of **1** should be similar to that of **2** because the central Co^{III} and Fe^{II} ions have the same electronic configurations. The experimental magnetic data of **1** again show a Curie behavior between room temperature ($\chi_{\text{M}}T = 26.1\text{ emu mol}^{-1}\text{ K}$) and 50 K. However, below $T = 50\text{ K}$, $\chi_{\text{M}}T$ increases up to $35\text{ emu mol}^{-1}\text{ K}$ at $T = 2\text{ K}$ indicating the occurrence of a weak ferromagnetic interaction instead of the antiferromagnetic one observed in **2** (Figure 3).^[14] Thus, the spin ground state can be evaluated as being larger or equal to 8, if an average g value of 2 is assumed. This behavior can be rationalized by an antiferromagnetic contribution similar to

that of **2**, and of an additional ferromagnetic contribution with larger magnitude. That $\chi_{\text{M}}T$ at low temperature does not reach the expected value for $S = 15$ ($120\text{ emu K mol}^{-1}$) may result from competition between the two contributions that leads to an intermediate spin state.

Following the assumption of Day and Mayoh about the origin of the ferromagnetic interaction in Prussian blue^[11] a comparison of the properties of **1** and **2** implies that the mixed-valence nature of compound **1**, and hence the presence of an intervalence band (absent in **2**), is the origin of this additional ferromagnetic contribution. A simple mechanism based on the interaction of the ground configuration with the excited configuration, generated by electron transfer from the low-spin Fe^{II} ion to one of the high-spin Fe^{III} ions, may account for this additional ferromagnetic contribution, but only if the exchange coupling within the excited configuration is ferromagnetic.

The six degenerate excited configurations resulting from charge transfer $(\text{L}_\text{S}\text{Fe}^{\text{III}})_\text{c}((\text{H}_\text{S}\text{Fe}^{\text{II}})(\text{H}_\text{S}\text{Fe}^{\text{III}})_5)_\text{p}$ (c and p refer to central and peripheral, respectively), which correspond to the transfer of an electron from the central low-spin Fe^{II} ion to one of the peripheral high-spin Fe^{III} ions through the t_{2g} orbitals, can mix with the ground configuration $(\text{L}_\text{S}\text{Fe}^{\text{III}})_\text{c}((\text{H}_\text{S}\text{Fe}^{\text{III}})_6)_\text{p}$. The electron transfer between the central low-spin Fe^{II} center and one of the peripheral high-spin Fe^{III} ions insures a parallel spin alignment between these two ions because the cost in energy is less when the moving electron retains its spin during the transfer process. In the excited configuration $(\text{L}_\text{S}\text{Fe}^{\text{III}})_\text{c}((\text{H}_\text{S}\text{Fe}^{\text{II}})(\text{H}_\text{S}\text{Fe}^{\text{III}})_5)_\text{p}$ the magnetic interaction between the central $\text{L}_\text{S}\text{Fe}^{\text{III}}$ and the peripheral $\text{H}_\text{S}\text{Fe}^{\text{III}}$ ions may be ferro- or antiferromagnetic. If this interaction is ferromagnetic, through mixing with the ground manifold a ferromagnetic state will be stabilized. If the same interaction is antiferromagnetic, an antiferromagnetic spin ground state will appear. Thus, in order to explain the ferromagnetic interaction observed in **1**, the magnetic coupling in the excited charge-transfer configuration between a low-spin Fe^{III} and a high-spin Fe^{III} ion must be ferromagnetic. Floriani et al. have reported that in the two-dimensional compound $[(\text{NET}_4)_2\{\text{Fe}^{\text{III}}(\text{salen})\}_2\{\text{Fe}^{\text{III}}(\text{CN})_6\}_n]$ (salen = N,N' -bis(salicylidene)-ethylenediamine anion) the interaction between low-spin and high-spin Fe^{III} ions is indeed ferromagnetic.^[15] Another example from our own work shows the same result in a tetranuclear compound containing a central low-spin Fe^{III} center and three peripheral high-spin Fe^{III} ions.^[16] Thus, the ferromagnetic exchange interaction observed in **1** is the result of the sum of two contributions: one antiferromagnetic arising from the pure ground-state configuration and the other ferromagnetic arising from the charge-transfer configuration that mixes with the ground-state configuration. The ferromagnetic contribution cannot be present in **2** because the charge transfer configuration $(\text{L}_\text{S}\text{Co}^{\text{IV}})_\text{c}((\text{H}_\text{S}\text{Fe}^{\text{II}})(\text{H}_\text{S}\text{Fe}^{\text{III}})_2)_\text{p}$ is very high in energy and no band associated with such a transfer was observed in the electronic spectrum of **2** below 50000 cm^{-1} .

The mechanism we present suggests that the magnitude of the ferromagnetic contribution is directly linked 1) to the magnitude of the ferromagnetic coupling within the charge transfer configuration, and 2) to the degree of mixing between

the charge-transfer and the ground-state configurations. The degree of mixing depends on the energy and the intensity of the intervalence band. The lower the energy of this band, the smaller the energy gap between the ground-state and the charge-transfer configurations is and thus the better the mixing is. The energy of the intervalence band depends on several parameters, including the stability of the Fe^{II} ion when complexed to the pentadentate ligand. Thus, by changing the nature of the pentadentate ligand we should be able to influence the electron transfer to favor an increase in the degree of mixing between the ground-state and the charge-transfer configurations. This would shift the intervalence band to lower energy. The result should be an enhancement in the magnitude of the ferromagnetic contribution. Recently two Fe^{III} complexes with different pentadentate ligands and the corresponding Prussian blue complexes have been prepared by us. The optical spectra of these compounds confirm our prediction concerning the shifting of the intervalence band (see Supporting Information).

Experimental Section

H₂L was prepared by mixing salicylaldehyde (0.1 mol) with bis(3-amino-propyl)methylamine (0.05 mol) in ethanol (100 mL). Evaporation of the solvent led to a yellow oil, yield 100%. ¹H NMR (200 MHz, CDCl₃, 20 °C, TMS): δ = 1.83 (q, *J* = 6.96 Hz, 4H), 2.20 (s, 3H), 2.41 (t, *J* = 7.08 Hz, 4H), 3.6 (t, *J* = 6.84 Hz, 4H), 6.85 (m, 4H), 7.24 (m, 4H), 8.31 (s, 2H).

[Fe(L)Cl] was obtained by adding a solution of FeCl₃·6H₂O (0.02 mol) in ethanol (50 mL) to a solution of H₂L (0.022 mol) in ethanol (30 mL). Triethylamine (0.044 mol) was added and the solution heated at 60 °C for 20 min. The black precipitate obtained was recrystallized from acetonitrile to give black needles, yield 60%. Elemental analysis calcd (%) for C₂₄H₂₅N₃O₂ClFe: C 56.96, H 5.65, N 9.49, Cl 8.06, Fe 12.51; found: C 56.79, H 5.68, N 9.41, Cl 8.01, Fe 12.60.

Compound **1** was obtained by adding a solution of K₄[Fe(CN)₆]·3H₂O (5 × 10^{−4} mol) in methanol/water (4/1, 40 mL) dropwise to a solution of [Fe(L)Cl] (3 × 10^{−3} mol) in methanol (50 mL). The microcrystalline powder formed was filtered, washed with cold methanol then water, and then dried under vacuum. Elemental analysis calcd (%) for C₁₃₂H₁₆₂N₂₄O₁₈Cl₂Fe₇: C 55.92, H 5.76, N 11.86, Cl 2.50, Fe 13.79; found: C 55.89, H 5.79, N 11.93, Cl 2.50, Fe 13.65. The IR spectrum contains all the bands expected from [Fe(L)Cl] as well as an additional band at 2079 cm^{−1}, which is assigned to the asymmetric elongation of the cyanide bridge. Thermogravimetric analysis confirms a mass loss between 20 and 150 °C corresponding to six water molecules per heptanuclear complex. Decomposition is observed above 190 °C and was confirmed by thermogravimetric studies. The same synthetic procedure was used with K₃[Co(CN)₆] and led to the formation of the neutral tetranuclear complex **2**. IR spectroscopy shows two bands in the cyanide region at 2160 and 2131 cm^{−1} which are assigned to bridging and nonbridging cyanide groups. Elemental analysis calcd (%) for C₆₉H₈₇N₁₅O₁₂Fe₃Co: C 53.62, H 5.68, N 13.60, Fe 10.87, Co 3.82; found: C 53.65, H 5.59, N 13.26, Fe 10.80, Co 3.69.

The Mössbauer samples were thin (~15 mg cm^{−2}); the source was 25 mCi ⁵⁷Co:Rh. The typical instrumental line width is ≈0.215 mms^{−1}. The spectrum were least-square fitted by lorentzian lines (single line + symmetrical doublet) with independent line widths, isomer shift data refer to metallic iron at 295 K. The electronic spectra were measured in CH₂Cl₂ using a Cary 5E spectrophotometer. The concentration of the solutions were [Fe(L)Cl] *c* = 3 × 10^{−4} mol L^{−1}, for **2** *c* = 10^{−4} mol L^{−1}, and for **1** *c* = 5 × 10^{−5} mol L^{−1}.

The magnetic measurements were carried out using a SQUID susceptometer. The susceptibility was performed in the 300–20 K temperature range with an applied field of 5 kOe (1 Oe = 10³ Am^{−1}) and below 20 K with **H** = 30 Oe in order to avoid saturation effects in compound **1** that are observed down to 500 Oe. Magnetization measurements with a different

applied field at a given temperature confirm the absence of ferromagnetic impurities that can be present in iron compounds.

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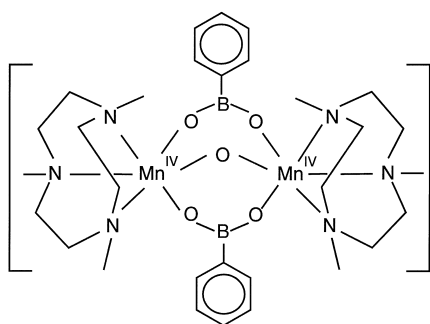
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Integer-Spin Multifrequency EPR Spectroscopy of a Ferromagnetically Coupled, Oxo-Bridged Mn^{IV}Mn^{IV} Model Complex**

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Conventional EPR spectroscopy (X-band: 9 GHz (0.3 cm⁻¹); Q-band: 35 GHz (1.2 cm⁻¹)) has been successfully used to determine the electronic structure of half-integer-spin paramagnets. It has also been applied for the characterization of transition metal ions, with a special focus on metal ion clusters present in biological systems. However, measurements at these frequencies are often insufficient to determine in detail the electronic properties of systems with integer spins or large-spin ground states, especially when the zero field splitting (ZFS) of these systems is larger than or comparable to the microwave quantum. The recent development of high-frequency and high-field EPR (HF-EPR) spectroscopy has allowed the spin Hamiltonian parameters of such compounds to be accurately determined.^[1]

We illustrate here the use of HF-EPR spectroscopy to study the symmetric, dinuclear, oxo-bridged Mn^{IV} complex [L₂Mn₂^{IV}(μ-O)(μ-phBO₂)₂](PF₆)₂ (**1**; L = 1,4,7-trimethyl-1,4,7-triazacyclononane, phBO₂ = phenyl boronic acid).^[2] This complex is particularly interesting as a model for the high oxidation states observed in several manganese-containing metalloproteins like class IV ribonucleotide reductase^[3] and the oxygen-evolving complex (OEC) of photosystem II.^[4] As reported previously,^[2] the two d³ Mn^{IV} ions are in distorted octahedral N₃O₃ environments (Scheme 1; av Mn...N 2.117, av Mn...O 1.824 Å). The metal sites with spins $S_1 = S_2 = \frac{3}{2}$ are ferromagnetically coupled by an exchange interaction, which leads to a rather unusual ground state for the dimer with a



Scheme 1. Schematic representation of the structure of complex **1**.

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total spin of $S=3$. From a measurement of the magnetic susceptibility of **1** at 1 T as a function of temperature, the coupling constant was determined to be $J = +10$ cm⁻¹ ($H_{\text{ex}} = -2JS_1S_2$). The ZFS parameter of Mn^{IV} in this analysis was set to zero.^[2] Variable-temperature variable-field (VTVB) magnetization measurements yield $|D_1| = |D_2| = 0.7 \pm 0.3$ cm⁻¹. The sensitivity of the technique with respect to the local ZFS parameters of the spin-coupled dimer results from the high spin multiplicity of the ground state. This leads to sizable deviations of the magnetization curves at low temperatures from the behavior of a Brillouin function (see Figure 4). The sign of D_i and the rhombicity parameter E/D_i could not be determined from the powder data.

Complex **1** exhibits in both frozen acetonitrile solution and in the solid state integer-spin EPR signals at Q-band and X-band frequencies at low temperature (Figure 1). The

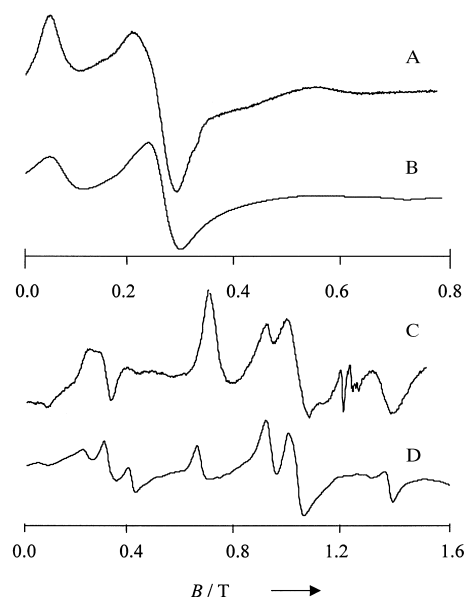


Figure 1. Experimental (A and C) and simulated spectra (B and D) of complex **1** in acetonitrile (0.01 M) recorded at 9.62 (A) and 34.02 GHz (C) at 12 K (parameters are given in the text).

X-band spectra were measured in the perpendicular mode as well as in the parallel mode. The spectra were analyzed by full diagonalization^[5] of the Hamiltonian for the $S=3$ spin ground state of the dimer [Eq. (1)]. The effective ZFS of $S=3$ is represented by the last two terms where $D_{(S=3)}$ and $E_{(S=3)}$ gauge the axial and the rhombic parts of the interaction, respectively.

$$H = \beta \mathbf{B} \mathbf{g} \mathbf{S} + D_{(S=3)} [S_z^2 - 1/3S(S+1) + E/D_{(S=3)} (S_x^2 - S_y^2)] \quad (1)$$

Based on the magnetization data the effective D value can be estimated by spin projection, assuming that the principal axes of the local interactions are colinear.^[6] $D_{(S=3)} = 0.2D_1 + 0.2D_2$. Additionally, the dipolar contributions are neglected in this approach, which is a safe approximation due to the large Mn...Mn separation of 318 pm. For the sake of simplicity, we also did not take into account the contribution of the J anisotropy. With $|D_1| = |D_2| \approx 0.7$ cm⁻¹ a value of

$|D_{(S=3)}| \approx 0.28 \text{ cm}^{-1}$ is obtained. Accordingly, the EPR spectra recorded at 9 and 35 GHz could be simulated reasonably well with effective axial ZFS parameters for the $S=3$ state in the range $0.15 < |D_{(S=3)}| < 0.25 \text{ cm}^{-1}$.

With increasing temperatures up to 150 K no low-frequency EPR signals could be observed from excited states of the coupled spin system. This is unexpected since the $S=2$ state is only about 60 cm^{-1} above the ground state ($\Delta E = 6 \text{ J}$), and the spin projection coefficient for the conversion of the intrinsic ZFS is equal to zero for the quintet. Therefore, a derivative spectrum should be observed around $g=2$. We assume that fast spin relaxation, including transitions between the spin multiplets, sets on as soon as excited multiplets are thermally populated and thus broadens the $S=2$ spectra.

Furthermore, the X- and Q-band spectra of the ground state are very broad and difficult to simulate in the usual spin-Hamiltonian approach, even if distributions of D and E/D are included to account for possible sample inhomogeneities. It might be that weak intermolecular spin couplings in the solid and in the concentrated solutions cause severe distortions of the spectra. Therefore, HF-EPR spectroscopy is essential to accurately analyze the zero-field parameters and the g tensor of this ferromagnetically coupled Mn^{IV} model compound with an $S=3$ spin ground state.

The HF-EPR experiments were performed on polycrystalline powder samples of complex **1**. The spectra were recorded at two different frequencies (190 and 285 GHz) in the temperature range of 5 to 145 K on a home-built spectrometer.^[7] The complete spectra obtained at 285 GHz and with measurement at 10 and 103 K are shown in Figure 2.^[8] With

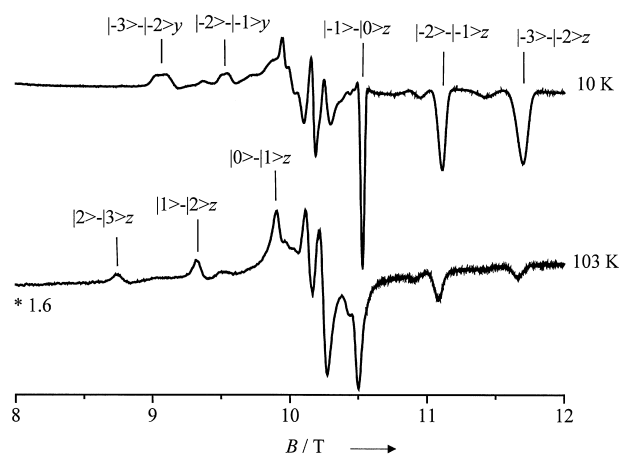


Figure 2. High-frequency EPR spectra (285 GHz) of polycrystalline complex **1** at 10 K (top) and 103 K (bottom). Particular EPR transitions of the $S=3$ multiplet for B_0 along the molecular z and y directions are labeled with the $|M_s\rangle$ quantum numbers of the respective ground and excited states and the magnetic orientation (z : B_0 along z ; y : B_0 along y). The spectrum recorded at 103 K is depicted with an amplification factor of 1.6 for clarity.

increasing temperature, three effects due to Boltzmann statistics are observed as expected: 1) The overall intensity of the spectrum decreases. 2) The relative intensities of the EPR transitions between different Zeeman levels (M_s , M_{s+1}) of the $S=3$ multiplet change. For instance, at 10 K the transition at 11.7 T is stronger than that at 11.1 T; the opposite

behavior is seen at 103 K. 3) Higher M_s levels are populated and, hence, additional transitions are observed.

An important feature of low-temperature HF-EPR spectra is that they directly provide the sign of the ZFS parameter D . This results from the fact that the strong fields of HF-EPR induce Zeeman splittings larger than kT at 4.2 K, which leads to significant differences in the populations of the M_s levels. Under this condition, if $D < 0$, the transitions along the z axis are expected to occur at the low-field side of the resonances centered around $g \approx 2$, while the reverse is true for $D > 0$.^[1e] Here the features associated with the z component of the $S=3$ multiplet are observed at high field at low temperature, which shows us that $D_{(S=3)}$ is positive. The assignment of the six derivative signals related to the expected g_z transitions, as indicated in Figure 2, was achieved from the temperature dependence of the spectra. At low temperature y transitions between the $-3 \rightarrow -2$ and $-2 \rightarrow -1$ levels are also observed at 9.1 and 9.5 T. Qualitative assignments for other transitions are not possible due to the fact that many spectral contributions corresponding to the different orientations overlap.

A first estimate of the D value can be obtained directly from the experimental spectrum recorded at 10 K. At high field limits the difference between the resonance fields of two subsequent z transitions is $2D/g_z\beta$. The corresponding resonances for the $M_s = -3, -2$, and -1 levels of **1** are observed at 11.7, 11.1, and 10.5 T, respectively (Figure 2). This yields a field difference of 0.6 T, corresponding to a $D_{(S=3)}$ value of about 0.28 cm^{-1} .

The HF-EPR spectra of **1** were simulated by using a full-matrix diagonalization procedure for the spin Hamiltonian [Eq. (1)].^[9] Since in this case the calculated transitions are very sensitive to all the parameters (D , E , and g) due to the relative strengths of the zero-field and Zeeman interactions at high field, the values and signs of the ZFS parameters $D_{(S=3)}$ and $E_{(S=3)}$ could be accurately determined, and the different components of the g tensor were obtained. The best simulated spectrum at 50 K is shown in Figure 3. The intensity, the

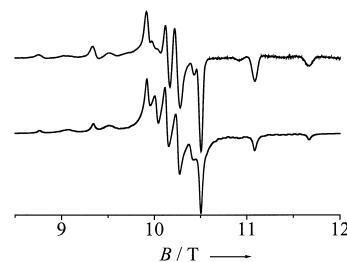


Figure 3. Experimental (top) and simulated powder HF-EPR spectrum (bottom) of polycrystalline complex **1** at 285 GHz and 50 K (parameters are given in the text).

position, and the shape of the simulated spectrum are in good agreement with the experimental data. The spin Hamiltonian parameters used for this simulation are $D_{(S=3)} = 0.27 \pm 0.01 \text{ cm}^{-1}$, $E_{(S=3)} = 0.05 \pm 0.01 \text{ cm}^{-1}$, $g_{x(S=3)} = 1.990 \pm 0.001$, $g_{y(S=3)} = 1.993 \pm 0.001$, and $g_{z(S=3)} = 1.994 \pm 0.001$. The magnitude of the rhombicity parameter $E/D_{(S=3)} = 0.185$ and of the corresponding single ion values reflect the asymmetric quasi-octahedral environment of the Mn^{IV} ion with three short Mn–O and three longer Mn–N bond lengths.

Biomimetic models of metalloenzymes may be helpful in determining the structure of the metal ion site and in elucidating the biological role of the metal cluster. It is also essential to study the models in solution. In an attempt to determine the electronic structure of **1** in solution, EPR measurements were performed at 285 GHz in acetonitrile (0.01M) at 5 K. The experimental data were simulated successfully with the same set of parameters determined above for the solid as well as with the low-frequency EPR data in acetonitrile (Figure 1). However, in solution the resolution is relatively poor due to broadening of the signals. We assume that this is caused by microinhomogeneities in the frozen matrix. The fact that the (average) ZFS parameters are the same in solution and in the solid state reveals identical coordination geometries of the manganese ions in the two states.

The magnetization data of solid **1** could be reevaluated^[10] by using the exact ZFS parameters from the HF-EPR data, which were converted into values for single ions: $D_1 = D_2 = 0.68 \text{ cm}^{-1}$ ($0.2 D_1 + 0.2 D_2 = D_{(S=3)}$), $E_1/D_1 = 0.19$ ($= E/D_{(S=3)}$). An excellent simulation of the experimental VTMB magnetization data and temperature dependence of the molar magnetic susceptibility was achieved with $J = +11.4 \text{ cm}^{-1}$ (Figure 4), if in addition to the explicit ZFS an intermolecular coupling was introduced by means of a mean-field approximation with a Weiss constant of $\Theta = 0.27 \text{ cm}^{-1}$. The effect of this coupling was not observed in the high-field spectra, presumably due to the strong fields which decouple the molecules.

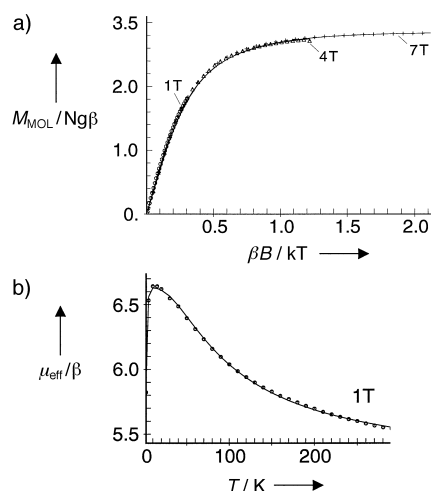


Figure 4. Static magnetization data of polycrystalline complex **1**. The solid lines are derived from a simulation based on a spin Hamiltonian description of exchange interaction, single-ion zero-field and Zeeman interactions (parameters are given in the text). a) M_{MOL} = magnetization per mole, B = applied magnetic field. b) μ_{eff} = effective magnetic moment.

Temperature- and frequency-dependent EPR investigations combined with VTBT magnetization measurements have been successfully applied to characterize the electronic structure of a spin-coupled system. The spin-Hamiltonian parameters of the $S=3$ ground state have been fully determined by simulation of the patterns obtained by HF-EPR on powders and frozen solutions. The ZFS is positive with a moderate value for $D_{(S=3)}$ and an unexpectedly high

value for $E_{(S=3)}$ ($E/D_{(S=3)} = 0.185$), which could not be deciphered from the low-frequency EPR studies. Considering the number of metal ion clusters that are present in enzymatic systems, but which have still not been fully characterized due to a lack of effective methods, the use of new spectroscopic techniques in biology is required. The ability to obtain high-quality spectra in solution makes the use of HF-EPR even more attractive, especially for proteins containing clusters whose characterization by classic EPR is insufficient or ineffective.

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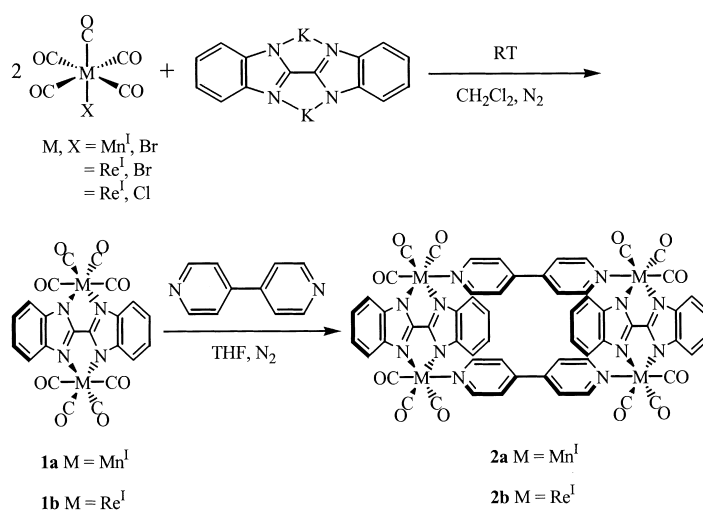
Luminescent Mesoporous Molecular Materials Based on Neutral Tetrametallic Rectangles**

Kurt D. Benkstein, Joseph T. Hupp,* and Charlotte L. Stern

There has been tremendous recent interest in the synthesis of discrete inorganic and organometallic cyclophanes as candidate host species for selective guest binding.^[1–7] We have previously reported on tetrametallic square cyclophanes, which function in the solid state as sensors for volatile organic compounds (VOCs) or as molecular sieves, and on other materials which behave as luminescent sensors for anions in solution.^[8, 9] Reasoning that a rectangular cavity would offer enhanced binding and selectivity for, for example, planar aromatic guest species, we sought to develop useful transition metal based “molecular rectangles”.^[10–12] While the first rectangles we made were either too small to accommodate guests or carried an undesired charge, they did give us insight into a synthetic strategy for the formation of mixed-ligand metallocyclophanes. (The charged compounds are of little value as building blocks for mesoporous materials because counterions block the channels formed by cavities; furthermore, because of their charge, the compounds are slightly water soluble, which precludes their use in aqueous environments.) Using a similar method, we developed a new family of metal-based molecular rectangles, that use a rigid, dianionic bisbenzimidazolate bridge—both to compensate for the cationic metal charge and to provide a cavity wide enough to serve as a host for small, planar, aromatic guest compounds.

The molecular rectangles were synthesized according to Scheme 1. The absence of long-chain oligomers, as opposed to macrocycles, was initially indicated by a) ¹H NMR spectroscopy, which showed only well resolved signals for each of the protons, b) FAB mass spectrometry, which showed no significant peaks at higher *m/z* ratios, and c) its high solubility product in several polar organic solvents (such as THF, CHCl₃, CH₂Cl₂, and acetone, but not in short-chain alcohols, CH₃CN, and hexanes).

Single-crystal X-ray diffraction (XRD) studies of **2a** yielded the ORTEP representation shown in Figure 1.^[13] The dimensions of the cavity, as defined by the Mn atoms, are 11.2 × 5.5 Å. However, the 4,4'-bipyridine (4,4'-bpy) bridges are bowed inward (presumably a packing effect), such that the cavity width at the center is only 3.9 Å. A similar



Scheme 1. Synthesis of bisbenzimidazolate-derived molecular rectangles.

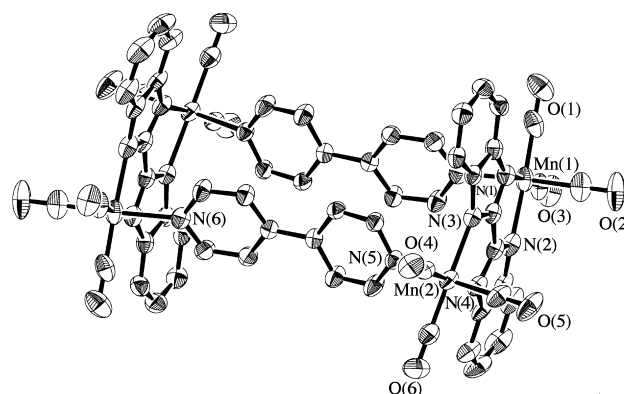


Figure 1. An ORTEP representation of **2a**, that shows a rectangular cavity of dimensions 11.2 × 5.5 Å, as defined by the metal atoms. For clarity, the hydrogen atoms have been omitted. Two THF molecules and a disordered THF/hexane site are also present in the unit cell but are not shown. The thermal ellipsoids represent 50% occupancy.

structure, based on $[(\text{CO})_3\text{Re}]_2(\text{bipyrimidine})^{2+}$ edges, previously yielded a cavity measuring 11.6 × 5.9 Å, without bowing.^[10b] The packing diagram (Figure 2) shows that a) the intramolecular cavity *openings* of all the molecules are similarly aligned and b) *intermolecular* vacancies (roughly rectangular), of nearly the same size (~10 × 6 Å), also exist—effectively doubling the number of sites potentially available for the uptake of guest molecules. From this diagram, both the intra- and the intermolecular effective cavity volumes are estimated to be about 600 Å³.

The UV/Vis absorption spectrum of **2b** shows a band in the high energy region of the visible region, which is absent in **1b**. We ascribe the extra band to an allowed $\text{Re} \leftrightarrow \text{bpy}$ singlet charge transfer. Unlike either **2a** or the bipyrimidine-edged rectangle,^[10b] **2b** luminesces in both the solution (for example, $\lambda_{\text{max}}(\text{THF}) = 617 \text{ nm}$) and the solid state ($\lambda_{\text{max}} = 572 \text{ nm}$). The emission lifetime τ varies with the (deoxygenated) solvent: $\tau(\text{CHCl}_3) = 238 \text{ ns}$ but $\tau(\text{THF}/\text{MeOH}) = \tau(\text{THF}/\text{MeOD}) = 26 \text{ ns}$. The comparatively long lifetimes and substantial emission Stokes shifts suggest the emission occurs from a predominantly triplet charge-transfer state, or states. The emission in solution is rapidly quenched dynamically by

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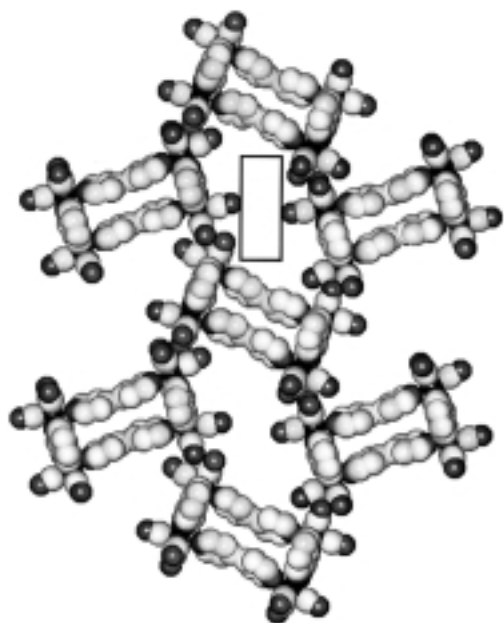


Figure 2. A space-filling model representation of a portion of the unit cell of **2a**, to show intra- and intermolecular (drawn box) cavities. For clarity, the solvent molecules have not been shown.

known reductants, such as hydroquinone and *p*-toluidine, which indicates diffusion-controlled, rather than binding, interactions. (Given the τ values, however, binding with an association constant of less than about 50 M^{-1} would be difficult to detect in this way.) The emission of evaporatively cast, solid, thin films of **2b** is similarly quenched upon exposure to *p*-toluidine vapor but enhanced by approximately 50% and shifted to higher energy upon exposure to THF vapor. The THF exposure also increases τ_{film} from ~ 620 ns to ~ 950 ns. This effect is reversible: After placing a film previously exposed to THF vapor under vacuum for several hours, the luminescent lifetime is again measured to be 620 ns.

Both the film-based emission studies and the crystal structure suggested that, as molecular solids, the rectangles might behave as high internal-surface area, mesoporous host materials. Quantitative assessments of the host behavior of **2a** toward selected VOCs as guests were obtained by quartz crystal microgravimetry (QCM) measurements.^[9, 14] These studies showed the following points. a) Film binding capacity is proportional to film thickness, as expected if guest uptake is based on permeation of the film interior rather than simple adsorption to the film exterior. b) At a sufficiently high aryl (guest) concentration (vapor pressure), the guest:host stoichiometry can exceed unity, with a hypothetical limiting ratio between 1:1 to 2:1 implied by fits to affinity curves (see Figure 3; for simplicity, curves were fit by assuming identical affinity constants for the uptake of the first and second guests). The high stoichiometry would seem to suggest that multiple sites are occupied by guest molecules, namely, both intra- and intermolecular cavities are being filled. c) Host–guest affinity constants can depend significantly on the peripheral composition of the aromatic guest (Table 1). Electron-withdrawing substituents diminish the affinity constant, whereas electron-donating substituents enhance it; this

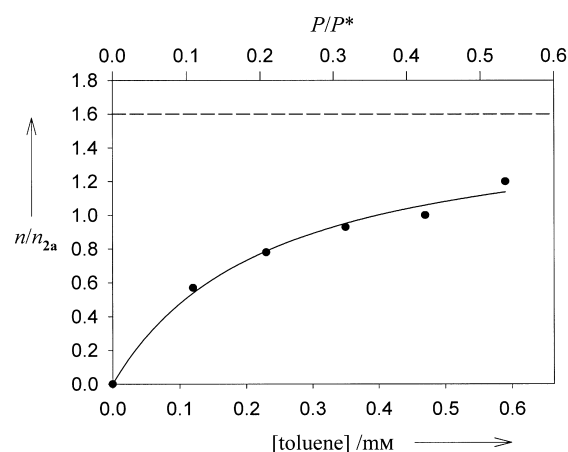


Figure 3. QCM-derived affinity data and best fit (solid line) for toluene uptake by a thin film of **2a**. The films were placed in vacuum for a minimum of eight hours prior to the measurement to remove encapsulated solvent molecules. The y axis shows the molar ratio of adsorbed guest toluene (n) to host **2a** (n_{2a}) in the film. From the fit line, the limiting host to guest ratio is ~ 1.6 (dashed line). For reference, the top axis provides the absolute pressure of toluene (P = pressure of toluene in the chamber; P^* = saturation vapor pressure of toluene at 24°C).

Table 1. Affinities of thin films of **2a** and a representative “molecular square” towards selected VOCs.

VOC	2a [M^{-1}]	$[\text{Re}(\text{CO})_3\text{Cl}(\eta\text{-pyrazine})]_4$ [M^{-1}] ^[a]
toluene	3200 ± 1900	332 ± 26
4-fluorotoluene	1400 ± 360	200 ± 42
benzene	740 ± 250 ^[b]	157 ± 8
fluorobenzene	420 ± 80	87 ± 8
hexafluorobenzene	460 ± 110	47 ± 8

[a] From ref. [9]. [b] Value for **2b** is $580 \pm 220\text{ M}^{-1}$.

could suggest a significant role for electron donor/acceptor (guest/host) interactions in inducing guest uptake. d) Affinity constants for planar aromatic guests are five- to ten-fold greater for the rectangle-based host material **2a** than for a closely related, square-based host material (Table 1). e) Within experimental error, replacement of Mn (**2a**) by Re (**2b**) does not change the affinity constants.

By analogy to N_2 uptake by zeolites and related non-molecular mesoporous materials, the available data for aromatic guest uptake by the solids based on the molecular rectangle can also be used to determine the effective internal surface areas. For example, a BET analysis of the QCM data for benzene uptake by **2a** yields internal surface areas of about $120\text{ m}^2\text{ g}^{-1}$ ($1.6 \times 10^5\text{ m}^2\text{ mol}^{-1}$, $150\text{ m}^2\text{ mL}^{-1}$), in which the area of only one face of the guest molecule has been used. If the probable encapsulating nature of the host–guest interaction is recognized (that is, both faces and portions of the edge of the guest molecule are in contact with the walls of the host cavity), the calculated internal surface area would be roughly doubled.

To conclude, a new family of molecular rectangles has been synthesized and characterized. The new family offers significant advantages over earlier metal-based rectangular host systems including a) overall charge neutrality, which facilitates solid state applications since potentially cavity-filling counterions are absent, b) luminescence, which provides a tag

for sensory applications, and c) the incorporation of Mn into a cyclophane framework, which provides a more economical building block than Re for nonluminescent applications.

Experimental Section

Bimetallic $[(\text{CO})_4\text{M}]_2\text{BiBzIm}$ edges^[15] (BzIm = benzimidazolate) were formed by stirring $[\text{M}(\text{CO})_5\text{X}]$ (M, X = Mn, Br or Re, Cl) with $\text{K}_2[\text{BiBzIm}]$ ^[16, 17] at ambient temperature in CH_2Cl_2 . At the reaction endpoint (monitored by FTIR), the solvent was partially removed and the desired $[(\text{CO})_4\text{M}]_2\text{BiBzIm}$ product precipitated as a yellow (**1a**) or white (**1b**) powder by addition of Et_2O (45 % yield).^[18] Rectangular cycles were obtained by reacting equimolar amounts of the bimetallic edge with 4,4'-bpy in refluxing THF for two days. Hexanes were added to the flask after cooling to room temperature. Further cooling provided orange-yellow crystals (**2a**) or a bright yellow powder (**2b**) at ~70 % yield.^[19, 20] Thin films of **2a** and **2b** were cast evaporatively from filtered THF/ CHCl_3 (1/1) solutions.

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-139036. 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).

The UV/Vis absorption spectra of **1b** and **2b** are found in the Supporting Information.

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- [20] **2b**: ¹H NMR (500 MHz, CDCl_3): $\delta = 8.13$ (d, $J = 6$ Hz, 8H), 7.83 (q, $J = 3$ Hz, 8H), 7.43 (q, $J = 3$ Hz, 8H), 6.77 (d, $J = 6$ Hz, 8H). FTIR (THF): $\tilde{\nu} = 2026$ (sh; CO), 2023, 1921, 1908 cm⁻¹. FAB-MS: calcd: $m/z = 1857.8$; found: 1858.1. Elemental analysis (**2b**·1 THF): calcd: C 39.83, H 2.09, N 8.71; found: C 39.50, H 2.44, N 8.12. Cyclic voltammetry: reversible reductions (versus ferrocene): -1.47 V ($\text{bpy}^{-0}/\text{bpy}^{-1}$), -1.70 V ($\text{bpy}^{-1}/\text{bpy}^{-2}$), -2.28 V, -2.43 V.

Enantioselective Synthesis of 4-Unsubstituted 3-Alkoxy- and 3-Aminoazetidin-2-ones from Formaldehyde *N,N*-Dialkylhydrazones**

Rosario Fernández, Ana Ferrete, José M. Lassaletta,* José M. Llera,* and Angeles Monge

The presence of the azetidin-2-one ring in several widely used families of antibiotics, which include the penicillins and carbapenems, has stimulated considerable activity directed at the stereocontrolled synthesis of β -lactams.^[1] One of the most popular methods is the [2+2] cycloaddition reaction of ketenes to imines (the Staudinger reaction).^[2] The particular

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case of 4-unsubstituted 2-azetidinones merits special attention for its presence in nocardicins and monobactams, as well as in other bioactive compounds such as tabtoxin^[3] and the pachystermine.^[4] In this case, however, the cycloaddition approach requires the use of formaldehyde-derived imines (methanimines), which tend to tri- and oligomerize under the reaction conditions.^[5] Hence, indirect, longer routes to these compounds are required.^[6]

During our recent work on the chemistry of formaldehyde *N,N*-dialkylhydrazones we have regularly observed their high stability as monomers, even at high temperatures and in the presence of bases and some Lewis acids.^[7] This fact and the availability of several hydrazones containing tunable chiral auxiliaries prompted us to explore the usefulness of these compounds, viewed now as electron-rich, *N*-aminosubstituted imines, for the asymmetric synthesis of β -lactams by cycloaddition to ketenes.^[8]

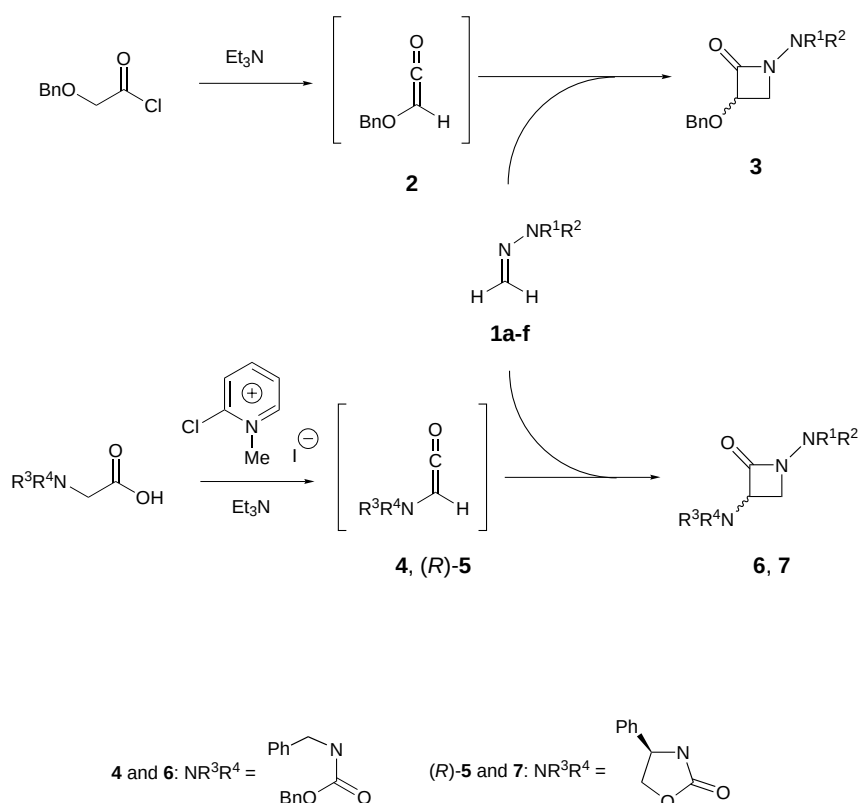
First experiments were performed using achiral hydrazones **1a** and **1b**, and in situ generated benzyloxyketene (**2**; Scheme 1) as model compounds. Reactions carried out in toluene led to the formation of the desired cycloadducts **3a**

and **3b** in 84 and 67% yield, respectively (Table 1, entries 1 and 2), with the first reaction (from **1a**) being a much cleaner reaction. This difference suggests that the higher nucleophilicity of **1b**^[7d,e] results in competing side reactions at its azomethine carbon. In addition, a strong influence of the solvent was recognized: use of chloroform, CH₂Cl₂, THF, or Et₂O resulted in the formation of complex mixtures, where 1-benzyloxy-3-(pyrrolidin-1-ylimino)-propan-2-one or its dimethyl analogue (1,2 adducts to the ketene) were found as major products.

Formaldehyde SAMP hydrazone **1c**^[7b] reacted with ketene **2** in a similar way (entry 3), but the low inductions observed prompted us to investigate the behavior of easily available hydrazones **1d**^[7e] and **1e**,^[7e] which have bigger (quaternary) residues at position 2. The higher steric hindrance in these reagents resulted not only in the expected increase of selectivity in the cycloaddition (d.r. values of 81:19 and 84:16 for **3d** and **3e**, respectively) but, also, in the inhibition of the above-mentioned side reactions, as demonstrated by the almost quantitative yields obtained (entries 4 and 5). The use of hydrazone **1e** had additional advantages: in this case, both

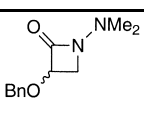
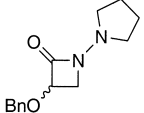
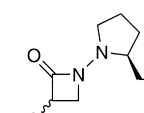
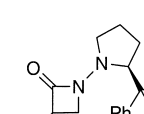
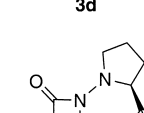
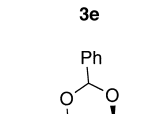
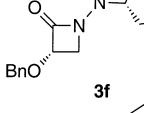
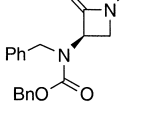
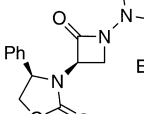
diastereoisomers (*R,S*)- and (*S,S*)-**3e** could be easily separated by flash chromatography, thus affording a satisfactory 80% yield of the major diastereomer (*R,S*)-**3e** in a single step. Finally, the behavior of the C₂-symmetric reagent **1f** was also investigated. The interest for this particular reagent arises from several remarkable singularities. First, a better stereoselectivity could be expected due to the benefits frequently observed for C₂-symmetric auxiliaries.^[9] Second, the presence of two neighboring groups at both sides (C-2 and C-5) of the C=N double bond and the rigidity conferred by the condensed 1,3 dioxane rings should hinder the planar conformation associated with the competing nucleophilic reactivity of the azomethine carbon.^[10] Third, the parent hydrazine can be prepared in bulk quantities from inexpensive D-mannitol in a few steps.^[11] A single isomer **3f** was detected in this case and isolated in 89% yield (entry 6). As expected considering the relative stereochemistry of **1e** and **1f**, **3f** had *S* configuration at C-3, the opposite configuration to the major isomer of **3e**.

The cycloaddition of hydrazones **1e** and **1f** to α -amino-



Scheme 1. [2+2] Cycloaddition reaction of formaldehyde *N,N*-dialkylhydrazones **1** to alkoxy and aminoketenes **2**, **4**, and (*R*)-**5**. Bn = Benzyl.

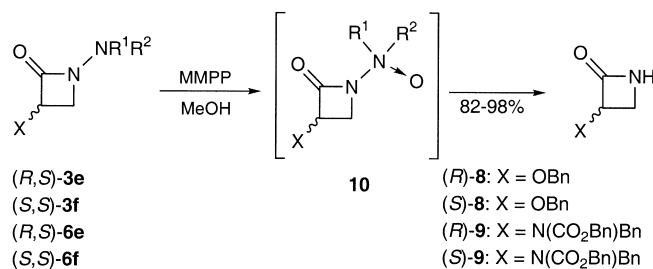
Table 1. [2+2] Cycloaddition of formaldehyde *N,N*-dialkylhydrazones with ketenes. Synthesis of 1-amino-3-alkoxyazetidin-2-ones and 1,3-diaminoazetidin-2-ones.

Entry	Ketene	Hydrazone	Cycloadduct	<i>T</i> ^[a]	<i>t</i> ^[b]	d.r. ^[c]	Configuration at C-3 ^[d]	Yield [%] ^[e]
1	2	1a	 3a	RT	48	–	–	84
2	2	1b	 3b	RT	26	–	–	57
				50	2.5	–	–	67
3	2	1c	 3c	RT	50	50:50 ^[f]	<i>R/S</i>	80
				80	2.5	50:50 ^[f]	<i>R/S</i>	89
4	2	1d	 3d	RT	6 d	85:15 ^[f]	<i>R</i>	85
				80	3.5	81:19 ^[f]		96
5	2	1e	 3e	RT	24	85:15	<i>R</i>	89 (75)
				80	1.5	84:16		96 (80)
6	2	1f	 3f	80	7	> 99:1	<i>S</i>	89
7	4	1e	 6e	80	23	82:18	<i>R</i>	93 (76)
8	(<i>R</i>)-5	1e	 7e	80	9	> 99:1	<i>R</i>	94
9	4	1f	 6f	60	8	> 99:1	<i>S</i>	81

[a] Units = °C. RT = Room temperature. [b] Units = hours unless otherwise stated. [c] Determined by ¹³C and ¹H NMR spectroscopy. [d] Configuration of the major isomer. [e] Yield after isolation by flash chromatography. Values in parentheses correspond to the pure (*de* = 98 %) major isomer. [f] Inseparable mixture of diastereomers.

ketenes **4** and (*R*)-**5** was also investigated. In this case, the best results were obtained by using suitable α -amino acid derivatives and 2-chloro-*N*-methyl pyridinium iodide^[12] for the generation of the ketene. Under these conditions, adduct **6e** was obtained in 93% yield as a 82:18 mixture of diastereomers (entry 7), which could also be separated by means of simple flash chromatography to yield adducts (*R,S*)- and (*S,S*)-**6e** in optically pure form. As expected, a “matched” double induction experiment using (*R*)-**5**^[13] afforded the corresponding adduct **7e** as a single diastereoisomer (entry 8). In addition, compound **6f**, which has the opposite *S* configuration at C-3, was also obtained in good yield as a single diastereoisomer from the reaction of **1f** with **4** (entry 9).

The key N–N bond cleavage needed for the release of the chiral auxiliaries of adducts **3** and **6** was unsuccessful by catalytic hydrogenolysis (Raney Ni, Pd/C, and Pd(OH)₂/C) or by Li/NH₃ under several conditions, while use of SmI₂^[14] required the presence of HMPA as cosolvent and gave moderate yields of product (**8**: 64–65% from **3e,f**; **9**: 42% from **6e**). Inspired in the Meisenheimer rearrangement^[15] of tertiary amine oxides, we decided to investigate the oxidative N–N bond cleavage promoted by magnesium monoperoxyphthalate (MMPP). This unprecedented deamination method efficiently afforded the desired products (82–89% yield; Scheme 2, Table 2), which are presumably formed by the homolytic cleavage of the N–N bond of intermediate hydrazide N(*sp*³) oxides **10**.^[16]



Scheme 2. MMPP-promoted oxidative N–N bond cleavage of compounds **3** and **6** to form compounds **8** and **9**.

Table 2. Oxidative N–N bond cleavage of adducts **3** and **6** promoted by MMPP. Synthesis of compounds **8** and **9**.

Adduct	X ^[a]	Product	Time [h]	Yield [%]	[α] _D ^[b]
(<i>R,S</i>)- 3e	OBn	(<i>R</i>)- 8	1	89	+54.9
3f	OBn	(<i>S</i>)- 8	6	82	–55.9
(<i>R,S</i>)- 6e	N(CO ₂ Bn)Bn	(<i>R</i>)- 9	1	83	+8.0
6f	N(CO ₂ Bn)Bn	(<i>S</i>)- 9	6	83	–8.8

[a] See Scheme 2. [b] (*c* = 1, CH₂Cl₂).

The absolute configuration of (*R,S*)-**3e** was determined by X-ray diffraction analysis^[17] (Figure 1) of the *p*-nitrobenzoyl derivative **12**, obtained from the former after hydrogenolysis ($\rightarrow$ **11**) and esterification (Scheme 3). The opposite 3*S* configuration of **3f** was determined by comparison of the optical rotation of (*R*)-**8** ([α]_D²⁰ = +54.9 (*c* = 1, CH₂Cl₂)) and (*S*)-**8** ([α]_D²⁰ = –55.9 (*c* = 0.9, CH₂Cl₂)), obtained from (*R,S*)-**3e** and **3f**, respectively. Finally, (*S*)-**9** ([α]_D²⁰ = –8.8 (*c* = 1, CH₂Cl₂)) was transformed into (*S*)-**13** (Scheme 4) and its optical

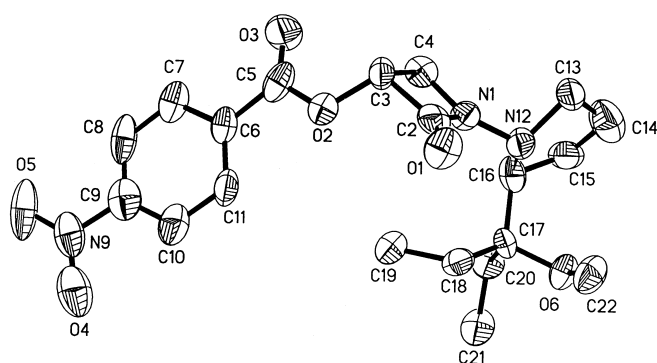
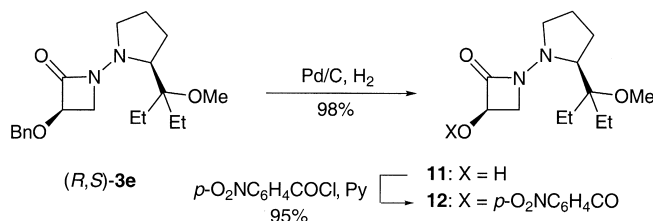
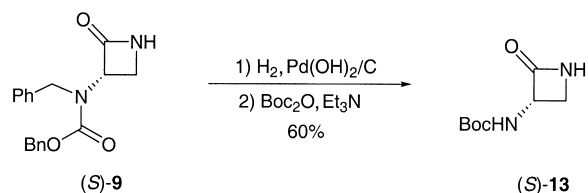


Figure 1. Crystal structure of (*R,S*)-**12**.



Scheme 3. Synthesis of (*R,S*)-**12**. Py = pyridine.



Scheme 4. Synthesis of (*S*)-**13**. Boc = *tert*-butoxycarbonyl.

rotation was compared to the literature data;^[18] (*R*)-**9** had the opposite sign ([α]_D²⁰ = +8.0 (*c* = 1, CH₂Cl₂)).

In summary, a new entry to enantiopure 4-unsubstituted 3-alkoxy and 3-amino β -lactams has been developed which consists of two efficient steps: (1) the [2+2] cycloaddition reaction of chiral formaldehyde *N,N*-dialkylhydrazones to alkoxy or aminoketenes, and (2) the MMPP-promoted oxidative N–N bond cleavage of the resulting hydrazides. Both *R*- and *S*-configured products can be easily synthesized starting from L-proline or D-mannitol derived hydrazones **1e** and **1f**, respectively. It is important to emphasize that the chiral auxiliaries act as N-protecting groups at the same time, thereby reducing the number of steps required to reach the final targets.

Experimental Section

Adducts **3**: Benzyloxyacetyl chloride (2 mmol) in toluene (5 mL) was added to a solution of hydrazone **1** (1 mmol) and Et₃N (4 mmol) in dry toluene (10 mL). The mixture was stirred at 80 °C until completion. Adducts **6** and **7**: 2-Chloro-*N*-methyl pyridinium iodide (0.82–1.65 mmol) and Et₃N (1.5–4.5 mmol) were added to a solution of the glycine derivative (0.75 or 1.5 mmol) in toluene (4.5 mL). **1** (0.3 mmol) was then added and the mixture was stirred at 80 °C until completion.

Compounds **8** and **9**: MMPP · 6 H₂O (0.9–1.8 mmol) was added to a solution of **3** or **6** (0.3 mmol) in MeOH (0.5–0.75 mL) and the mixture was stirred at RT until completion.

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(excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-135522. 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 Highly Conductive Macrocycle-Linked Metallophthalocyanine Polymer**

Richard P. Kingsborough and Timothy M. Swager*

Phthalocyanines are among the most extensively investigated chemical species because of their uses in chemical sensors,^[1] low-dimensional conductors,^[2–6] nonlinear optics,^[7] liquid crystals,^[8–12] as well as their utility as catalysts and dyes.^[13] Clearly, these properties are derived from the exceptional stability and delocalized electronic nature of the macrocycle. The vast majority of metallophthalocyanine-containing polymers are composed of axially connected phthalocyanines that possess conductivities (σ) of 10⁻³–10⁻¹ S cm⁻¹ when oxidatively doped.^[5, 14–17] These polymers, however, cannot be exploited for catalytic applications because of the lack of vacant coordination sites. Previous work directed toward the preparation of one- and two-dimensional and macrocycle-linked phthalocyanine polymers resulted in moderately conductive (σ = 10⁻⁸–10⁻² S cm⁻¹), intractable materials that have not been studied electrochemically.^[18–21] Electrochemical polymerization of designed phthalocyanine macrocycles offers a number of advantages over existing systems, including the deposition of thin electroactive films wherein the electronic states of the macrocycle π system mix with those of the polymer backbone. After considering all the desirable attributes that phthalocyanine ligands offer we chose them as ideal moieties from which to design electroactive metal-containing polymers.^[22–26] Herein we report the synthesis of a novel highly electroactive polythiophene–metallophthalocyanine hybrid material that exhibits conductivities more than three orders of magnitude higher than previously synthesized macrocycle-connected polymers.

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Our synthetic procedures were designed to favor the preparation of macrocycles with electrochemically polymerizable thiophene moieties on alternating subunits. This arrangement should give nearly linear polymer backbones with the metal center in direct electronic communication with the conjugated polymer backbone (Scheme 1). Hoffman and co-workers have recently developed methodologies to preferentially prepare *trans*-[M{pz(A₂;B₂)}] (where pz(A;B) denotes the porphyrazines contain two differently substituted heterocyclic rings) porphyrazines through the use of steric bulk on one of the condensation components.^[27] We have further endowed our macrocycle polymers with rigid, three-dimensional scaffolds to prevent the cofacial aggregation of the macrocycles which would otherwise limit monomer solubility and the accessibility of ligands to the metal centers in the polymer. For this scaffold we utilize iptycene groups, which have previously been demonstrated to create porous highly soluble materials.^[28, 29]

The synthesis of the iptycene-containing phthalocyanine monomers is shown in Scheme 1. Quinone **1** was converted into **2** by reaction with bromine in chloroform. Reduction of the quinone **2** to the hydroquinone **3** was carried out using Na₂S₂O₄ in a biphasic Et₂O/H₂O solvent system. Alkylation of the hydroquinone **3** afforded **4** in high yield. Cyanation of **4** with CuCN in DMF yielded the dicyano derivative **5** in moderate yields. Conversion into the diiminoisoindoline **6** was carried out by bubbling ammonia through a suspension of **5** in ethylene glycol at 140 °C with a catalytic amount of Na for 5 h. Compound **6** (A) was then cyclized then with 4-iodophthalonitrile^[30] (B) in a refluxing Mg(OBu)₂ suspension in BuOH. After trifluoroacetic acid treatment, the free bases of the three isomers, 2H[pz(A₃;B)], *cis*-2H[pz(A₂;B₂)], and *trans*-2H[pz(A₂;B₂)] were obtained. Consistent with our synthetic design the alternating mixed-ring phthalocyanines (precursors to **9-Ni**) were the major products. Numerous attempts to separate these isomers failed. Subsequently, the crude mixture was metalated with [Ni(acac)₂] in refluxing THF. The crude metallophthalocyanine mixture was then treated with 2-tributylstannyl-3,4-ethylenedioxythiophene in DMF at 60 °C in

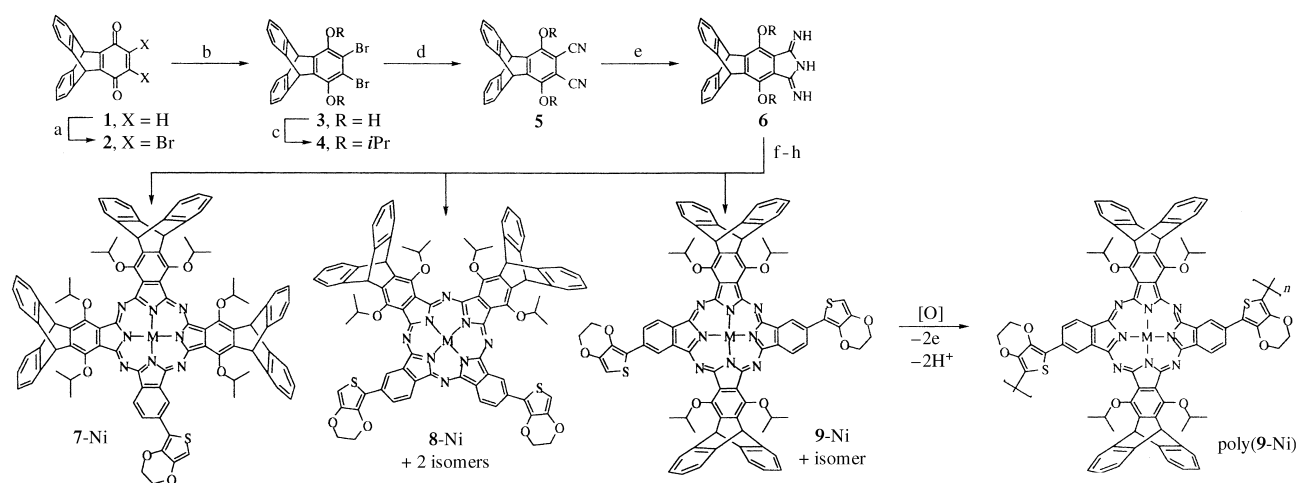
the presence of a catalytic amount of [PdCl₂(PPh₃)₂]. Separation of the three isomers **7-Ni**, **8-Ni**, and **9-Ni** (see Table 1) was easily accomplished by flash chromatography. Compound **7-Ni** with a single thiophene group is incapable of forming polymers and was not studied further. Corey–Pauling–Koltun (CPK) space-filling models indicate that the iptycene architecture should create a pocket at the metal center in all the metal containing species.

The electrochemical characteristics of both the *cis* and *trans* isomers and their polymers were investigated. Electropoly-

Table 1. Spectroscopic and analytical data for selected compounds.^[a]

7-Ni: ¹H NMR (500 MHz, CDCl₃): δ = 9.64 (s, 2H), 9.26 (d, <i>J</i> = 8 Hz, 2H), 8.45 (d, <i>J</i> = 7.5 Hz, 2H), 7.61 (dd, <i>J</i> = 5.25, 3 Hz, 4H), 7.08 (dd, <i>J</i> = 5.75, 2.5 Hz, 4H), 6.51 (s, 2H), 6.45 (s, 4H), 5.83 (sept, <i>J</i> = 6 Hz, 4H), 4.55–4.54 (m, 4H), 4.43–4.42 (m, 4H), 1.79 (d, <i>J</i> = 5.5 Hz, 6H), 1.78 (d, <i>J</i> = 5.5 Hz, 6H), 1.76 (d, <i>J</i> = 5.5 Hz, 6H), 1.76 (d, <i>J</i> = 5.5 Hz, 6H); ¹³C NMR (125 MHz, CDCl₃): δ = 147.0, 146.9, 146.8, 146.5, 146.4, 145.9, 145.55, 145.5, 145.46, 143.6, 143.54, 143.52, 143.4, 143.2, 140.0, 138.7, 135.8, 135.5, 129.3, 129.0, 128.9, 128.8, 127.9, 127.0, 126.98, 125.0, 123.1, 119.8, 118.6, 99.9, 77.9, 65.7, 65.3, 49.8, 30.4, 24.4, 24.3; UV/Vis (CH₂Cl₂) λ_{max} [nm] (lgε) = 319 (4.71), 730 (4.94); MS: <i>m/z</i>: 1588 ([<i>M</i>+H]⁺); HR-MS (FAB): calcd for C₉₈H₈₁N₈NiO₈S: 1587.5252 ([<i>M</i>+H]⁺); found: 1587.5288 ([<i>M</i>+H]⁺) 8-Ni: ¹H NMR (300 MHz, CDCl₃): δ = 9.62 (s, 1H), 9.51 (s, 1H), 9.30–9.05 (m, 2H), 8.42–8.16 (m, 2H), 7.68–7.61 (m, 4H), 7.13–7.08 (m, 4H), 6.49–6.45 (m, 6H), 5.90–5.77 (m, 2H), 5.43–5.35 (m, 2H), 4.58–4.32 (m, 8H), 1.82–1.79 (m, 6H), 1.70 (d, <i>J</i> = 5.7 Hz, 6H), 1.50–1.45 (m, 12H); suitable ¹³C NMR spectra could not be obtained; UV/Vis (CH₂Cl₂) λ_{max} [nm] (lgε) = 318 (4.42), 718 (4.73); MS: <i>m/z</i>: 1436 ([<i>M</i>+H]⁺); HR-MS (FAB): calcd for C₈₄H₆₅N₈NiO₈S₂: 1435.3720 ([<i>M</i>+H]⁺); found: 1435.3673 ([<i>M</i>+H]⁺) 9-Ni: ¹H NMR (500 MHz, CDCl₃): δ = 9.61 (s, 1H), 9.26 (d, <i>J</i> = 7.5 Hz, 1H), 9.50 (dd, <i>J</i> = 8, 1.5 Hz, 1H), 7.65 (dd, <i>J</i> = 5.25, 3 Hz, 4H), 7.63–7.60 (m, 8H), 7.11 (dd, <i>J</i> = 5.25, 3 Hz, 4H), 7.10–7.07 (m, 8H), 6.52 (s, 1H), 6.46 (s, 2H), 6.44 (s, 2H), 6.42 (s, 2H), 5.85 (sept, <i>J</i> = 6.5 Hz, 1H), 5.78 (sept, <i>J</i> = 6.5 Hz, 1H), 5.32 (spt, <i>J</i> = 6.5 Hz, 2H), 5.14 (sept, <i>J</i> = 6.5 Hz, 2H), 4.56–4.54 (m, 2H), 4.42–4.41 (m, 2H), 1.81 (d, <i>J</i> = 6 Hz, 6H), 1.78, (d, <i>J</i> = 6 Hz, 6H), 1.46 (d, <i>J</i> = 6 Hz, 12H), 1.40 (d, <i>J</i> = 6 Hz, 12H); ¹³C NMR (125 MHz, CDCl₃): δ = 145.89, 145.86, 145.80, 145.6, 145.55, 145.50, 142.7, 139.4, 134.6, 129.2, 128.2, 128.1, 125.5, 125.4, 124.5, 124.46, 124.4, 77.4, 65.2, 64.8, 49.3, 49.1, 24.0, 23.9, 23.3, 23.2; UV/Vis (CH₂Cl₂) λ_{max} [nm] (lgε) = 325 (4.65), 715 (5.05); MS: <i>m/z</i>: 1436 ([<i>M</i>+H]⁺); HR-MS (FAB): calcd for C₈₄H₆₅N₈NiO₈S₂: 1435.3720 ([<i>M</i>+H]⁺); found: 1435.3688 ([<i>M</i>+H]⁺)

[a] For full experimental details and characterization for the compounds reported see Supporting Information (7 pages).



Scheme 1. a) Br₂ (2 equiv), CHCl₃, RT, 3 d (87%); b) Na₂S₂O₄ (4 equiv), 1:1 Et₂O:H₂O, RT, 24 h (90%); c) K₂CO₃ (5 equiv), (CH₃)₂CHBr (8 equiv), DMF, 60 °C, 3 d (93%); d) CuCN (6 equiv) DMF, 140 °C, 2 d (71%); e) cat. Na, NH₃(g), ethylene glycol, 140 °C, 5 h (87%); f) Mg(OBu)₂, BuOH, reflux, 12 h then 4-iodophthalonitrile, 1 d; g) [Ni(acac)₂], THF, reflux, 12 h; h) [Bu₃SnEDOT], [PdCl₂(PPh₃)₂], DMF, 60 °C, 12 h.

merization of **9**-Ni on 2 μm -interdigitated microelectrodes^[31–34] resulted in the deposition of dark green films of poly(**9**-Ni). The polymerization proceeds by oxidative coupling of the pendant thiophenes (Scheme 1), there is extensive literature precedent for this process,^[2] and we find that related macrocycles without pendant thiophenes do not oxidatively polymerize. The cyclic voltammogram of this polymer in fresh (monomer free) electrolyte on the interdigitated microelectrodes (Pt button electrodes give identical results) is shown in Figure 1.^[36]

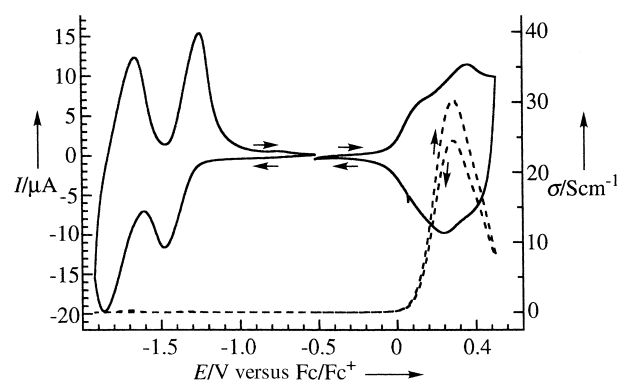


Figure 1. Cyclic voltammograms (solid lines) and conductivity profiles (dashed lines) of poly(**9**-Ni) in 0.1M Bu₄NPF₆/CH₂Cl₂ on 2 μm -interdigitated microelectrodes. Sweep rates are 100 mV s⁻¹ for cyclic voltammograms and 5 mV s⁻¹ with a 40 mV offset potential for conductivity measurement.

The cyclic voltammogram of poly(**9**-Ni) displays prominent features in the reductive region at -1.35 and -1.75 V (Figure 1). Based upon comparisons of cyclic voltammograms with those of the *cis* derivative **8**-Ni, we assign the first one-electron wave to a Ni⁺/Ni²⁺ redox process and the second to a ligand-centered process. The oxidative region of the cyclic voltammogram of poly(**9**-Ni) consists of a single broad redox wave centered at 0.3 V with a leading shoulder at 0.1 V. The conductivity trace obtained *in situ*^[35] (Figure 1) shows significant conduction associated only with the polymer backbone and not the metal-centered and ligand-centered reductive processes. This later fact is consistent with our previous studies that demonstrate the need for redox-matching of the polymer with square planar Co species to produce high levels of metal centered electroactivity and redox conductivity.^[22a] The maximum conductivity is observed at the half-wave potential of the polymer film ($\sigma_{\text{max}} = 30 \text{ Scm}^{-1}$) and decreases rapidly at higher applied potentials.

Complex **8**-Ni did not afford films when subjected to the standard electrochemical polymerization conditions. During this process, oxidation of the ethylenedioxythiophene, EDOT, moieties is observed along with a ligand-based oxidation. However, the expected reduction waves associated with an immobilized polymer film were not detected, perhaps because of the higher solubility of the polymer. Cyclic voltammograms of the monomeric species in solution closely resemble those of **9**-Ni. Two metal- and ligand-based reductive processes are observed, with the first corresponding almost exactly to that

of **9**-Ni, which allows (see above) for the assignment as the Ni⁺/Ni²⁺ redox process.

We have prepared a highly conductive phthalocyanine-containing hybrid polymer. This polymer shows both metal-centered electroactivity as well as high conductivity. The Ni⁺/Ni²⁺ redox wave does not contribute to the conductivity.

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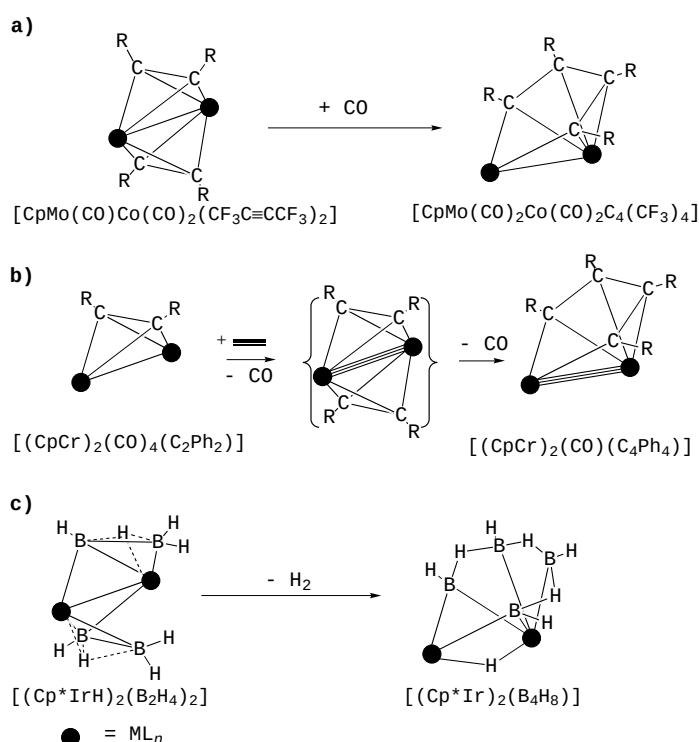
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 [36] Unless noted all potentials are referenced to the Fc/Fc⁺ redox couple.

Symmetrical Scission of the Coordinated Tetraborane in [(Cp*ReH₂)₂B₄H₄] on CO Addition and Reassociation of the Coordinated Diboranes on H₂ Loss**

Sundargopal Ghosh, Xinjian Lei, Christopher L. Cahill, and Thomas P. Fehlner*

The formation and scission of C–H and C–C bonds within the coordination sphere of a metal lie at the heart of organometallic chemistry.^[1] For example, the cleavage of alkynes coordinated to multinuclear metal sites into separated coordinated alkylidyne fragments^[2] exhibits more than one mode of scission: dissociation of a dimetal ethyne complex (dimetallatetrahedron) into two methylidyne complexes^[3] or ligand loss from a *nido*-trimetallaalkyne cluster to yield a *closo*-trimetalladialkylidyne cluster.^[4] The linking of alkynes at dimetal sites has been documented and, pertinent to this work, are the two systems outlined in Scheme 1 a and b. In the first, a 46 cluster valence electron (cve) bitetrahedral complex is converted into a 48 cve pentagonal-pyramidal complex containing a C₄ fragment.^[5] In the other, a μ -alkyne complex is converted into a 44 cve pentagonal-pyramidal complex with a localized Cr≡Cr bond via a postulated 46 cve bitetrahedral intermediate.^[6] Both generate a C₄ fragment from two C₂ fragments.

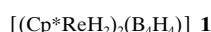
In principle, the incorporation of transition metals into the chemistry of p-block elements other than carbon permits similar manipulation of homonuclear bonding.^[7] The literature contains numerous examples for boron,^[8–11] but other elements illustrate the possibilities as well.^[12] In our own work, the elimination of H₂ from 50 cve [(Cp*IrH)₂(B₂H₄)₂] leads to the 48 cve complex [(Cp*Ir)₂(B₄H₈)] in which a B₄ fragment is generated from the fusion of two B₂ fragments (Scheme 1 c).^[13] The structure of the latter is analogous to that



Scheme 1. Examples of C–C bond formation promoted by a) CO addition to a Mo,Co complex, b) CO loss from (and C₂Ph₂ addition to) a Cr₂ complex, and c) B–B bond formation promoted by H₂ loss from an Ir₂ complex.

of the product in Scheme 1 although one reaction (C–C bond formation) is driven by ligand addition and the other (B–B bond formation) by ligand loss. Here we describe a related dirhenium system in which both B–B bond formation on ligand loss and B–B bond breaking on ligand addition are demonstrated.

The direct synthesis of **1**, from [Cp*ReCl₄] and LiBH₄, permits an examination of the chemistry of its derivatives.^[14] Thus, the chemistry of compounds of molecular formula



$[(\text{Cp}^*\text{M})_2\text{B}_4\text{H}_8]$ as M varies from Cr^[15] to Re^[16] (and Ru^[17] to Ir^[13]) unambiguously reveals the role of the transition metal. We have reported that the reaction of 42 cve [(Cp*Cr)₂(B₄H₈)] with CO leads to 44 cve [(Cp*Cr(CO))₂(B₄H₆)] in good yield (Scheme 2).^[15] Now, the same reaction with the rhenium analogue has been explored.

Reaction of **1** with 1 atm of CO and mild heating leads (via an intermediate **2** see below) to the 44 cve complex **3**, which has been characterized spectroscopically and crystallographi-

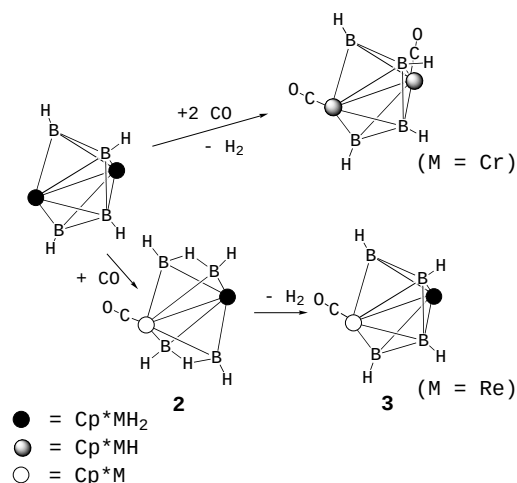


cally (Figure 1).^[18] The core structure deduced from the data based on the electron counting rules^[19, 20] is consistent with the solid-state structure. The close analogy with the behavior of the unsaturated complex [(Cp*Cr)₂B₄H₈] is evident—each loses H₂ and adds an appropriate number of CO ligands to the

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[**] This work was supported by the National Science Foundation. Cp = $\eta^5\text{-C}_5\text{Me}_5$.



Scheme 2. A comparison of the consequences of the addition of CO to [(Cp*Cr)₂B₄H₈] and to **1**.

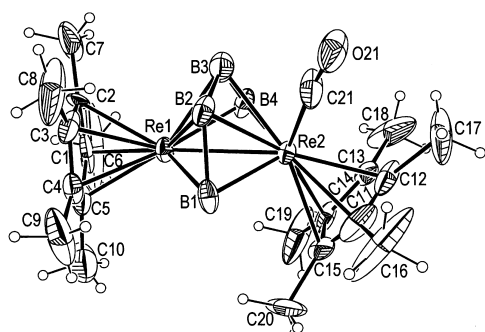


Figure 1. Molecular structure of **3**. Selected bond lengths [Å]: Re1-B1 2.11(2), Re1-B2 2.17(2), Re1-B4 2.26(2), Re1-B3 2.28(2), Re1-Re2 2.7819(8), Re2-B1 2.12(2), Re2-B4 2.17(2), Re2-B2 2.29(2), Re2-B3 2.39(2), B3-B4 1.67(3), B2-B3 1.70(3), B1-B2 1.85(3).

metal centers to achieve the 44 cve count required for a saturated bicapped tetrahedral cluster.

Investigation of the reaction by NMR spectroscopy at room temperature revealed the formation of a metastable intermediate which, when heated, converted cleanly into **3**. Full spectroscopic characterization of this intermediate presented us with a structural puzzle. Either there were two species present or the compound was much more complex than was reasonable to expect. An unambiguous explanation eluded us until an X-ray structure study revealed that there were two molecules of **2**, which differed in geometry, in the crystallo-



graphic asymmetric unit (Figure 2).^[18] There is precedence for such a situation, for example [(Cp*RuCl₂)₂]^[21] and [Cp₂WOs(CO)₇{C₂(C₆H₄Me)₂}].^[22] The assumption of an approximately 1:1 ratio of these two isomers in solution permits the mass spectrometric, infrared, and ¹H, ¹¹B, and ¹³C NMR data to be satisfactorily rationalized. The spectra are nearly temperature independent (room temperature and

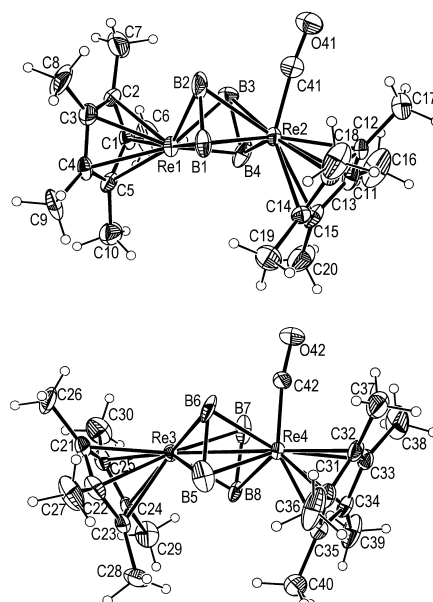
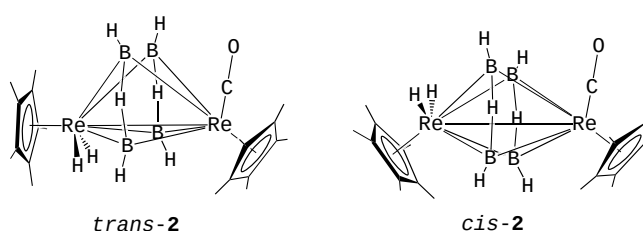


Figure 2. Molecular structure of (top) *trans*-**2** and (bottom) *cis*-**2**. Selected bond lengths [Å]: *trans*-**2**: Re1-B3 2.178(15), Re1-B4 2.205(16), Re1-B2 2.213(17), Re1-B1 2.376(19), Re1-Re2 2.7730(7), Re2-B4 2.194(15), Re2-B1 2.235(15), Re2-B3 2.264(17), Re2-B2 2.355(16), B3-B4 1.63(2), B1-B2 1.72(3), B2-B3 2.32, B1-B4 3.51; *cis*-**2**: Re3-B8 2.125(18), Re3-B5 2.158(14), Re3-B7 2.336(18), Re3-B6 2.417(15), Re3-Re4 2.8274(7), Re4-B5 2.219(16), Re4-B6 2.29(2), Re4-B8 2.298(16), Re4-B7 2.314(14), B7-B8 1.70(3), B5-B6 1.72(3), B5-B8 2.94, B6-B7 3.34.

below) this requires a slow interconversion of the isomeric forms and suggests that the isomers arise from kinetic control of the addition of CO to **1** (likely) or that they have identical free energies (possible but less likely).

As shown in Figure 2 and Scheme 3, the essential difference between the structures of the two isomers of **2** is the disposition of the ancillary ligands relative to the Re–Re axis. This disposition is *cisoid* (angles Re–Re–Cp*(c) = 149, 154°; Cp*(c) = centroid of Cp* ring) in one case and *transoid*



Scheme 3. A comparison of the structures of the isomers of **2**.

(angles Re–Re–Cp*(c) = 167, 134°) in the other. Thus, the two Re hydride units seen in the ¹H NMR spectra are likewise *cis* and *trans*, respectively, to the CO ligand. Hence, *cis*-**2** probably arises from CO attack *cis* to the “butterfly” face of **1** while *trans*-**2** arises from *trans* attack. Secondary differences are the positioning of the B₂ ligands relative to the Re–Re axis (the B...B distance corresponding to the bond broken in going from **1** to **2** is 2.94 Å in the *cis* isomer and 2.32 Å in the *trans* isomer) and the Re–Re distances (2.8274(7) Å in the *cis*

isomer and 2.7730(7) in the *trans* isomer). Like *trans*-**2**, the Cp* ligands in **3** are *trans* (angles Re-Re-Cp*(c) = 167, 132°; Re-Re = 2.7819(8) Å)

The characterization of **2** as an intermediate in the conversion of **1** into **3** reveals a complexity hidden in the analogous chromium system. As shown in Scheme 2, the addition of CO to **1** precedes H₂ loss. The extra pair of cve causes B-B bond breakage and migration of 2H that leads to two B₂H₃ ligands and a 46 cve bitetrahedral cluster geometry. Elimination of H₂ results in B-B bond reformation and a 44 cve bicapped tetrahedral geometry as found in **1**. Presumably a similar pathway exists for the chromium analogue; however, addition of CO to the metal center is much less facile and at the temperature required for reasonable reaction times any intermediates are rapidly converted into the 44 cve product. With the same ancillary ligands one expects an associative reaction to be more facile for Re than Cr; hence, the lower entrance barrier to intermediate **2**, relative to the exit barrier to **3**, permits its isolation.

In this boron chemistry the relationship to C-C bond cleavage and formation in coordinated alkynes is clear; however, the distinctive character of boron is shown in the analogous processes involving B-B bonds. For example, the ready formation of B-H-B bridge bonds accommodates the addition of CO to **1** and is an essential part of the pathway for the substitution of H₂ by CO.

Experimental Section

2: Standard air sensitive techniques were used. Compound **1** (0.12 g, 0.17 mmol) in hexane (10 ml) saturated with CO at 1 atm was stirred for 15 min at room temperature. The solvent was removed in vacuo, the residue extracted with hexane, and filtered through celite. The filtrate was concentrated and crystallization at -40 °C produced air sensitive, light yellow needles of **2** (0.09 g, 75%) contaminated with [Cp*ReH₆] (5–15%). X-ray quality crystals were grown by vapor diffusion—hexane solution of **2** in inner tube, toluene in outer tube at 7 °C. Selected data for *cis*-**2** and *trans*-**2** in solution. MS (EI): *m/z*: 722, isotopic pattern for 2Re, 4B, 21C, 10 atoms; calcd for weighted average of isotopomers lying within the instrument resolution: 724.2410; observed: 724.2398; IR (KBr): $\tilde{\nu}$ = 2510w, 2472w (B-H), 1923s, 1889s (Re-CO) cm⁻¹; ¹¹B NMR (C₆D₆, 22 °C): δ = 13.6 (d, *J*(B,H) = 163 Hz, 1B), -1.9 (d, *J*(B,H) = 151 Hz, 1B), -7.7 (d, *J*(B,H) = 153 Hz, 1B), -20.1 (d, *J*(B,H) = 161 Hz, 1B); ¹H NMR (C₆D₆, 22 °C): δ = 2.83 (partially collapsed quartet (pcq), 2BHt (*t*=terminal), 2.55 (pcq, 2BHt), 1.88 (s, 15H; Cp*), 1.85 (s, 15H; Cp*), 1.84 (s, 15H; Cp*), 1.82 (s, 15H; Cp*), -0.15 (pcq, 2BHt), -0.18 (pcq, 2BHt), -2.01 (brs, 4H; 4B-H-B), -12.55 (brs, 2H; 2B-H-Re), -14.42 (s, 2H; 2Re-H); ¹³C NMR (C₆D₆, 22 °C): δ = 206.7, 204.1 (Re-CO), 95.9, 94.5, 92.4, 89.2 (C₅(CH₃)₅), 13.1, 13.04, 12.3, 11.7 (C₅(CH₃)₅).

3: Thermolysis of a light yellow solution of **2** (0.05 g, 0.07 mmol) in benzene (12 ml) at 75 °C generated a red solution with complete conversion into a single product (¹¹B NMR $\approx$ 78% yield) after 5 h. Workup as for **2** gave crystalline **3**. MS (EI): *m/z*: 720, isotopic pattern for 2Re, 4B, 21C, 10 atoms; calcd for weighted average of isotopomers lying within the instrument resolution: 722.2254; observed: 722.2239; IR (KBr): $\tilde{\nu}$ = 2504w (B-H), 1926s (Re-CO) cm⁻¹; ¹¹B NMR (C₆D₆, 22 °C): δ = 87.2 (d, *J*(B,H) = 147 Hz, 2B), -11.7 (d, *J*(B,H) = 121 Hz, 2B); ¹H NMR (C₆D₆, 22 °C): δ = 9.96 (pcq, 2BHt), 1.92 (s, 15H; Cp*), 1.77 (s, 15H; Cp*), -0.43 (pcq, 2BHt), -13.12 (s, 2H; 2Re-H); ¹³C NMR (C₆D₆, 22 °C): δ = 217.6 (Re-CO), 97.9, 95.6 (C₅(CH₃)₅), 13.1, 10.6 (C₅(CH₃)₅); elemental analysis calcd (%) for C₂₁H₃₀Re₂B₄O: C 34.26, H 4.92; found: C 34.49, H 5.01.

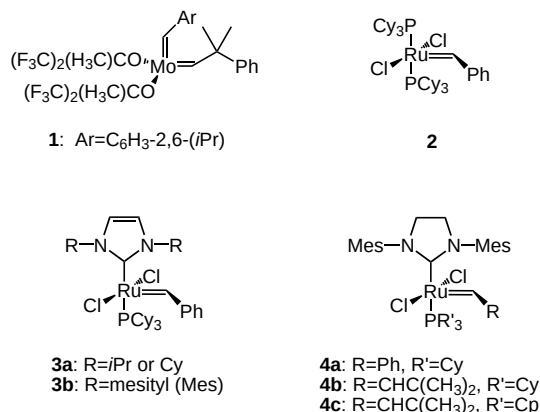
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- [18] Crystal structure data. Data were collected with MoK α radiation on a Bruker SMART CCD diffractometer at 294 K. Structure solutions were proceeded by direct methods, and subsequent refinements were carried out using SHELXTL. The hydrogen atoms of the Cp* groups were refined isotropically with an idealized riding model while the hydrogen atoms of B-H and Re-H were not found and not included in the final refinement. Structure of **2**: *M_r* = 722.15, monoclinic, space group *P*2₁/*c*, *a* = 16.1604(10), *b* = 14.9037(10), *c* = 20.2280(13) Å, β = 98.1780(10)°, *V* = 4822.4(5) Å³, *Z* = 8, ρ_{calcd} = 1.989 Mg m⁻³. The refinement converged to final values of *R*1 = 0.0470, *wR*2 = 0.0985 for 8398 observed unique reflections (*I* > 2 σ (*I*)). Structure of **3**: *M_r* = 720.14, monoclinic, space group *P*2₁/*c*, *a* = 8.1687(9), *b* = 14.575(2), *c* = 20.674(2) Å, β = 100.770(2)°, *V* = 2418.1(5) Å³, *Z* = 4, ρ_{calcd} = 1.978 Mg m⁻³. The refinement converged to final values of *R*1 = 0.0500, *wR*2 = 0.1042 for 4186 observed unique reflections (*I* > 2 σ (*I*)). 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-140717 (**2**) and -140718 (**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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Highly Efficient Ring-Opening Metathesis Polymerization (ROMP) Using New Ruthenium Catalysts Containing N-Heterocyclic Carbene Ligands**

Christopher W. Bielawski and Robert H. Grubbs*

Ring-opening metathesis polymerization (ROMP) has become an effective tool for the preparation of a wide variety of macromolecular architectures.^[1] To date, the most widely used catalysts for ROMP and other metathesis reactions are the well-defined complexes based on molybdenum^[2] (**1**) and ruthenium^[3] (**2**). The former complex displays much higher



activity than the latter, thus permitting polymerization of many sterically hindered or electronically deactivated cyclic olefins.^[1a, 2] However, the latter catalyst is stable under ambient conditions and tolerates a much larger range of protic and polar functional groups including alcohols, acids, and aldehydes.^[1a, 4]

Replacement of one of the phosphane ligands with a more electron-donating N-heterocyclic carbene ligand produced ruthenium complexes **3a,b** which displayed dramatically improved metathesis activity, thermal stability, and inertness towards oxygen and moisture when compared to **2**.^[5] Recently, complexes^[6, 7] **4a–c** have been prepared which utilize N-heterocyclic carbene ligands with saturated backbones. These complexes display catalytic activity in ring-closing metathesis^[6–8] (RCM) and cross-metathesis^[7, 9] (CM) that not only exceed that of **3a, b** in many cases, but also begin to rival molybdenum complex **1**, while maintaining the functional group compatibility of **2**. Herein, we report that complexes **3a, b** and **4a–c** are *more active* than **1** in ROMP and demonstrate their utility in the synthesis of a variety of polymeric structures.

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An effective method for comparing relative activities between various catalysts is through monitoring the ROMP of a low-strain cyclic olefin such as *cis,cis*-cycloocta-1,5-diene (COD).^[5c–d, 10, 11] The ROMP of COD was initiated with **1**, **3b**, and **4a–c** and the percent monomer converted to polymer was monitored over time using ¹H NMR spectroscopy (Figure 1).^[12] The polymerization rate of COD when initiated with

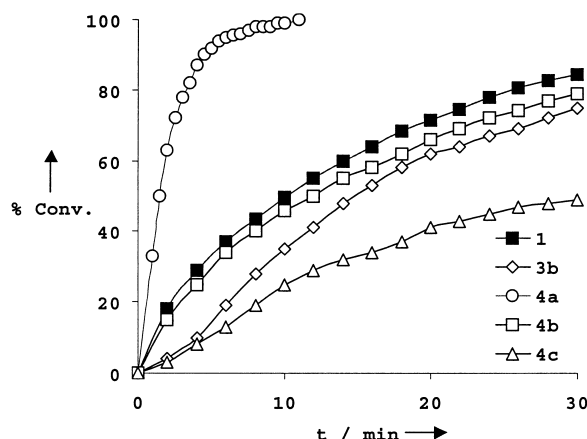


Figure 1. ROMP of COD using catalysts **1**, **3b**, and **4a–c**. Conditions: 20 °C, monomer/catalyst ratio 300:1, [catalyst] = 0.5 mM, CD₂Cl₂ as solvent. Conversion determined by ¹H NMR spectroscopy.

4a was found to be significantly higher than when initiated with the molybdenum complex **1** at 20 °C. In addition to activity trends observed in RCM and CM,^[6–9] complex **4a**, which contains a saturated N-heterocyclic carbene ligand, also displayed increased activity in ROMP relative to its unsaturated analogue (**3b**). When elevated temperatures (50 °C) were employed, complexes **3b**, **4b**, and **4c** also exhibited activities greater than **1** (Figure 2). Interestingly, the activity

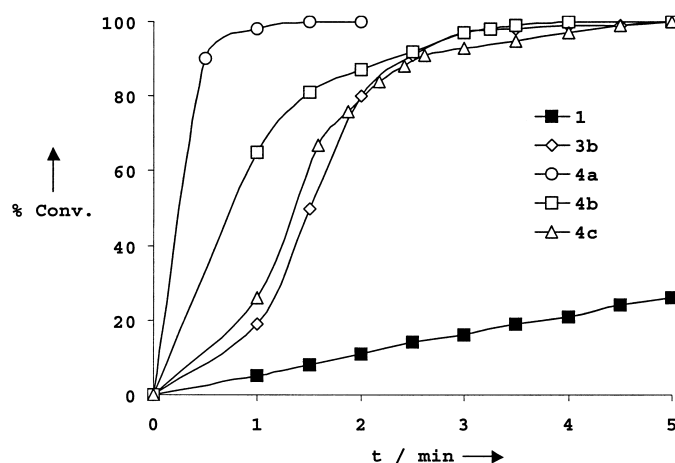


Figure 2. ROMP of COD using catalysts **1**, **3b**, and **4a–c**. Conditions: 50 °C, monomer/catalyst ratio 300:1, [catalyst] = 0.5 mM, CD₂Cl₂ as solvent. Conversion determined by ¹H NMR spectroscopy.

of the various ruthenium complexes appears to be related to both the phosphane ligands and alkylidene moiety substitution. For example, the discrepancy in polymerization rates between **4a** and **4b** may be related to the initiation rate,^[13]

since the propagating species resulting from both catalysts is identical. Thus, the bulkier benzylidene ligand may facilitate phosphane dissociation to a greater extent than the dimethylvinyl carbene ligand, thereby enhancing initiation. In addition, the bulkier tricyclohexyl phosphane (PCy₃) in **4b** may dissociate to a higher degree than the tricyclopentyl phosphane (PCp₃) in **4c**,^[14] thereby providing a relatively higher concentration of the highly active (phosphane dissociated) species.

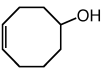
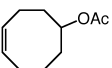
As shown in Table 1, catalysts **4a–c** polymerized a variety of low-strain cyclic olefins with extremely low catalyst loadings (up to a monomer/catalyst ratio of 100 000).^[15] In agreement with the results obtained above, elevated temperatures (50 °C) generally increased the yields of polymer while reducing reaction times. Monomers such as 5-hydroxy- or 5-acetoxycyclooctene were also efficiently polymerized, providing access to polymers with a high degree of backbone functionality.^[16, 17] In general, ¹H NMR spectroscopy indicated a predominately (75–95 %) *trans*-olefin microstructure in these polymers. As expected for an equilibrium-controlled polymerization in which chain transfer occurs, longer polymerization times resulted in higher *trans*-olefin values.^[1a]

Close examination of Table 1 reveals that these highly active ruthenium systems do not form well-defined polymeric structures.^[1a] The polydispersity indices (PDIs) of the resulting polymers are relatively high and strongly suggest that significant chain transfer occurred during the polymerization. The percent *trans* olefin in the polymer backbone gradually increased with time which suggests that secondary metathesis isomerizations are also occurring. In addition, monitoring the ROMP of COD (50 equivalents) using catalyst **4a** by ¹H NMR spectroscopy (CD₂Cl₂, 25 °C) indicated that less than 5 % of the catalyst initiated before the ROMP was complete (<1 min). After 15 min at 55 °C, complete conversion of unreacted initiator to propagating species ($\delta = 18.60$) through chain transfer was observed. The slow initiation rate explains the relatively high experimental molecular weights when

compared to their theoretical values (based on initial monomer/catalyst ratios).

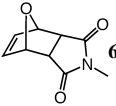
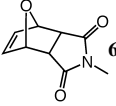
However, the molecular weights were easily regulated through the inclusion of acyclic olefins which effectively act as chain transfer agents (CTAs; Table 1).^[1] This technique is extremely useful when poorly soluble polymers are obtained by polymerizing monomers such as cyclooctene in bulk. Including functionalized CTAs during the ROMP of cyclic olefins has been shown to afford end-functionalized (telechelic) polymers,^[18] which are useful as intermediates in the syntheses of triblock copolymers and in the formation of polymeric networks.^[19] Owing to its high functional group tolerance, complex **2** has been the catalyst of choice when preparing telechelic polymers using this approach; however, only low-strain monomers have been used.^[1a, 18] When 1,4-diacetoxy-2-butene was included as a CTA during the **4a**-catalyzed ROMP of highly strained monomers such as norbornene (**5**) and *N*-methyl-7-oxanorbornenedicarbimide (**6**),^[20] acetoxy end-terminated polymer was obtained (Table 2). Excellent agreement between theoretical and experimental molecular weights (based on the initial monomer/CTA ratio) were observed when norbornene was employed as the monomer and led to the formation of bis(acetoxy) end-terminated polymer. However, in the case of the oxanorbornene derivative **6**, the CTA did not completely incorporate and produced polymer with a higher-than-expected molecular weight. The monomer is a cyclic ether that may coordinate to the catalyst and sufficiently attenuate its ability to transfer polymer chains. Nevertheless, the molecular weight could still be regulated and an acetoxy endgroup was observed on the polymer chains. These results are particularly notable since end-functionalized polymers composed of highly strained monomers are relatively difficult to obtain using other methods. For example, a metathesis degradation approach using tungsten-based metathesis catalysts has been used to prepare telechelic poly(oxanorbornene)s and poly(norbornene)s.^[21] However, the catalyst's tolerable range of func-

Table 1. ROMP of various low-strain cyclic olefins.^[a]

Monomer	M/C ^[b]	T [°C]	t [h]	Yield [%]	M _n ^[c]	PDI ^[c]	<i>trans</i> [%] ^[d]
	100 000	55	0.5	85	112 400	2.3	70
	10 000	25	24	85	92 900	2.5	85
	25 000 ^[e]	55	24	89	10 700	2.1	90
	100 000	55	< 0.1	[g]	[g]	[g]	[h]
	10 000	25	0.5	[g]	[g]	[g]	[h]
	25 000 ^[e, f]	55	24	75	2 200	1.6	85
	100 000	55	< 0.1	[g]	[g]	[g]	[h]
	10 000	25	0.5	[g]	[g]	[g]	[h]
	25 000 ^[e, f]	55	24	85	2 600	2.3	85
	10 000	55	< 0.1	50	103 900	2.8	85
	1000	25	1	60	79 300	3.2	90
	1000	25	24	50	23 000	2.5	50
	1000	25	24	52	9 000	2.5	90

[a] Bulk polymerizations using catalyst **4c**. Catalyst **4a** gives similar results. [b] Initial monomer/catalyst ratio. [c] Determined by CH₂Cl₂ or THF GPC and results are reported relative to poly(styrene) standards. [d] Percent *trans* olefin in the polymer backbone, as determined by ¹H and ¹³C NMR analysis. [e] 1,4-Diacetoxy-cis-2-butene was included as a CTA. Monomer/CTA ratio 80:1. [f] Monomer/CTA ratio 10:1. [Monomer]₀ = 4.5 M in C₂H₄Cl₂. [g] Polymer was insoluble. [h] Not determined.

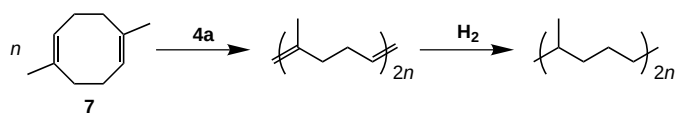
Table 2. Synthesis of acetoxy end-terminated polymers composed of highly strained monomers.^[a]

Monomer	M/CTA ^[b]	Yield [%]	X_n (NMR) ^[c]	M_n (GPC) ^[d]	PDI ^[d]
 5	5	95	6	800	2.0
 5	20	97	20	2100	2.0
 6	1	90	[e]	4200	2.0
 6	10	98	[e]	12500	1.9

[a] General polymerization conditions: $T = 55^\circ\text{C}$; 12 h; $\text{C}_2\text{H}_4\text{Cl}_2$ as solvent; $[\mathbf{4a}]_0 = 10\text{--}30\text{ mM}$; $[\text{monomer}] = 3\text{--}5\text{ M}$; nitrogen atmosphere. 1,4-bis(acetoxy)-*cis*-2-butene was included as CTA. [b] Initial monomer/CTA ratio. [c] Average number of monomer units per polymer chain. Determined by endgroup analysis using ^1H NMR spectroscopy and assumes the number of functional groups per polymer chain is exactly two. [d] Determined by CH_2Cl_2 GPC and results are reported relative to poly(styrene) standards. [e] Not determined.

tional groups limits the choice of CTAs. The “pulsed addition” approach has also been employed using catalysts **1** and **2**, but requires carefully timed additions of monomer and/or CTA, thereby reducing the ease of performing these polymerizations.^[22]

As shown in Scheme 1, **4a** initiated the ROMP of 1,5-dimethyl-1,5-cyclooctadiene (**7**),^[23] a sterically hindered, tri-substituted cyclic olefin, affording poly(isoprene) ($M_n = 10000$, $\text{PDI} = 2.3$) in 90 % yield (monomer/catalyst ratio



Scheme 1. Synthesis of an ethylene-propylene copolymer by ROMP of 1,5-dimethyl-1,5-cyclooctadiene (**7**) followed by hydrogenation.

1000:1, 55°C , 24 h). Subsequent hydrogenation using *p*-toluenesulfonhydrazide as the hydrogen source afforded an ethylene-propylene copolymer (as determined by NMR analysis) in quantitative yield.^[24] Previously, a six-step synthesis was necessary to obtain a similar copolymer by a metathetical route.^[25] To the best of our knowledge, this is the first ROMP of this monomer.

We have demonstrated that complexes **4a–c** are high-performance ROMP catalysts that can be used to synthesize a variety of polymeric materials including functionalized, telechelic, and trisubstituted polymers. Importantly, these catalysts are late transition metal complexes and demonstrate high functional group tolerance. Through careful tuning of the ligand environment and reaction conditions, these catalysts display metathetical activity that exceeds both previous ruthenium-based catalysts and the early transition metal catalyst **1**.

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The Phenylsulfonyl Group as an *endo* Stereochemical Controller in Intramolecular Pauson–Khand Reactions of 3-Oxygenated 1,6-Enynes**

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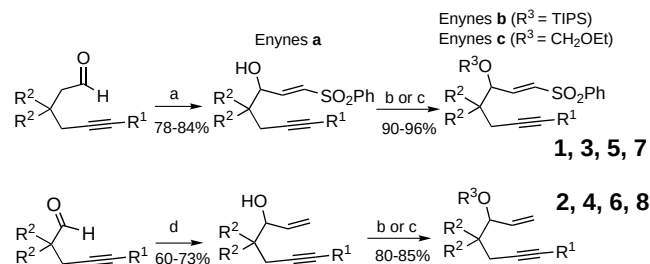
Dedicated to Prof. José Barluenga on the occasion of his 60th birthday

The intramolecular Pauson–Khand (PK) reaction of enynes has become one of the most powerful tools for the construction of cyclopentenone-fused bicyclic compounds. In particular, due to the reliability and efficiency of the cyclization of 1,6-enynes, this reaction has been widely applied to the synthesis of complex products with bicyclo[3.3.0]octane skeletons.^[1] In the case of 3-substituted 1,6-enynes, it is well known since the pioneering work of Magnus et al.^[2] that the reaction occurs with moderate to high *exo* stereoselectivity^[3] (the substituent at the allylic position on the starting enyne ends up on the less sterically crowded *exo* face of the bicyclic product).

Although it is generally assumed that electron-poor alkenes are unsuitable substrates for PK reactions,^[1,4] we recently reported that certain 1-sulfinyl-1,6-enynes undergo intramolecular PK cyclizations in acceptable yields and in a highly stereoselective manner.^[5] Encouraged by these results, we envisioned the use of other electron-poor sulfur-substituted olefins, such as vinyl sulfones.^[6] Here we report that the readily available racemic or enantiopure 1,6-enynes with γ -oxygenated α,β -unsaturated phenylsulfone structures are not

only excellent substrates in intramolecular PK reactions, but also that cyclization takes place with a reversal selectivity, that is, *endo* instead of *exo* selectivity.^[7]

Following our usual method of synthesis of (*E*)- γ -hydroxy- α,β -unsaturated phenyl sulfones,^[8] several differently substituted 1-phenylsulfonyl-3-hydroxy-1,6-enynes (substrates **1a**, **3a**, **5a**, and **7a**^[9]) were prepared in one step by the piperidine-promoted condensation of the corresponding aldehydes with phenylsulfonyl *p*-tolylsulfonylmethane in acetonitrile at 0 °C (78–84% yield, Scheme 1). Next, to study the effect of



Scheme 1. Synthesis of the starting 1,6-enynes **1–8**. a) $\text{PhSO}_2\text{CH}_2\text{SO}_2\text{Tol}$, piperidine, CH_3CN , 0 °C; b) TIPSOTf, 2,6-lutidine, CH_2Cl_2 , RT; c) ClCH_2OEt , DIPEA, CH_2Cl_2 , RT; d) vinylmagnesium bromide, THF, –78 °C. TIPSOTf = triisopropylsilyl trifluoromethanesulfonate; DIPEA = *N,N*-diisopropylethylamine.

substitution at the γ -position, the hydroxy group of these enynes was protected as the triisopropylsilyl (TIPS) ether (substrates **b**) and ethoxymethyl ketal derivatives (substrates **c**). To evaluate precisely the effect of the sulfonyl group on the reactivity and stereoselectivity of the PK reaction, the corresponding 1-unsubstituted 3-oxygenated 1,6-enynes were readily prepared by addition of vinylmagnesium bromide to the appropriate aldehyde (synthesis of alcohols **2a**, **4a**, **6a**, and **8a**^[9]) followed by protection of the hydroxy group (substrates **2b–c**, **4b–c**, **6b–c**, and **8b–c**) (Scheme 1).

Table 1 summarizes the results of the PK cyclizations of enynes **1–8** under the usual conditions with an amine *N*-oxide promoter:^[10] initial formation of the alkyne dicobalt complex by reaction with $[\text{Co}_2(\text{CO})_8]$ in CH_2Cl_2 at room temperature followed by addition of 6 equiv of trimethylamine *N*-oxide (TMANO). Incomplete conversion was observed under these standard conditions with enynes **7**, and so the reaction was performed in the presence of TMANO and molecular sieves^[11] (entries 15, 17, and 19).

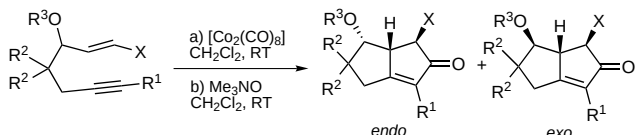
Remarkably, in spite of the electron-poor character of the C–C double bond of the vinyl sulfones **1**, **3**, **5**, and **7**, these compounds reacted completely after 2–3 h to give the corresponding bicyclic PK adducts, in most cases as the only detectable compounds (^1H NMR) after removal of the cobalt by-products by filtration through Celite. From a synthetic viewpoint, it is noteworthy that the yields of PK products from 1-sulfonylated 1,6-enynes (70–79% yields for cyclopentenones **9**, **11**, **13**, and **15**) are generally higher than those obtained from the corresponding 1-unsubstituted enynes (cyclopentenones **10**, **12**, **14**, and **16**; 38–79% yield), and that there is no significant decrease in efficiency on going from the 4,4-dimethyl series **5** and **7** (Thorpe–Ingold effect) to the 4-unsubstituted series **1** and **3**.^[12]

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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. Pauson–Khand reactions of enynes **1–8**.



Entry	Enyne	X	R ¹	R ²	R ³	Product	<i>endo/exo</i> ^[a]	Yield ^[b] [%] (<i>endo</i> ^[c] [%])
1	1b	SO ₂ Ph	H	H	TIPS	9b	92/8	74 (66)
2	2b	H	H	H	TIPS	10b	46/54	54
3	1c	SO ₂ Ph	H	H	CH ₂ OEt	9c	> 98 / < 2	76 (76)
4	2c	H	H	H	CH ₂ OEt	10c	28/72	72
5	3b	SO ₂ Ph	Me	H	TIPS	11b	91/9	73 (63)
6	4b	H	Me	H	TIPS	12b	32/68	46
7	3c	SO ₂ Ph	Me	H	CH ₂ OEt	11c	93/7	77 (71)
8	4c	H	Me	H	CH ₂ OEt	12c	25/75	38
9	5a	SO ₂ Ph	H	Me	H	13a	80/20	60 ^[d]
10	6a	H	H	Me	H	14a	36/64	50
11	5b	SO ₂ Ph	H	Me	TIPS	13b	39/61	71 (25)
12	6b	H	H	Me	TIPS	14b	10/90	63
13	5c	SO ₂ Ph	H	Me	CH ₂ OEt	13c	67/33	74 (47)
14	6c	H	H	Me	CH ₂ OEt	14c	20/80	79
15 ^[e]	7a	SO ₂ Ph	Ph	Me	H	15a	76/24	75 ^[d]
16	8a	H	Ph	Me	H	16a	17/83	42
17 ^[e]	7b	SO ₂ Ph	Ph	Me	TIPS	15b	94/6	78 ^[d]
18	8b	H	Ph	Me	TIPS	16b	< 2 / > 98	45
19 ^[e]	7c	SO ₂ Ph	Ph	Me	CH ₂ OEt	15c	87/13	79 ^[d]
20	8c	H	Ph	Me	CH ₂ OEt	16c	16/84	46

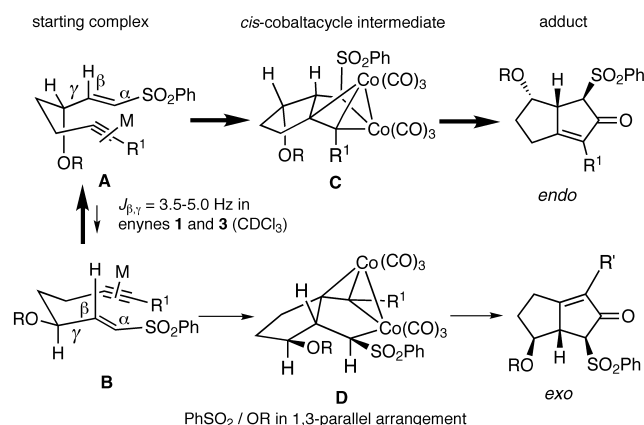
[a] Determined by ¹H NMR spectroscopy after removal of the cobalt by-products by filtration. [b] Of pure *exo* + *endo* adducts after chromatography on silica gel. [c] In parentheses: yield of pure *endo*-4-sulfonylcyclopentenone after chromatography. [d] The *endo* + *exo* adducts could not be separated by chromatography on silica gel. [e] Reaction conditions: Me₃NO (6 equiv), molecular sieves (4 Å), toluene, RT.

However, the most outstanding result concerns the stereoselectivity of the cyclization. As expected for allylically substituted 1,6-enynes, the PK reactions of enynes **2** and **6** were moderately *exo* selective, and, in agreement with the model proposed by Magnus et al.,^[2] which predicts a higher *exo* selectivity with increasing size of the substituent at the alkyne terminus, the PK reactions of the alkyne-substituted series **4** and **8** were somewhat more *exo* selective than those of **2** and **6**, respectively. Interestingly, the PK reactions of the sulfonylated enynes **1**, **3**, **5**, and **7** were *endo* selective in most cases (values in bold in Table 1), even in the alkyne-substituted series **3** and **7**, which proves the strong capacity of the phenylsulfonyl group to reverse the “natural” *exo* selectivity of the process. Thus, in the four sulfonylated series the *endo*-bicyclo[3.3.0]oct-1-en-3-one was obtained with high (entries 7, 9, and 17) or complete stereoselectivity (entry 3), whereby the pronounced reversal of stereoselectivity in the case of the pairs of enynes **1c/2c** (entries 3 and 4), **3c/4c** (entries 7 and 8) and **7b/8b** (entries 17 and 18) is especially remarkable. Furthermore, apart from the adducts **15**, the rest of *endo/exo* mixtures of 4-sulfonylcyclopentenones (adducts **9**, **11**, and **13**) were readily separated by simple chromatography on silica gel.

The *exo/endo* configuration of the sulfonylated cyclopentenones **9**, **11**, **13**, and **15** was evident from spectroscopic data.^[13] In addition, the homogeneity of the stereochemical assignments was confirmed in several cases by hydrolysis of the *O*-protected PK adducts (products **b,c**) to the corresponding alcohols (products **a**), and by chemical correlation between sulfonylated and unsulfonylated cyclopentenones. Thus, the

reductive desulfonylation of *endo*-**9b**, *endo*-**9c**, and *endo*-**13b** with activated zinc (NH₄Cl, THF/H₂O, RT) furnished in excellent yields *endo*-**10b** (93 %), *endo*-**10c** (94 %), and *endo*-**14b** (91 %), respectively. In addition, the structure of *endo*-**9b** was unequivocally proved by X-ray diffraction.^[14]

Although the assumed multi-step mechanism of the PK reaction makes rationalization of the stereochemical outcome difficult, two main factors are usually invoked in diastereoselective PK cyclizations: the conformational preferences of the starting complex prior to metal-lacycle formation^[7, 15] and the thermodynamic stability of the putative key *cis*-cobaltacycle intermediates.^[2, 16] In the case of the 1-sulfonylated-1,6-enynes, both effects could operate in the same direction, providing a reasonable explanation for the observed major formation of the *endo* isomer (Scheme 2). Thus, as we had

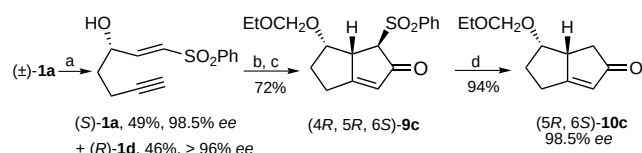


Scheme 2. Proposed mechanism for the observed *endo*-selective reaction.

noticed in other γ -oxygenated α,β -unsaturated phenyl sulfones,^[17] the conformation **A** (OR and H _{α} in 1,3-parallel arrangement) would probably be more stable than conformation **B** (H _{α} and H _{γ} in 1,3-parallel arrangement), as is deduced from the low value of $J_{\beta\gamma}$ (3.5–5.0 Hz, CDCl₃) in the starting enynes **1** and **3**. This leads to the *cis*-cobaltacycle **C**, in which the OR and SO₂Ph groups are located on opposite faces of the bicyclic structure, which finally affords the *endo* adduct. In contrast, the less stable conformation **B** (H _{β} and H _{γ} in *anti* relationship) would produce the presumably less stable *cis*-cobaltacycle **D** (intermediate in the formation of the *exo*

adduct), which is likely destabilized with respect to **C** due to the 1,3-parallel steric interaction between the OR and SO₂Ph groups (Scheme 2).

For the application of the results shown in Table 1 to the synthesis of enantiopure 6-oxygenated *endo*-bicyclo[3.3.0]oct-1-en-3-ones, optically pure 1-sulfonyl-1,6-enynes were required. Some years ago we described a practical lipase-mediated kinetic resolution of a wide structural variety of (±)-γ-hydroxy-α,β-unsaturated sulfones based on their highly enantioselective acetylation with lipase PS (*Pseudomonas cepacia* lipase) as catalyst in an organic solvent.^[18] Pleasingly, under these conditions the reaction of (±)-**1a** stopped at 50 % conversion (48 h in toluene as solvent) and afforded 49 % of the alcohol (*S*)-**1a**^[19] and 46 % of the acetate (*R*)-**1d**^[19] after flash chromatography, both in very high optical purity (98.5 % *ee* for (*S*)-**1a** (HPLC, Chiralpak AS) and > 96 % *ee* for (*R*)-**1d** (¹H NMR, [Pr(hfc)₃]). Protection of (*S*)-**1a** as ketal (*S*)-**1c**^[19] and subsequent PK cyclization afforded (4*R*,5*R*,6*S*)-**9c**^[19] as the only isolated product (72 % yield). Finally, zinc-mediated reductive desulfonylation furnished the enantiomerically pure *endo*-substituted cyclopentenone (5*R*,6*S*)-**10c**^[19] (94 % yield, 98.5 % *ee* (HPLC, Chiralcel OD); Scheme 3).



Scheme 3. Enantioselective synthesis of 6-oxygenated *endo*-bicyclo[3.3.0]oct-1-en-3-ones. a) Lipase PS, vinyl acetate, molecular sieves, toluene, RT; b) ClCH₂OEt, DIPEA, CH₂Cl₂, RT; c) [Co₂(CO)₈], CH₂Cl₂, RT; TMANO, RT; d) Zn, NH₄Cl, THF/H₂O, RT.

In summary, we have demonstrated that α,β-unsaturated sulfones can be useful substrates in intramolecular PK reactions. Interestingly, unlike the usual stereochemical behavior of allylic substituted 1,6-enynes, the intramolecular PK cyclizations of differently substituted (*E*)-1-phenylsulfonyl-3-oxygenated-1,6-enynes occur with moderate to high *endo* selectivity. As these sulfonylated enynes are readily available in both racemic and enantiopure forms, and the sulfonyl group can be easily removed from the cyclopentenone products, this overall procedure represents an efficient stereocomplementary Pauson–Khand approach to the asymmetric synthesis of 6-substituted bicyclo[3.3.0]oct-1-en-3-ones. The application of this procedure to the stereoselective synthesis of triquinanes is underway.

Experimental Section

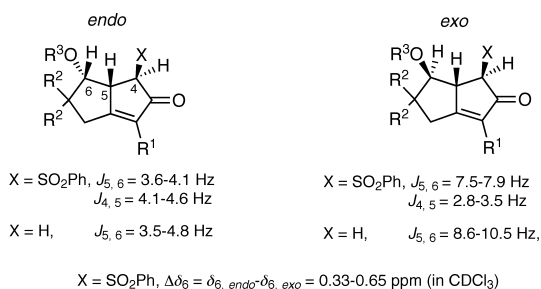
(4*R*,5*R*,6*S*)-**9c**: A solution of enyne (*S*)-**1c** (187 mg, 0.61 mmol) in CH₂Cl₂ (5 mL) was added dropwise to a stirred solution of [Co₂(CO)₈] (250 mg, 0.73 mmol) in CH₂Cl₂ (5 mL) under argon atmosphere at room temperature (RT). The solution was stirred for 10 min, and TMANO (407 mg, 3.66 mmol) was added in one portion. The resulting solution was stirred for 3 h at RT and filtered through a pad of Celite, which was washed with diethyl ether (30 mL). The combined solvents were evaporated and the residue was purified by flash chromatography (hexane/ethyl acetate 5/1) to afford 155 mg of (4*R*,5*R*,6*S*)-**9c** (76 %) as a white solid. M.p. 73–74 °C; [α]_D²⁰ = +241 (*c* = 0.4, CHCl₃); ¹H NMR (CDCl₃): δ = 7.99–7.96 (m, 2H), 7.70–7.55 (m, 3H), 5.92 (m, 1H), 4.65 (m, AB, 2H), 4.28 (t, *J* = 4.1 Hz, 1H),

4.23 (d, *J* = 4.4 Hz, 1H), 3.59 (m, 1H), 3.51 (m, 2H), 2.71 (m, 2H), 2.24 (m, 2H), 1.21 (t, *J* = 7.0 Hz, 3H); ¹³C NMR (CDCl₃): δ = 198.1, 185.0, 138.6, 134.0, 129.2, 129.1, 125.0, 93.8, 76.3, 68.3, 63.7, 53.2, 32.5, 24.3, 15.1; analysis calcd for C₁₇H₂₀O₅S: C 60.70, H 5.99, S 9.53; found: C 60.46, H 6.42, S 9.92.

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- [6] Vinyl sulfones had never been successfully used in PK reactions. As a related reported precedent, the treatment of divinyl sulfone with alkyne dicobalt complexes did not give the cyclopentenone PK product (I. U. Khand, P. L. Pauson, *Heterocycles* **1978**, *11*, 59) in accordance with the accepted behavior of alkenes with electron-withdrawing substituents (see ref. [4]).
- [7] To the best of our knowledge, the only reported case of *endo* selectivity in a PK reaction of an allylic substituted 1,6-enyne concerns a specific 3,4-disubstituted enyne: J. A. Casalnuovo, R. W. Scott, E. A. Harwood, N. E. Schore, *Tetrahedron Lett.* **1994**, *35*, 1153. Similarly, for a study of the *endo/exo* selectivity in 3,4-disubstituted-1,7-enynes, see C. Mukai, J. S. Kim, H. Sonobe, M. Hanaoka, *J. Org. Chem.* **1999**, *64*, 6822.
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- [10] Very similar yields and diastereoselectivities were obtained in the thermal PK reaction (CH_3CN , 80°C) of the dicobalt complexes of the enynes **1** and **3**.
- [11] The combination TMANO/molecular sieves has been recently reported as an efficient promoter in PK reactions: L. Pérez-Serrano, L. Casarrubios, G. Domínguez, J. Pérez-Castells, *Org. Lett.* **1999**, *1*, 1187.
- [12] Except for the free alcohols **1a** and **3a**, which proved to be rather unreactive in PK reactions. For instance, the alkyne dicobalt hexacarbonyl complex of **1a** did not react at all under thermal (CH_3CN , 80°C) or TMANO-promoted conditions (CH_2Cl_2 , RT). A very low yield of PK products **9a** (25 % yield, *endo:exo* = 85/15) was obtained when the reaction was performed in the presence of TMANO and molecular sieves. In this case, instead of the PK cyclopentenone, the main product was the corresponding exocyclic 1,3-diene (see ref. [4]).
- [13] As is usual in other 6-substituted bicyclo[3.3.0]octen-3-ones (for example, ref. [2c]), the coupling constant between H_5 and H_6 is a very simple and excellent criterion for stereochemical diagnosis. Thus, $J_{5,6}$ is much smaller in the *endo* isomers ($J_{5,6}$ = 3.6–4.8 Hz, H_5/H_6 in *cis* relationship) than in the *exo* isomers ($J_{5,6}$ = 7.5–10.5 Hz, H_5/H_6 in *trans* arrangement). Also, a characteristic trend was observed for the chemical shift of H_6 in the C-4 sulfonylated adducts: H_6 is significantly more deshielded in the *endo* isomer than in the *exo* isomer (see below), in accordance with the strong deshielding effect of the phenylsulfonyl group on the hydrogen atom in the 1,3-parallel arrangement. Additionally, these stereochemical assignments have been confirmed by NOESY experiments on the pairs of isomers *endo*/*exo* **9b** and *endo:exo* **13c**.



- [14] 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-141942. 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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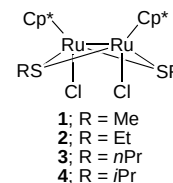
Cyclization of Terminal Diynes Catalyzed by Thiolate-Bridged Diruthenium Complexes: A Simple Synthetic Route to *endo*-Macrocyclic (*Z*)-1-En-3-yne**

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Homogeneous catalysis by polynuclear transition metal complexes has been receiving much attention because the multimetallic sites are expected to provide unique reaction sites for activation and transformation of substrate molecules. These sites are anticipated to differ significantly from those of conventional monometallic centers coordinated by ancillary ligands.^[1] Toward this end, our studies have long been focused on the synthesis and reactivity of polynuclear noble-metal complexes with bridging sulfur ligands.^[2] Recently, we found that the thiolate-bridged diruthenium complexes $[\text{Cp}^*\text{RuCl}(\mu_2\text{-SR})_2\text{RuCp}^*\text{Cl}]$ (**1–3**) ($\text{Cp}^* = \eta^5\text{-C}_5\text{Me}_5$; $\text{R} = \text{Me}$, Et , $n\text{Pr}$) catalyze the head-to-head *Z* dimerization of terminal alkynes containing straight-chain aliphatic and functional groups.^[3] Although mononuclear ruthenium complexes such as $[\text{Cp}^*\text{Ru}(\text{L})\text{H}_3]$ ($\text{L} = \text{phosphane}$) also catalyze the dimerization of terminal alkynes, such a highly regio- and stereoselective dimerization of a wide range of terminal alkynes has never been realized.^[4] We have now extended our study of the catalytic activity of the diruthenium complexes to include the cyclization of terminal diynes. Preliminary results are described here.

Trost and co-workers have reported the palladium-catalyzed cyclization of α,ω -diynes to give the corresponding *exo*-macrocyclic 1-en-3-yne in moderate yields and high regio- and stereoselectivities.^[5] In sharp contrast, synthetic routes to *endo*-macrocyclic 1-en-3-yne are extremely limited.^[6] Actually, six steps are necessary to prepare (*Z*)-1-cyclododecen-3-yne from cyclododecanone and the total yield is less than 5 %.^[6] To the best of our knowledge, there is no report of a synthetic method for *endo*-macrocyclic (*Z*)-1-en-3-yne from α,ω -diynes.^[7]

Treatment of 1,15-hexadecadiyne^[8] (**6a**) in methanol in the presence of $[\text{Cp}^*\text{RuCl}(\mu_2\text{-SMe})_2\text{RuCp}^*\text{Cl}]$ (**1**) (10 mol %) at



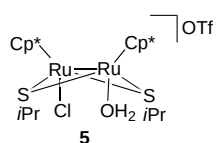
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60 °C for 18 h afforded (Z)-1-cyclohexadecen-3-yne (**7a**) in 72% yield as determined by gas-liquid chromatography (GLC). A dilute solution of **6a** (0.002 mol L⁻¹) was required to obtain **7a** in high yield. This new compound (**7a**) was fully characterized by NMR and IR spectroscopy, GC-MS, and elemental analysis (see Experimental Section). No regio- or stereoisomers of **7a** were detected by ¹H NMR spectroscopy or by GLC. However, dimers of **6a** were obtained in approximately 25% yield by GLC. Interestingly, addition of NH₄BF₄ significantly improved both the catalytic activity and the selectivity (94% yield; Table 1; entry 1).^[9] As observed in the previous dimerization of terminal alkynes^[3] the bridging thiolato ligands in the diruthenium complex were found to strongly influence the catalytic activity. The complex with the sterically demanding SiPr group [Cp*RuCl(μ₂-SiPr)₂-RuCp*Cl] (**4**) exhibited only low catalytic activity, whereas the complexes with SEt and SnPr groups (**2** and **3**, respectively) showed almost the same catalytic activity as **1**. Other diruthenium complexes such as [Cp*RuCl(μ₂-SiPr)₂RuCp*(OH₂)]-OTf (**5**), [Cp*RuCl(μ₂-SH)₂-RuCp*Cl], and [Cp*RuCl(μ₂-Cl)₂-RuCp*Cl] were ineffective.



Reactions of other α,ω-diynes (**6**) in the presence of **1** and NH₄BF₄ have been investigated. The cyclization reactions of 1,13-tetradecadiyne (**6b**) and 1,11-dodecadiyne (**6c**) proceeded rapidly to afford the corresponding (Z)-1-en-3-yne (**7b** and **7c**) in 69 and 48% yields, respectively (Table 1; entries 2 and 3). In contrast, the reaction of 1,9-decadiyne (**6d**) under identical conditions did not occur smoothly. A longer reaction time was required to produce (Z)-1-cyclodecen-3-yne (**7d**) in 41% yield (Table 1; entry 4). Noteworthy is that the 16-, 14-, and 10-membered ring products (**7a**, **7b** and **7d**) are new compounds. On the other hand, no cyclization of 1,7-octadiyne (**6e**) occurred, although after 24 h **6e** was almost completely consumed (Table 1; entry 5). The cyclization of the unsymmetrical diyne **8** under the same reaction conditions led to the formation of a mixture of two regioisomers (Z)-1-en-3-yne **9** in 31% yield (isomer ratio 2:1; Table 1; entry 6), whereas a 13-membered ring product **11** was obtained from a symmetrical diyne **10** in 72% yield (Table 1; entry 7). Furthermore, cyclization of terminal diynes with a phenyl or ferrocenyl moiety (**12** and **14**, respectively) proceeded smoothly to give the corresponding *endo*-macrocyclic (Z)-1-en-3-yne (**13** and **15**) in moderate to high yields (Table 1; entries 8–11). All the cyclized products were fully charac-

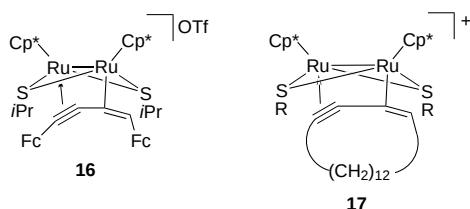
Table 1. Ruthenium-catalyzed cyclization of α,ω-diynes.^[a]

Entry	α,ω-Diynes	Time [h]	Conv. [%] ^[b]	Product	Yield [%] ^[c]
1	6a <i>n</i> = 12	1	> 99	7a <i>n</i> = 12	78 (94)
2	6b <i>n</i> = 10	1	> 99	7b <i>n</i> = 10	69 (81)
3	6c <i>n</i> = 8	1	> 99	7c <i>n</i> = 8	48 (60)
4	6d <i>n</i> = 6	18	87	7d <i>n</i> = 6	41 (50)
5	6e <i>n</i> = 4	24	90	7e <i>n</i> = 4	0 (0)
6	8	48	75	9	31 (48) ^[d]
7	10	24	> 99	11	72
8	12a <i>n</i> = 2	24	> 99	13a <i>n</i> = 2	73
9	12b <i>n</i> = 3	72	–	13b <i>n</i> = 3	35
10	14a <i>n</i> = 2	24	–	15a <i>n</i> = 2	52
11	14b <i>n</i> = 3	72	–	15b <i>n</i> = 3	24

[a] All the reactions were carried out with α,ω-diyne (0.25 mmol), **1** (0.025 mmol), and NH₄BF₄ (0.05 mmol) in methanol (125 mL) at 60 °C. [b] Determined by GLC. [c] Yield of isolated product. The value in parenthesis is the yield determined by GLC. [d] Isomer ratio 2:1.

terized by spectroscopic methods, MS, and elemental analysis. Their configuration was determined from the vinyl coupling constants in the ^1H NMR spectra, which are diagnostic of *cis* couplings. The molecular structure of one of the typical *endo*-macrocyclic (*Z*)-1-en-3-yne, **15a**, was unambiguously determined by X-ray analysis, and an ORTEP drawing is shown in the Supporting Information.^[10]

We previously reported the reaction of complex **5** with ferrocenylacetylene at ambient temperature to form the butenyne complex $[\text{Cp}^*\text{Ru}(\mu_2\text{-C}(\text{=CHFc})\text{C}\equiv\text{CFc})(\mu_2\text{-SiPr})_2\text{-RuCp}^*]\text{OTf}$ (**16**; Fc = ferrocenyl), which catalyzes the stereoselective di- and trimerization of ferrocenylacetylene at 60°C .^[11] The ^1H NMR analysis of the reaction mixture of **1** and **6a** at room temperature or 60°C did not show the formation of such intermediate complexes. However, the present cyclization of α,ω -diynes is also considered to involve a butenyne intermediate **17** similar to **16**. Noteworthy is that **7a** was not obtained from the cyclization of **6a** by using $[\text{Ir}(\text{biph})(\text{PMe}_3)_3\text{Cl}]$ (biph = biphenyl-2,2'-diyl), which is known to be effective for the selective head-to-head *Z* dimerization of aliphatic terminal alkynes.^[12]



In summary, we have a novel cyclization of α,ω -diynes, which is catalyzed by the thiolate-bridged diruthenium complexes **1**, **2**, and **3** to produce the corresponding *endo*-cyclic (*Z*)-1-en-3-yne in moderate to high yields with complete stereoselectivities.

Experimental Section

Typical procedure for the cyclization: NH_4BF_4 (5.5 mg, 0.05 mmol), and **6a** (55 mg, 0.25 mmol) were added to a solution of complex **1** (16 mg, 0.025 mmol) in dry MeOH (125 mL). The reaction mixture was stirred at 60°C for 1 h. The GLC analysis using naphthalene (10 mg) as an internal standard showed the formation of **7a** in 94% yield. For the isolation of **7a**, the solvent was removed under reduced pressure and the residue was purified by HPLC (CHCl_3 eluent) to give pure **7a** (43 mg, 0.20 mmol, 78%); ^1H NMR (400 MHz, CDCl_3): δ = 1.34–1.50 (brm, 20H, $-\text{CH}_2-$), 2.29–2.40 (m, 4H, $\text{CH}_2\text{C}\equiv\text{C}$, $-\text{CH}=\text{CHCH}_2-$), 5.44 (d, 1H, J = 10.7 Hz, $-\text{CH}=\text{CHCH}_2-$), 5.80 (dt, 1H, J = 10.7, 7.7 Hz, $-\text{CH}=\text{CHCH}_2-$); ^{13}C NMR (100 MHz, CDCl_3): δ = 19.2 (t), 25.8 (t), 25.8 (t), 26.5 (t), 26.5 (t), 26.6 (t), 26.9 (t), 27.1 (t), 27.2 (t), 27.6 (t), 28.1 (t), 29.8 (t), 77.8 (s), 94.1 (s), 109.5 (d), 142.5 (d); GC-MS: m/z (%) 218 (17) [M^+], 189 (1), 161 (5), 147 (9), 133 (17), 105 (26), 91 (67), 79 (100), 67 (61), 41 (91); IR (neat): $\tilde{\nu}$ (cm^{-1}) = 1617 ($\text{C}=\text{C}$), 2211 ($\text{C}\equiv\text{C}$); elemental analysis calcd for $\text{C}_{16}\text{H}_{26}$: C 88.00, H 12.00; found: C 87.63, H 11.89.

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- [9] The reaction of **1** with NH_4BF_4 is considered to afford a cationic, thiolate-bridged diruthenium complex with a vacant site.
- [10] 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-137921. 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 Semiconducting Lamella Polymer $[\{\text{Ag}(\text{C}_5\text{H}_4\text{NS})\}_n]$ with a Graphite-Like Array of Silver(I) Ions and Its Analogue with a Layered Structure**

Weiping Su, Maochun Hong,* Jiabao Weng, Rong Cao,* and Shaofang Lu

A great deal of work has recently been devoted to inorganic–organic hybrid-framework assemblies such as multilayer perovskites,^[1] metal phosphonates^[2] and metal–ligand networks^[3] because of their potential as functional solid materials. The diversity of organic components used has resulted in numerous inorganic–organic hybrid frameworks with fascinating structural topologies.^[1–4] By carefully selecting the organic components one hopes to tune the physical properties of this type of compound by tailoring their structures and thus realize various applications, including electrical conductivity,^[4a,b] magnetism,^[4c] catalysis,^[4d,e] shape specificity,^[4f] and ion exchange.^[4g,h] Structural isomers of coordination polymers can be prepared by controlling the synthetic conditions such as temperature and medium.^[5] By using different thiolate ligands containing N-donor groups we

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have synthesized several silver thiolate polymers with different architectures.^[6] Herein, we demonstrate that the formation of silver(I) thiolate polymers, $[\text{Ag}(\text{C}_5\text{H}_4\text{NS})]_n$ (**1**) ($\text{C}_5\text{H}_4\text{NS}$ = pyridine-2-thiolate (PyS^-)) with a graphite-like array of silver(I) ions and $[\text{Ag}_5(\text{C}_5\text{H}_4\text{NS})_4(\text{C}_5\text{H}_5\text{NS})\text{BF}_4]_n$ (**2**) ($\text{C}_5\text{H}_5\text{NS}$ = 1*H*-pyridine-2-thione (HPyS)) with a layered structure, depends on the reaction temperature and the solvents used. In contrast to **2** and $[\text{Ag}_6(\text{PyS})_6]_n$ (**3**) which has a one-dimensional chain structure, and other silver(I)-thiolate polymers synthesized in our laboratory,^[6] all of which are insulators, **1** is a semiconductor.

The reaction of AgBF_4 with pyridine-2-thiol in DMF/ H_2O (20/1) at 0°C followed by crystallization by the slow diffusion of Et_2O into the solution resulted in the growth of a large amount of colorless, thin sheetlike hexagonal crystals of **1**. Elemental analysis showed the formula of the product to be $[\text{Ag}(\text{C}_5\text{H}_4\text{NS})]_n$. An X-ray diffraction study confirmed the structure to be a layered polymer $[\text{Ag}(\text{C}_5\text{H}_4\text{NS})]_n$ with a graphite-like array of silver(I) ions.^[7] Polymer **1** has a two-dimensional lamella structure wherein the silver atoms are linked by PyS^- ligands to form inorganic layers and the pyridyl groups of PyS^- ligands protrude into the interlayer region (Figure 1). The interlayer distance is 17.17 Å. Each

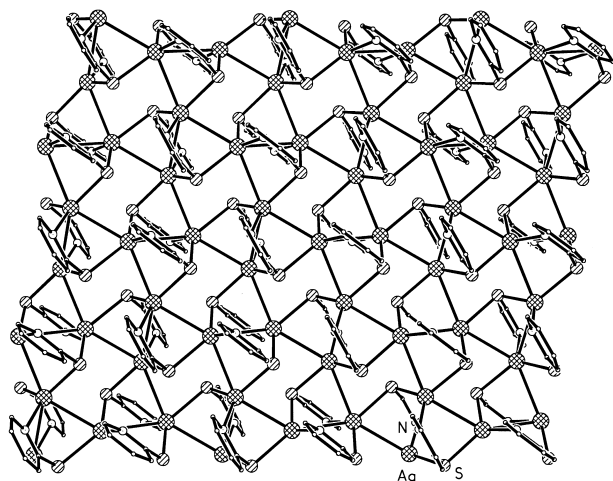


Figure 1. A view of the lamella structure in **1**.

PyS^- ligand in **1** acts as a μ_3 bridge to link two silver atoms through an S atom, and ligates another silver atom through an N atom with Ag–S bond distances of 2.424 and 2.647 Å and an Ag–N bond distance of 2.428 Å. Each silver atom is tri-coordinated by two S atoms and one N atom from three different PyS^- ligands in a distorted planar trigonal fashion (S–Ag–N angles are 103.4 and $125(1)^\circ$, and S–Ag–S is $127.0(2)^\circ$). The silver atoms of each layer are nearly coplanar (the deviation being within 0.13 Å), and they arrange to form a graphite-like hexagonal motif (Figure 2). The Ag_6 hexagon is somewhat distorted because of the different Ag–Ag–Ag angles (73.81 , 140.40 , and 142.20°). The three adjacent Ag–Ag bond lengths (3.215 , 3.215 , and 3.250 Å) are significantly shorter than the van der Waals contact distance (3.40 Å).^[8]

The crystalline product of **1** is very stable in air at room temperature. However, when the thin hexagonal crystals of **1** were soaked in DMF at room temperature (about 30°C), they transformed over four weeks into colorless needlelike crystals.

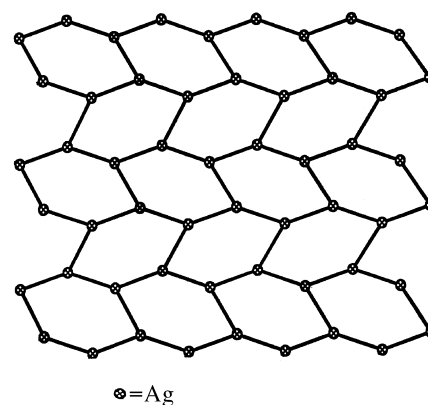


Figure 2. The graphite-like hexagonal motif of the array of silver(I) ions in **1**.

Single-crystal X-ray diffraction analysis showed this new product to be an isomer of **1**, $[\text{Ag}_6(\text{PyS})_6]_n$ (**3**), which has been prepared in our laboratory by a different method,^[6] this transformation of **1** into **3** indicates that **1** is metastable.

The results described above suggest that the reaction of Ag^+ with PySH might be temperature and solvent dependent. Thus the reaction conditions were changed; at room temperature the reaction of AgBF_4 and pyridine-2-thiol (1:1) in CH_3CN followed by addition of 0.5 mL of H_2O gave a clear solution. This solution was kept at room temperature for three days and gave pale yellow crystals of **2**. The structure of **2** (Figure 3),^[7] also has an extended two-dimensional motif but

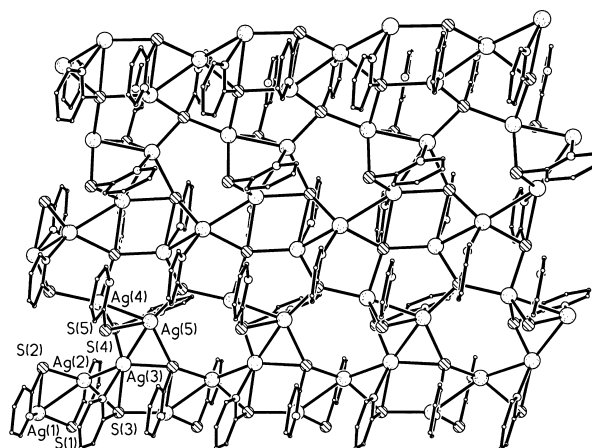
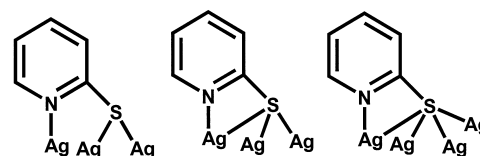


Figure 3. A view of the layered structure in **2**.

quite different from that of **1**. The BF_4^- ions are embedded in the interlayer region. Pyridine-2-thiol exists in the forms pyridine-2-thiolate and 1*H*-pyridine-2-thione, where pyridine-2-thiolate can take the three different coordination modes shown in Scheme 1 while 1*H*-pyridine-2-thione acts only as a μ_2 bridge to link two silver atoms through its sulfur atom.



Scheme 1. Coordination modes of pyridine-2-thiolate in **2**.

In the infinite solid-state structure of **2**, the silver centers are found in three coordination environments, that is, AgNS_2 in a distorted planar trigonal geometry, and AgNS_3 , and AgS_4 in a distorted tetrahedral geometry. The Ag–N and Ag–S bond lengths range from 2.28 to 2.44 Å, and from 2.45 to 2.90 Å, respectively. Unlike the array of silver ions in **1**, that in **2** is irregular and the adjacent Ag–Ag bond lengths range from 2.995 to 3.810 Å. As discussed below, the difference in structure between **1** and **2** results in their different conductivities.

An infinite hexagonal array of silver ions has recently been discovered by Shimizu et al. in the structure of silver benzenesulfonate.^[9] The formation of a silver(I) layer with a uniform motif in different coordination polymers is likely to be because of Ag–Ag interactions, that is, argentophilicity.^[10] This rational is supported by the specific orientation of the Ag–Ag interaction and by the observed inter-metallic distances that are close to those of ligand-unsupported Ag–Ag interactions.^[11] According to Jansen,^[10d] the evidence for interaction or bonding between the formally closed-shell d^{10} cations in extended solid structures is provided not only by characteristic uniform structural features but also by their physical properties such as a large red-shift of the absorption in their UV/Vis spectra and by electrical conductivity. Therefore, the conductivity of **1**, **2**, and other related polymers synthesized in our laboratory^[6] was studied. Determination of the conductivity of **1** (powder sample from ground crystals) demonstrated that its electrical conductivity is $2.04 \times 10^{-5} \text{ Scm}^{-1}$ at 298 K and increases as the temperature rises (Figure 4), which indicates that **1** is a semiconductor. However, the conductivity studies of **2**, **3**, and other silver(I) thiolate polymers showed that their electrical conductivities

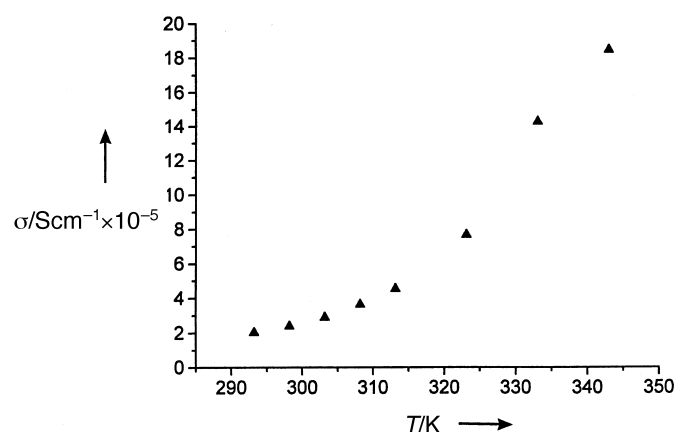


Figure 4. Temperature dependence of the electrical conductivity of **1**.

are in the range of 10^{-12} to $10^{-15} \text{ Scm}^{-1}$ at room temperature and independent of the temperature, which indicates that they are insulators. By comparison of the polymer structures the semiconductivity of **1** can be attributed to its characteristic structural feature, the graphite-like array of silver(I) ions. In turn, this semiconductivity provides evidence for Ag–Ag interactions in **1**.

In summary, two layered silver(I) thiolate polymers were obtained from the reaction of silver salts with pyridine-2-thiol under different conditions, which suggests that the reaction

conditions can influence the formation of silver thiolate polymers. The semiconductivity of **1** that distinguishes it from other silver(I) thiolate polymers results from its lamella structure with a graphite-like array of silver(I) ions. The semiconductivity provides evidence for Ag–Ag interactions that have been considered as a factor directing the formation of the hexagonal array of silver ions. Since hexagonal structural units of MoS_2 can be used to construct nested inorganic fullerenes and nanotubes^[12] a goal of our research is to assemble analogous aggregates by using Ag_6 hexagons as building blocks.

Experimental Section

1: AgBF_4 (0.195 g, 1 mmol) was dissolved in DMF (10 mL) and added to a solution of pyridine-2-thiol (0.11 g, 1 mmol) in DMF (10 mL) in an ice bath. The mixture was stirred for 30 min at 0°C , then H_2O (0.5 mL) was added. Colorless, thin sheetlike hexagonal crystals of **1** were obtained after allowing Et_2O vapor to diffuse into the resulting solution at 0°C for 2 weeks. IR (KBr): $\tilde{\nu} = 3021, 1559, 1443, 1414, 1126, 766, 721 \text{ cm}^{-1}$. Elemental analysis calcd (%) for $\text{C}_5\text{H}_4\text{AgNS}$: C 27.52, H 1.83, N 6.42; found: C 27.69, H 1.90, N 6.38. By using the experimental procedure above **1** could also be formed from the reaction of AgNO_3 with pyridine-2-thiol.

2: AgBF_4 (0.19 g, 1 mmol) was added to a solution of pyridine-2-thiol (0.11 g, 1 mmol) in CH_3CN (20 mL). The mixture was stirred for 0.5 h, then H_2O (0.5 mL) was added. The resulting solution was kept at room temperature for 3 d and gave pale yellow crystals of **2**. IR (KBr): $\tilde{\nu} = 3213 - 2991, 1579, 1416, 1130, 1084, 1070, 1003, 777, 746, 721 \text{ cm}^{-1}$. Elemental analysis calcd (%) for $\text{C}_{25}\text{H}_{21}\text{Ag}_5\text{BF}_4\text{N}_5\text{S}_5$: C 25.47, H 1.78, N 5.94; found: C 25.37, H 1.82, N 6.02.

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- [7] Crystal data for **1**: Crystal dimensions $0.06 \times 0.45 \times 0.45 \text{ mm}$, formula $\text{C}_5\text{H}_4\text{NAgS}$, $M_r = 233.60$, monoclinic, space group $\text{C}2/c$, $a = 34.335(7)$, $b = 7.0167(14)$, $c = 6.0833(12) \text{ Å}$, $\beta = 97.98^\circ$, $V = 1451.4 \text{ Å}^3$, $Z = 8$, $R(\text{wR}) = 0.079(0.180)$ for 837 reflections with $F \geq 2.0\sigma(F_o)$. The intensity data were collected on an Enraf-Nonius CAD4 diffractometer with graphite-monochromated MoK_α radiation at room temperature. Crystal data for **2**: Crystal dimensions $0.35 \times 0.30 \times 0.06 \text{ mm}$, formula $\text{C}_{25}\text{H}_{21}\text{Ag}_5\text{BF}_4\text{N}_5\text{S}_5$, $M_r = 1177.93$, monoclinic, space group Pc , $a = 7.0206(7)$, $b = 13.7308(13)$, $c = 16.9225(16) \text{ Å}$, $\beta = 93.133^\circ$, $V = 1628.9(3) \text{ Å}^3$, $Z = 2$, $R(\text{wR}) = 0.0498(0.1336)$ for 5566 reflections with

$F \geq 2.0\sigma(F_o)$. The intensity data were collected on a Bruker CCD diffractometer with graphite-monochromated $\text{MoK}\alpha$ radiation at room temperature. 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-141837 for **1** and -141836 for **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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Controlled Assembly of Polyoxometalate Chains from Lacunary Building Blocks and Lanthanide-Cation Linkers**

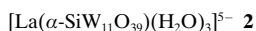
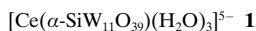
Masahiro Sadakane, Michael H. Dickman, and Michael T. Pope*

Polyoxometalates, in addition to their considerable potential as catalysts and catalytic precursors, and their applications in biochemistry and medicine,^[1] are attracting increased attention as components of supramolecular complexes and materials.^[2]

In recent years, a number of very large polyoxometalate anions have been synthesized and structurally characterized. Beginning with the “big wheel” Mo_{154} anion, Müller’s group have reported several giant mixed-valence polyoxomolybdates with cyclic (Mo_{176}),^[3] icosahedral (Mo_{132}),^[4] capped cyclic (Mo_{248}),^[5] and “basket” (Mo_{116})^[6] architectures. In polytungstate chemistry we have described quite different structures (for example W_{148}),^[7] and Zubietta and co-workers have reported one- and two-dimensional polymeric oxometalates in several organic-inorganic hybrid materials.^[8] Although these substances and materials can be synthesized in modest to good yield from monometallic components in one-pot reactions, it is of course generally true that the composition and structure of the products could not have been predicted. However, once structural principles are recognized, syntheses of targeted species can be designed, as has

been demonstrated by the work of Müller and co-workers with the large polymolybdates.

In the course of our investigations into the construction of large inorganic clusters from lacunary polyoxotungstate anions with lanthanide (Ln) or actinide cations as assembling groups,^[9] we report a new simple strategy to yield infinite one-dimensional polyoxometalates that are recrystallizable from aqueous solution. Two types of polymer chain are exemplified in the structures of the ammonium salts of **1** and **2**. These are 1:1 complexes of the type first reported by Peacock and



Weakley,^[10] and they can be isolated by mixing solutions of the lacunary polytungstates with approximately 3.8 equivalents of $\text{Ln}(\text{NO}_3)_3$, and recrystallization from aqueous ammonium chloride. The excess $\text{Ln}(\text{NO}_3)_3$ ensures high yields of the desired product. Decreasing the amount of $\text{Ln}(\text{NO}_3)_3$ results in the formation of the 1:2 complex ($[\text{Ln}(\alpha\text{-SiW}_{11}\text{O}_{39})_2]^{13-}$) as a side product. If present, this 1:2 complex can be removed by recrystallization.

As shown in Figures 1 and 2, the anions are polymeric in the solid state. The $\alpha\text{-SiW}_{11}\text{O}_{39}$ units are connected by the

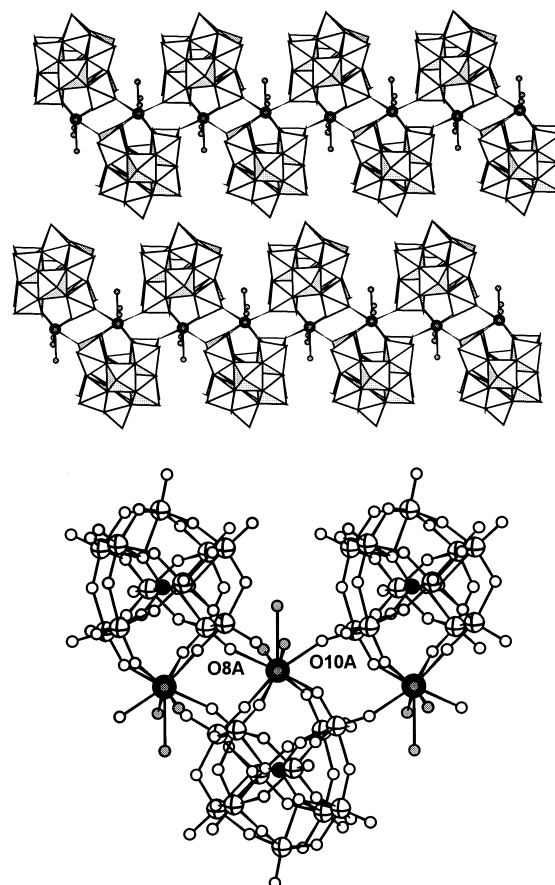


Figure 1. Structure of **1** Top: Polyhedral representation (black circle: cerium and gray circle: water oxygen). Bottom: Ball-and-stick representation (large black circle: cerium, small black circle: silicon, open circle: oxygen, gray circle: water oxygen, and crossed circle: tungsten). The chain is parallel to the crystallographic *a* axis.

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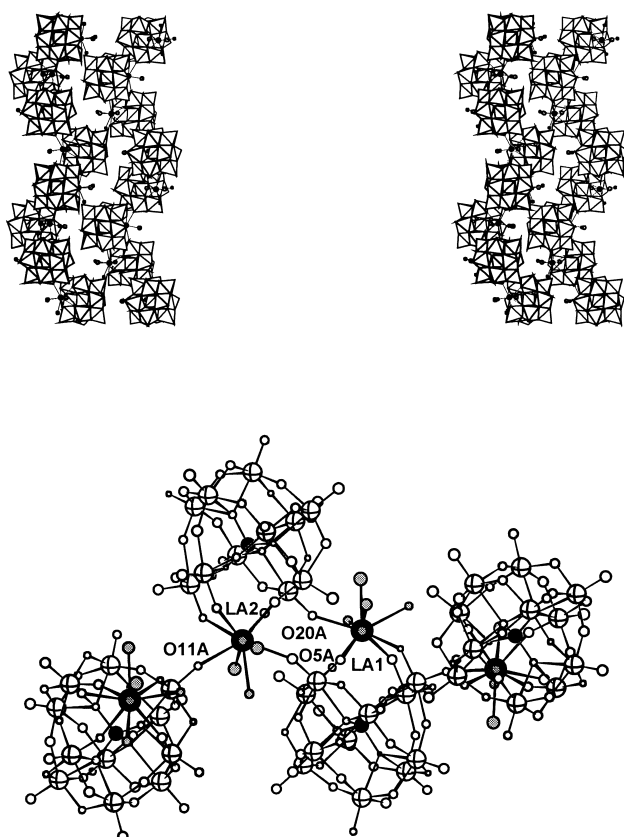


Figure 2. Structure of **2** Top: Stereoview of the polyhedral representation. Bottom: Ball-and-stick representation (large black circle: lanthanum, small black circle: silicon, open circle: oxygen, gray circle: water oxygen, and crossed circle: tungsten).

lanthanide cations, and the structure of the polymer depends on the identity of the connecting cation. The cerium complex **1** is a polymer with an inversion center between the two $[\text{Ce}(\alpha\text{-SiW}_{11}\text{O}_{39})(\text{H}_2\text{O})_3]^{5-}$ units in the unit cell (Figure 1), and the lanthanum complex **2** is a polymer with no inversion center (Figure 2). Both lanthanide cations are coordinated by nine oxygen atoms in a distorted monocapped square-antiprism environment. The four oxygen atoms that form one uncapped face are the oxygen atoms that surround the vacant site of the lacunary anion. In **1** the cerium cations are additionally coordinated by two neighboring $\alpha\text{-SiW}_{11}\text{O}_{39}$ units, through terminal oxygen atoms (O8A and O10A), and by three water oxygen atoms (gray circles, Figure 1). In the lanthanum complex **2**, on the other hand, two kinds of lanthanum atoms (LA1 and LA2 in Figure 2) alternate in the chain; LA1 is coordinated by only one additional $\alpha\text{-SiW}_{11}\text{O}_{39}$ unit through O20A, while LA2 is coordinated by two additional $\alpha\text{-SiW}_{11}\text{O}_{39}$ units through O5A and O11A. The average bond length between the lanthanide cation and oxygen atom (2.54(17) Å for **1** and 2.56(5) Å for **2**) is comparable to the sum of the Ln–O ionic radii for 9-coordinate Ln^{3+} and 2-coordinate O^{2-} ions (2.546 Å for Ce–O and 2.566 Å for La–O).^[11] The larger standard deviation for the average Ce–O bond length is a consequence of the longer “capping water” distance (2.98(4) Å) compared with the corresponding La–O bond lengths (2.69(2) and 2.58(2) Å). The interatomic

distances between the lanthanide centers are 6.554 and 6.596 Å for **1**; 6.880 and 7.644 Å for **2**.

The poly- $[\text{Ce}(\alpha\text{-SiW}_{11}\text{O}_{39})(\text{H}_2\text{O})_3]$ anion chains in **1** are arranged parallel to the crystallographic *a* axis (Figure 3, upper), whereas the bulkier poly- $[\text{La}(\alpha\text{-SiW}_{11}\text{O}_{39})(\text{H}_2\text{O})_3]$ anion chains in **2** mesh together in both the *ac* and *ab* planes (Figures 2 and 3).

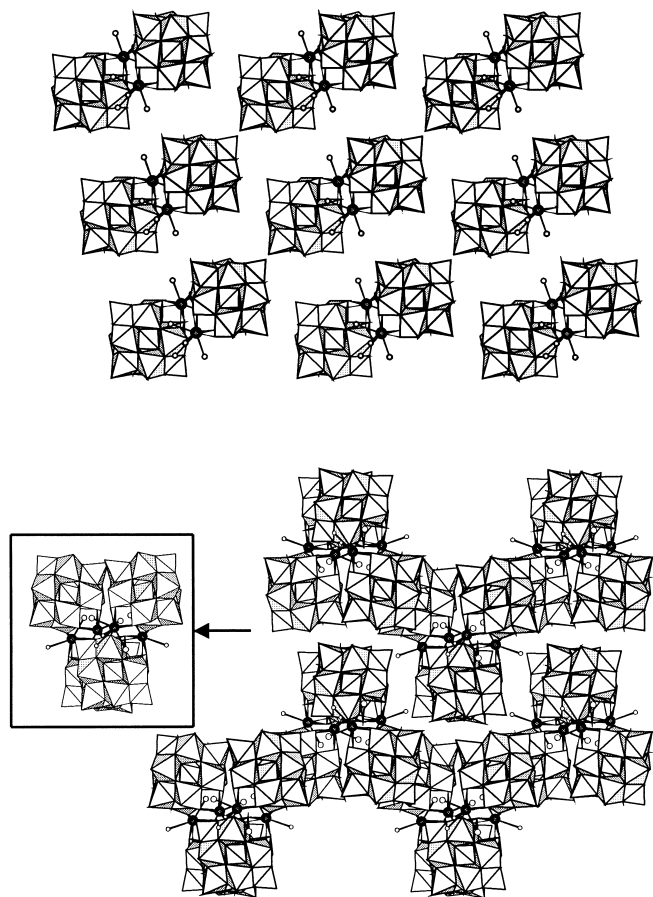


Figure 3. Packing of anions in planes perpendicular to chain axis. Top: **1** viewed down the crystallographic *a* axis. Bottom: **2** viewed down the crystallographic *c* axis. The *a* axis is horizontal. The inset shows a view down a single anion chain of **2**.

Although **1** and **2** exist in polymeric form in the solid state, there is good evidence to show that in aqueous solution they dissociate into the monomeric building blocks, $[\text{Ln}(\alpha\text{-SiW}_{11}\text{O}_{39})(\text{H}_2\text{O})_n]^{5-}$. The ^{183}W NMR spectra summarized in Table 1 show the characteristic six-line pattern consistent with the C_s symmetry of the $\alpha\text{-SiW}_{11}\text{O}_{39}$ group.^[12] The differences in the chemical shifts observed in the spectra of solutions of **1**, **2**, the free $\alpha\text{-SiW}_{11}\text{O}_{39}$ anion, and the previously established 1:2 complexes^[12c, 13] $[\text{Ln}(\alpha\text{-SiW}_{11}\text{O}_{39})_2]^{13-}$ demonstrate the identity and integrity of each species in solution.

Experimental Section

Crystals of the ammonium salts of **1** and **2** were isolated as follows: A hot (80 °C) solution of $\text{K}_8\text{SiW}_{11}\text{O}_{39} \cdot x\text{H}_2\text{O}$ (2.28 g, ca. 0.76 mmol) in water (10 mL) was added slowly to a hot (80 °C) stirred solution of $\text{Ln}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (Ln = Ce: 1.26 g, 2.90 mmol or La: 1.21 g, 2.79 mmol) in water (10 mL), over 30 min. After the solution had been stirred at this temper-

Table 1. ^{183}W NMR chemical shifts.

Complex	Chemical shift [ppm]
1 ^[a]	326.3, 110.0, –104.8, –115.1, –132.4, –161.8
2 ^[b]	–110.5, –117.3, –139.7, –144.8, –171.5, –173.7
$[\alpha\text{-SiW}_{11}\text{O}_{39}]^{8-}$ ^[c]	–105.2, –106.0, –120.5, –121.6, –143.5, –173.5
$[\text{Ce}(\alpha\text{-SiW}_{11}\text{O}_{39})_2]^{13-}$ ^[d]	313.6, 139.0, –101.3, –116.2, –130.1, –163.4
$[\text{La}(\alpha\text{-SiW}_{11}\text{O}_{39})_2]^{13-}$ ^[e]	–107.2, –117.7, –132.7, –137.7, –159.6, –172.0

[a] Ammonium salt (0.3 g) in D_2O (2.5 mL). [b] Lithium salts were prepared in situ, because of the low solubility of the ammonium salt in D_2O . Lithium salt of $[\alpha\text{-SiW}_{11}\text{O}_{39}]^{8-}$ (1.5 g) in D_2O (5.0 mL) + 1 equiv of $\text{La}(\text{NO}_3)_3$. [c] Lithium salt of $[\alpha\text{-SiW}_{11}\text{O}_{39}]^{8-}$ (1.5 g) in D_2O (5.0 mL). [d] Ammonium salt (1.0 g) in D_2O (2.5 mL). [e] Lithium salt of $[\alpha\text{-SiW}_{11}\text{O}_{39}]^{8-}$ (1.5 g) in D_2O (5.0 mL) + 0.5 equiv of $\text{La}(\text{NO}_3)_3$.

ature for 5 min, a solution of 4 M NH_4Cl (30 mL) at the same temperature was added the reaction mixture allowed to cool to room temperature and filtered. Addition of a threefold excess of ethanol to the cooled filtrate yielded a precipitate (for **1** 1.84 g, 84.5%; for **2** 1.97 g, 84.2% based on $[\text{SiW}_{11}\text{O}_{39}]^{8-}$), which was collected by filtration and recrystallized twice from hot 2 M NH_4Cl solution to give 1.03 g (**1**) and 1.14 g (**2**) corresponding to yields of about 48%.

Elemental analysis (KANTI Technologies, Inc., Amherst, NY, USA) of air-dried materials calcd % for $(\text{NH}_4)_5[\text{Ce}(\text{SiW}_{11}\text{O}_{39})] \cdot 7\text{H}_2\text{O} \cdot (\text{NH}_4\text{Cl})_{0.5}$ (**1**): N 2.52, H 1.19, Ce 4.58, Si 0.92, W 66.15, Cl 0.57; found: N 2.41, H 1.32, Ce 4.06, Si 0.75, W 66.66, Cl 0.64; calcd % for $(\text{NH}_4)_5[\text{La}(\text{SiW}_{11}\text{O}_{39})] \cdot 5\text{H}_2\text{O} \cdot \text{KCl}$ (**2**): N 2.30, H 0.99, La 4.53, Si 0.92, W 65.90, K 1.27, Cl 1.16; found: N 2.32, H 1.10, La 4.51, Si 0.87, W 65.90, K 1.20, Cl 1.19; IR (KBr disk) in metal–oxygen stretching region: for **1**: $\tilde{\nu} = 1003$ (m), 954 (s), 895 (vs), 830 (s), 797 (s) cm^{-1} , and for **2**: $\tilde{\nu} = 1006$ (m), 947 (s), 891 (vs), 833 (s), 794 (s) cm^{-1} .

Crystal structure analysis: for **1** (orange block), $(\text{NH}_4)_5[\text{Ce}(\text{SiW}_{11}\text{O}_{39})] \cdot 11\text{H}_2\text{O}$, (although chloride ions in **1**, and potassium and chloride ions in **2** were found in the elemental analyses, these elements were not found in the crystal structures, this is either because of disorder or differences between the crystal and bulk sample. Less water was found in the elemental analyses than the crystal structures, because the bulk samples were air-dried), crystal dimension $0.30 \times 0.12 \times 0.05$ mm, crystal system triclinic, space group $P\bar{1}$, $a = 11.7451(1)$, $b = 12.3956(1)$, $c = 18.2487(2)$ Å, $\alpha = 76.78$, $\beta = 80.990(1)$, $\gamma = 86.291(1)^\circ$, $V = 2554.70(4)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 4.034$ Mg m^{–3}, $2\theta_{\text{max}} = 56.64^\circ$, total measured reflections 15454, independent reflections 11152, $R_{\text{int}} = 0.0850$, $\mu = 25.659$ mm^{–1}, analytical absorption correction, min./max. transmission 0.0332/0.3811, $R_1 = 0.0929$ for 8076 reflections with $I > 2\sigma(I)$ and $wR_2 = 0.3084$ for all reflections, 354 parameters, highest residual electron density 8.980 e Å^{–3}; and for **2** (colorless block), formula $[(\text{NH}_4)_5[\text{La}(\text{SiW}_{11}\text{O}_{39})] \cdot 10\text{H}_2\text{O}]_2$, crystal dimension $0.16 \times 0.16 \times 0.20$ mm, crystal system orthorhombic, space group $Pca2_1$, $a = 22.7517(1)$, $b = 17.3293(2)$, $c = 24.3516(2)$ Å, $V = 9601.13(14)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 4.267$ Mg m^{–3}, $2\theta_{\text{max}} = 56.52^\circ$, total measured reflections 103602, independent reflections 23092, $R_{\text{int}} = 0.1200$, empirical absorption correction based on measured intensities of equivalent reflections at different ϕ and ω values ($\mu = 27.249$ mm^{–1}, min./max. transmission 0.0377/0.0963),^[14] $R_1 = 0.0694$ for 18276 reflections with $I > 2\sigma(I)$ and $wR_2 = 0.1650$ for all reflections, 690 parameters, highest residual electron density 9.413 e Å^{–3}.

Single-crystal X-ray analyses were performed on a Bruker–Siemens SMART CCD single-crystal diffractometer equipped with a Mo anode and graphite monochromator ($K\alpha$ wavelength = 0.71073 Å). An approximate sphere of data was collected using ω scans. Crystals were mounted on a glass fiber under mineral oil to prevent water loss, and placed in a nitrogen stream at 173(2) K. Routine Lorentz-polarization corrections were applied. All structures were solved using direct methods and refined against F^2 over all independent reflections using the routines included in the SHELXTL-PC software suite.^[15] No hydrogen atoms were included. Nitrogen atoms of ammonium cations were modeled as oxygen atoms because nitrogen atoms could not be distinguished from oxygen atoms. Bond valence sum calculations^[16] did not clearly indicate any protonated oxygen atoms in the anion other than the water coordinated to lanthanide ions. Further details on the crystal structure investigations may be obtained from the Fachinformationszentrum Karlsruhe, 76344 Eggenstein-Leopolds-

hafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depositary number CSD-411198 (**1**) and CDD-411199 (**2**).

^{183}W NMR was recorded at 12.504 MHz on a Bruker AM 300WB spectrometer with 2 M Na_2WO_4 as an external standard at room temperature.

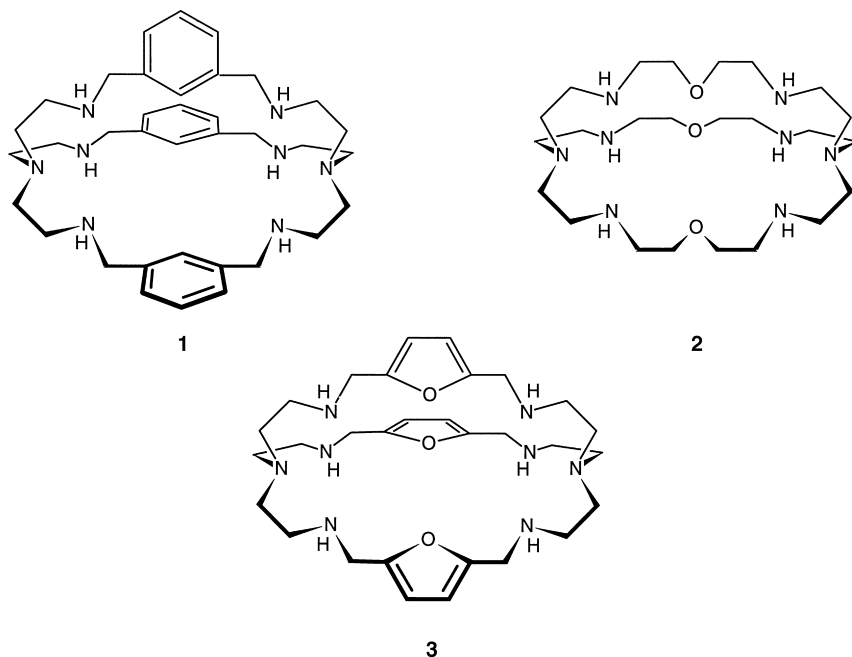
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Halide-Ion Encapsulation by a Flexible Dicopper(II) Bis-Tren Cryptate

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Homodimetallic complexes of bis-tren cryptands such as **1–3** are suitable receptors for anionic substrates. Each metal center (e.g., Cu^{II}) is coordinated by a tren subunit according to a trigonal bipyramidal stereochemistry, thus leaving an axial site vacant and available for the coordination of an anion.



The dicopper(II) complex of the bis-tren cryptand **1**, which contains rigid 3,5-xylyl spacers, encapsulates ambidentate anions such as N₃[−] and NCO[−], and the structural details of the inclusion adducts have been determined by X-ray diffraction studies. The anion collinearly bridges the two metal centers by coordination of its terminal donor atoms.^[1] Moreover, the log *K* values for the inclusion equilibrium in an aqueous solution buffered at pH 8 [Eq. (1)] were determined by



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spectrophotometric titration experiments.^[2] A sharp selectivity exists that is not related to the shape of the anion, but to its “bite length”, that is, the distance between the two donor atoms. In particular, the most favorable inclusion is observed with the N₃[−] ion, which exhibits the correct bite length to position its terminal nitrogen atoms in the vacant coordination sites of the two Cu^{II} centers without inducing any endothermic rearrangement of the polyamine cage. Monoatomic anions such as halides are not encapsulated by the cryptate [Cu₂^{II}(**1**)]⁴⁺, even if added in a large excess. Failure to include halide ions seems to be attributable to the mechanical resistance of the rigid cage framework to adjusting its cavity to the size requirements of the monoatomic guest anion. In

contrast, encapsulation of chloride ions by the cryptate [Cu₂^{II}(**2**)]⁴⁺ has been hypothesized on the basis of potentiometric titration experiments carried out in the absence and presence of NaCl.^[3] Inclusion of chloride ions and bridging of the two Cu^{II} centers should be favored by the flexible nature of the CH₂CH₂OCH₂CH₂ spacers. Halide bridging, both in the solid state and in solution, was observed in the dicopper(II) complex of the very flexible octamine ligand *N,N',N'',N'''*-tetrakis-(2-aminoethylcyclam).^[4, 5]

Among other bis-tren cryptands containing rigid spacers, we noticed the peculiar behavior of the dicopper(II) complex of **3**, in which the two tripodal tetramine subunits are linked by 2,5-dimethylfuran spacers. In particular, the complex [Cu₂^{II}(**3**)(OH)]³⁺ was isolated and structurally characterized,^[6] in which an OH[−] ion bridges the two metal centers. The Cu^{II}...Cu^{II} distance of 3.87 Å is much smaller than that observed for the com-

plexes [Cu₂^{II}(**1**)(X)]³⁺ (X = N₃[−]: 6.10 Å; NCO[−]: 6.18 Å). Hence, we thought that [Cu₂^{II}(**3**)]⁴⁺ might be able to encapsulate monoatomic anions of comparable or larger size and exhibit a different selectivity pattern than [Cu₂^{II}(**1**)]⁴⁺.

We first investigated the formation of the dicopper(II) cryptate of **3** in aqueous solution by carrying out potentiometric titration experiments on a solution of **3** and Cu^{II} in a 1:2 molar ratio (25 °C, 0.1 M NaClO₄). The log *K* values for the species present at the equilibrium were calculated, and their abundance over the pH range 2–12 is illustrated in Figure 1.

Two major dimetallic species are present in the pH 4–11 region: a [Cu₂^{II}L]⁴⁺ complex in the range pH 4–7 (maximum concentration: 85 %, at pH 5; indicated in the graph by 120) and a [Cu₂^{II}L(OH)]³⁺ complex in the range pH 6–11 (maximum concentration: 97 %, at pH 8, indicated by 12-1). A solution adjusted to pH 8 (ca. 100 % of the complex [Cu₂^{II}L(OH)]³⁺), emerald green in color, displays similar spectral features (λ_{max} = 362 nm; ε = 6500 M^{−1} cm^{−1}) to those observed for a solution of the crystalline [Cu₂^{II}L(OH)]·(ClO₄)₃ in MeCN (360 nm, 4700 M^{−1} cm^{−1}), which was structurally characterized.^[7] The development of the band centered at 362 nm, pertinent to the emerald green color, was

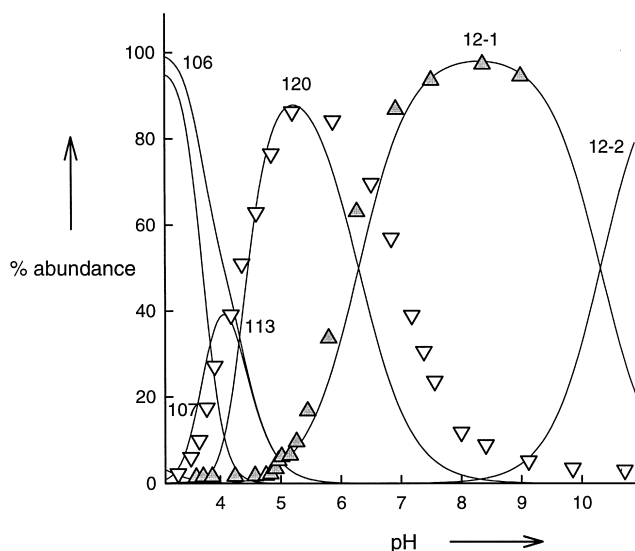


Figure 1. Percentage concentration of the species present at equilibrium in the system **3**/ Cu^{II} (ligand:metal 1:2) in a 0.1M aqueous solution of NaClO_4 at 25°C: the figures in the triads indicate the number of molecules of **3**, Cu^{2+} , and H^+ contained in each species; – 1 indicates the OH^- ion; for example, 12 – 1 symbolizes $[\text{Cu}_2^{\text{II}}(\text{3})(\text{OH})]^{3+}$. $\blacktriangle$ indicate the absorbance of the band centered at 362 nm (pertinent to the green complex $[\text{Cu}_2^{\text{II}}(\text{3})(\text{OH})]^{3+}$; arbitrary units; limiting value of molar absorbance is $6500\text{M}^{-1}\text{cm}^{-1}$). $\triangle$ indicate the absorbance of the band centered at 410 nm, measured in the presence of a tenfold excess of Cl^- (pertinent to the yellow complex $[\text{Cu}_2^{\text{II}}(\text{3})(\text{Cl})]^{3+}$; arbitrary units; limiting value of molar absorbance is $12600\text{M}^{-1}\text{cm}^{-1}$).

then monitored at varying pH by spectrophotometric titration: the molar absorbance profile superimposes well on the percentage concentration profile of the $[\text{Cu}_2^{\text{II}}\text{L}(\text{OH})]^{3+}$ species (see $\blacktriangle$ in Figure 1). All these results suggest that in the aqueous dicopper(II) cryptate the OH^- ion is also included within the cavity. On the other hand, in the absence of coordinating anions, the intermetallic cavity in the $[\text{Cu}_2^{\text{II}}\text{L}]^{4+}$ complex is thought to be “void” or, more realistically, occupied by water molecules or the anion of the background electrolyte, that is, ClO_4^- .

The binding tendencies of the dicopper(II) cryptates $[\text{Cu}_2^{\text{II}}\text{L}]^{4+}$ and $[\text{Cu}_2^{\text{II}}\text{L}(\text{OH})]^{3+}$ towards anions were investigated by spectrophotometric titration at varying pH in the presence of the envisaged anion X^- . Remarkable spectral effects were observed when X^- was a halide. For Cl^- , Br^- , and I^- at $\text{pH} > 3$, the solution became bright yellow, and an intense band developed above 400 nm in the spectrum (Cl^- , $\lambda_{\text{max}} = 410$; Br^- , 430 nm; I^- , 440 nm). For example, the values of the absorbance at 410 nm as a function of pH for a 10^{-3}M solution of dicopper(II) cryptate containing 10^{-2}M NaCl are shown in Figure 1 ($\triangle$). The band develops in the pH range in which the empty cryptate $[\text{Cu}_2^{\text{II}}\text{L}]^{4+}$ is present, and this suggests that in the presence of Cl^- ions a strong interaction is established between the halide ion and the metal center(s). Hence, the intense absorption band should result from a $\text{Cl}^- \rightarrow \text{Cu}^{\text{II}}$ charge-transfer transition. Similar absorbance versus pH plots were observed for solutions with 10^{-2}M bromide or iodide ions.

Yellow crystals of $[\text{Cu}_2^{\text{II}}(\text{3})(\text{Br})](\text{ClO}_4)_3$ suitable for X-ray diffraction studies were obtained on slow evaporation of the bromide-containing solution adjusted to pH 5.2 (Figure 2).^[8]

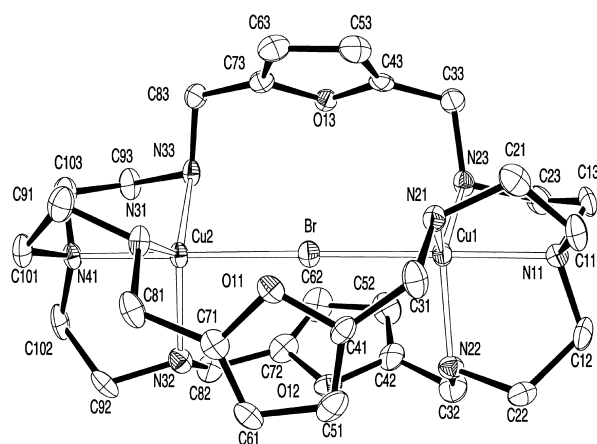


Figure 2. ORTEP plot (30% probability thermal ellipsoids) of $[\text{Cu}_2^{\text{II}}(\text{3})(\text{Br})]^{3+}$. Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Cu1–Br 2.432(1), Cu2–Br 2.435(1), Cu–N11 2.048(7), Cu1–N21 2.092(7), Cu1–N22 2.096(7), Cu1–N23 2.091(8), Cu2–N41 2.046(7), Cu2–N31 2.070(7), Cu2–N32 2.131(8), Cu2–N33 2.103(7), Cu1–Cu2 4.866(2); Cu1–Br–Cu2 179.4(1), Br–Cu1–N11 179.4(2), Br–Cu2–N41 179.4(1), N21–Cu1–N22 119.0(3), N21–Cu1–N23 123.7(3), N22–Cu1–N23 115.9(3), N31–Cu2–N32 117.3(3), N31–Cu2–N33 124.8(3), N32–Cu2–N33 116.3(3).

The Br^- ion is encapsulated within the cage and bridges the two metal centers. Cu1 and Cu2 exhibit a trigonal-bipyramidal geometry and are slightly displaced from the least-squares plane of the three secondary amino nitrogen atoms of each tren subunit towards the halide ion (0.145(1) and 0.157(1) Å, respectively). The Cu1...Cu2 distance is 4.86 Å, and the Cu1–Br–Cu2 angle is 179.4°. Hence, the bright yellow color and the intense absorption band around 400 nm can be ascribed to the inclusion of the halide ion within the dicopper(II) cryptate. Notably, the halide inclusion complex is stable only in a restricted pH range, as indicated by the bell-shaped profile of the absorbance of $[\text{Cu}_2^{\text{II}}(\text{3})(\text{Cl})]^{3+}$ (Figure 1). The same profiles, centered at pH 5.2, were also observed for the Br^- and I^- derivatives. The bright yellow solution turns emerald green on increasing the pH; this indicates that the halide ion is replaced by OH^- . Anion replacement is fast and reversible, and the solution can be made to turn yellow and green consecutively by the sequential addition of a few drops of standard base and acid.

To quantify the binding tendencies of the dicopper(II) cryptate towards halide ions and to assess selectivity effects, a solution containing **3** and two equivalents of Cu^{II} and buffered to pH 5.2 was titrated with a standard solution of sodium halide NaX. The pale blue solution (color of the void complex) turned bright yellow on addition of X^- . Figure 3 shows the family of spectra recorded during the titration with chloride ion. The titration profile in the inset corresponds to the formation of a 1:1 inclusion complex, and the log *K* value for the equilibrium $[\text{Cu}_2^{\text{II}}(\text{3})]^{4+} + \text{Cl}^- \rightleftharpoons [\text{Cu}_2^{\text{II}}(\text{3})(\text{Cl})]^{3+}$ at pH 5.2, as obtained by a nonlinear least-squares procedure, is 3.98 ± 0.02 . The limiting value of the absorption band at 410 nm is especially high ($\epsilon = 12600\text{M}^{-1}\text{cm}^{-1}$). Analogous titration profiles were obtained for bromide and iodide ions: Br^- : $\log K = 3.01 \pm 0.01$, $\epsilon = 10800\text{M}^{-1}\text{cm}^{-1}$; I^- : $\log K = 2.39 \pm 0.02$, $\epsilon = 950\text{M}^{-1}\text{cm}^{-1}$. Surprisingly, titration with NaF did not cause any development of the yellow color. However,

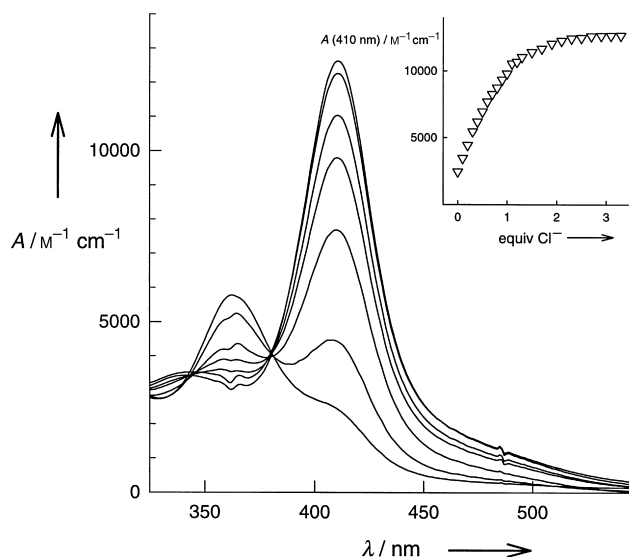


Figure 3. Titration of a solution containing $[\text{Cu}_2^{\text{II}}(\mathbf{3})]^{4+}$ with Cl^- , adjusted to pH 5.25 (0.05 M 2-morpholinoethanesulfonic acid buffer). The growing band centered at 410 nm corresponds to the formation of the inclusion complex $[\text{Cu}_2^{\text{II}}(\mathbf{3})(\text{Cl})]^{3+}$. The titration profile in the inset gave a $\log K$ value of 3.98 ± 0.02 for the equilibrium $[\text{Cu}_2^{\text{II}}(\mathbf{3})]^{4+} + \text{Cl}^- \rightleftharpoons [\text{Cu}_2^{\text{II}}(\mathbf{3})(\text{Cl})]^{3+}$.

it must be considered that the absorption band has a halide-to-metal charge-transfer (CT) character, and its energy must therefore increase with increasing electronegativity of the halogen atom. It is possible that the CT transition for the complex $[\text{Cu}_2^{\text{II}}(\mathbf{3})(\text{F})]^{3+}$, because of the high electronegativity of fluorine, is markedly shifted to lower wavelengths, in particular in the UV portion of the spectrum, where it is obscured by the intense amine-to-metal CT bands. Significant spectral modifications in the UV region were observed in the titration of the cryptate solution buffered at pH 5.2 with NaF. A saturation 1:1 profile was obtained, and the $\log K$ value was determined to be 3.20 ± 0.02 .

The $\log K$ versus halide ion radius plot shown in Figure 4 reveals selectivity for chloride ions. The effect of anion size is rather moderate, ranging within an interval of 1.2 log units. However, the unique feature of the $[\text{Cu}_2^{\text{II}}(\mathbf{3})]^{4+}$ cryptate is its

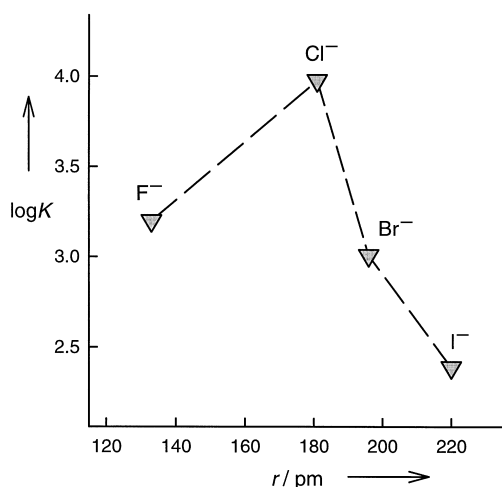


Figure 4. Selectivity pattern for the $[\text{Cu}_2^{\text{II}}(\mathbf{3})]^{4+} + \text{X}^- \rightleftharpoons [\text{Cu}_2^{\text{II}}(\mathbf{3})(\text{X})]^{3+}$ equilibrium (X^- = halide anion).

ability to accommodate anions of varying size and shape. Not only does it firmly encapsulate OH^- and halide ions, but it is also able to include linear triatomic anions such as N_3^- and NCS^- . In particular, when titrating a solution of $[\text{Cu}_2^{\text{II}}(\mathbf{3})]^{4+}$ adjusted to pH 5.2 with NaN_3 , the pale blue solution became intense olive green, and the plot of the absorbance of the band which developed at 386 nm versus equivalents of N_3^- added indicated the formation of a 1:1 adduct with $\log K = 4.70 \pm 0.06$. The development of a CT band ($\epsilon = 6100 \text{ M}^{-1} \text{ cm}^{-1}$) as well as the 1:1 stoichiometry strongly suggest that the N_3^- ion is included within the cage. Similar behavior was observed with NCS^- ($\log K = 4.28 \pm 0.03$). Thus, it appears that the $[\text{Cu}_2^{\text{II}}(\mathbf{3})]^{4+}$ complex displays an extreme versatility, being able to contract and to expand its cavity to include (and form intense metal–ligand interactions with) anions of various sizes and shape, from the small hydroxide to the large thiocyanate ions. A closer inspection of available structural data suggests that the flexibility of $[\text{Cu}_2^{\text{II}}(\mathbf{3})]^{4+}$ is associated with the mobility of the 2,5-dimethylfuran spacers. In particular, the $\text{Cu}^{\text{II}} \cdots \text{Cu}^{\text{II}}$ distance in both $[\text{Cu}_2^{\text{II}}(\mathbf{3})\text{Br}]^{3+}$ and $[\text{Cu}_2^{\text{II}}(\mathbf{3})\text{OH}]^{3+}$ complexes can be varied at a low energy cost by adjusting the torsion angles along the chain arms adjacent to the 2,5-dimethylfuran spacer. Indeed, dramatic differences are observed in the $\text{NH}-\text{CH}_2-\text{C}-\text{O}$ torsion angles in the two complexes: in the μ -bromo derivative the average value of such torsion angles is $-69(1)^\circ$ for the NH groups coordinated to one copper(II) center and $57(1)^\circ$ for the remaining NH groups; in the μ -hydroxo dicopper(II) complex the $\text{NH}-\text{CH}_2-\text{C}-\text{O}$ torsion angles vary from $-68(1)$ to $76(1)^\circ$ for the NH groups coordinated to one copper(II) center, and from $75(1)$ to $-58(1)^\circ$ for the NH groups coordinated to the other copper(II) center. In other words, each $\text{NH}-\text{CH}_2-\text{C}-\text{O}$ group behaves as a spring, which controls the length of the intermetallic cavity and can be operated through the variation of the magnitude of the corresponding dihedral angle. Apparently, an analogous spring mechanism can not be exhibited by the arms adjacent to the phenyl spacer in $[\text{Cu}_2^{\text{II}}(\mathbf{2})]^{4+}$, which does not contract its cavity and does not include monoatomic anions. It is possible that the $[\text{Cu}_2^{\text{II}}(\mathbf{3})]^{4+}$ cage can establish further unique bonding interactions which favor halide inclusion. In this regard, the intramolecular distances between C(4), O(1), and C(7) of each furan ring and the Br atom ($\text{O} \cdots \text{Br}$ 3.172(6)–3.212(6) Å, $\text{C} \cdots \text{Br}$ 3.339(8)–3.420(9) Å) are distinctly shorter than the sum of the van der Waals radii (3.37 and 3.55 Å, respectively). Hence, weak yet distinct interactions exist between the furan oxygen atoms and the encapsulated bromide ion in $[\text{Cu}_2^{\text{II}}(\mathbf{3})\text{Br}]^{3+}$. These interactions cannot be established in the $[\text{Cu}_2^{\text{II}}(\mathbf{2})]^{4+}$ cage, which contains phenyl spacers, and may help to balance endothermic effects, if any, associated with cage contraction.

In conclusion, $[\text{Cu}_2^{\text{II}}(\mathbf{3})]^{4+}$ displays a special affinity towards halide ions, which are firmly encapsulated in a 1:1 stoichiometry. Halide inclusion can be detected visually by the appearance of an intense, bright yellow color, and instrumentally by the development of a very strong absorption band centered at 410–440 nm. In this regard, $[\text{Cu}_2^{\text{II}}(\mathbf{3})]^{4+}$ is an efficient colorimetric sensor of halide ions and joins the recently developed family of colorimetric sensors for inorganic anions.^[13]

Experimental Section

Cage **3** was prepared as described.^[7] Setup and procedures for spectrophotometric and potentiometric titration experiments have been reported elsewhere.^[14] Equilibrium constants were determined by a nonlinear least-squares method.^[15]

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Unprecedented Expansion of the Pore Size and Volume of Periodic Mesoporous Silica**

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The synthesis of materials with designed pore structures is of paramount importance for many applications in areas such as catalysis, adsorption, and separation.^[1] In designing such materials, several characteristics of the pore structure may be addressed, which include their shape, size, and connectivity. Since the discovery of the M41S periodic mesoporous silicas in the early 1990s,^[2] a number of synthesis strategies to control their pore size have been developed. With only a few exceptions,^[3, 4] all of the reported investigations dealt with MCM-41 silica whose pore structure consists of hexagonally packed unidimensional cylindrical channels ($p6mm$). In the latter case, the pore sizes may be adjusted from the lower end of the mesoporous range (about 2.0–2.5 nm) to well into the macroporous range. A summary of these approaches along with pertinent references is provided elsewhere.^[5] In our earlier work,^[6, 7] we found that treatment of as-synthesized 3.5 nm pore MCM-41 silica with long chain trialkylamines or *N,N*-dimethylalkylamines, typically at 100–130 °C for 2–3 days, increased the pore size to a maximum of about 11 nm. The pore volume also increased threefold from 0.8 to 2.4 $\text{cm}^3 \text{g}^{-1}$. In this paper, we report an unprecedented pore size expansion from approximately 3.15 to 25 nm with conservation of the surface area and pore wall thickness.

The filled symbol in Figure 1 shows the nitrogen adsorption–desorption isotherm for MCM-41BT (BT = before treatment) with a sharp nitrogen condensation step at a

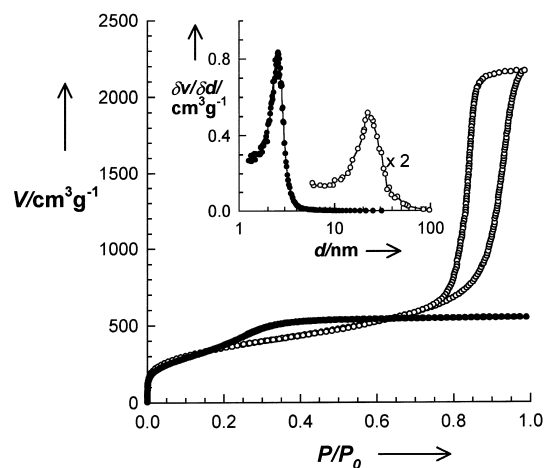


Figure 1. Nitrogen adsorption–desorption isotherms for MCM-41BT (●) and MCM-41AT (○). Inset: Corresponding PSDs ($\times 2$ indicates enhanced by a factor of 2).

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relative pressure of 0.2, characteristic of mesoporous materials with a narrow pore-size distribution (PSD). The PSD (Figure 1, inset) exhibited a maximum at 3.15 nm. The BET (Brunauer-Emmett-Teller) surface area and the total pore volume were $1316 \text{ m}^2 \text{ g}^{-1}$ and $0.86 \text{ cm}^3 \text{ g}^{-1}$. Figure 2 shows a

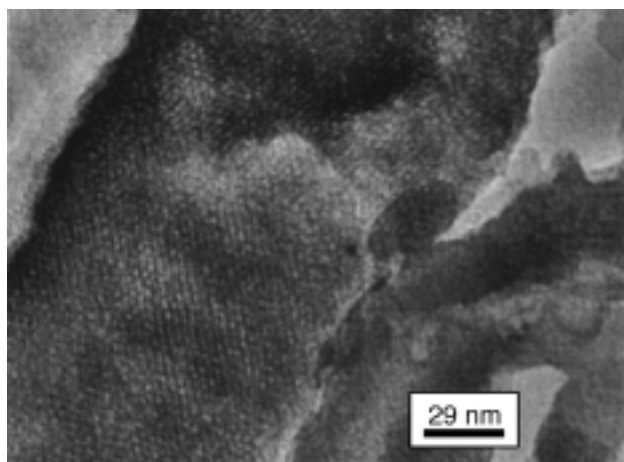


Figure 2. TEM image of MCM-41BT.

typical transmission electron micrograph (TEM) image for calcined MCM-41BT, which confirms the presence of hexagonally packed channels. As for the morphology of this material, scanning electron microscopy (SEM) showed it consisted of faceted tubules of approximate dimensions $45 \times 10 \mu\text{m}$ (Figure 3, above).

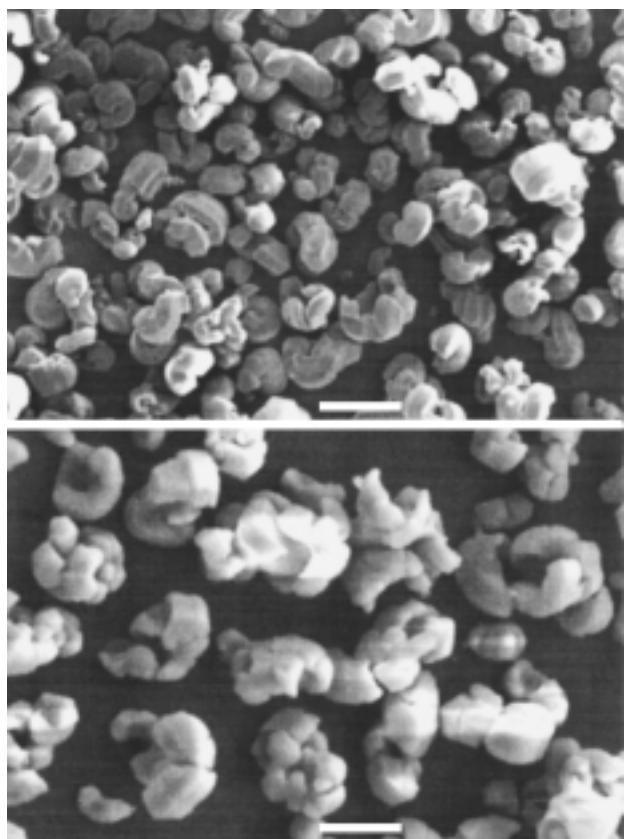
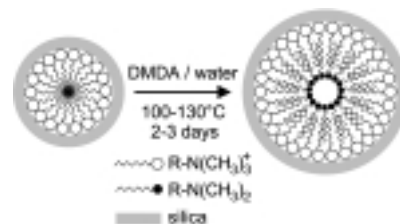


Figure 3. SEM image of MCM-41BT (above) and MCM-41AT (below). The scale bar represents $100 \mu\text{m}$.

The nitrogen adsorption-desorption isotherm for the material after treatment in the presence of *N,N*-dimethyldimethylamine (DMDA) at 120°C for 48 h, MCM-41AT (AT = after treatment), is shown as open symbols in Figure 1. The nitrogen condensation and evaporation steps were shifted strongly to higher relative pressures, which indicates the occurrence of an unprecedented pore size expansion. The PSD was relatively narrow with a maximum at 25 nm (Figure 1, inset). The pore volume also increased tremendously, from 0.85 to $3.31 \text{ cm}^3 \text{ g}^{-1}$, whereas the surface area remained almost constant at $1268 \text{ m}^2 \text{ g}^{-1}$. This indicates that the pore wall thickness did not undergo any significant changes and shows the extreme structural flexibility of the MCM-41 silica. The pore structure, however, was no longer ordered as inferred from the absence of X-ray diffraction (XRD) lines and the lack of sufficient contrast to obtain good quality TEM images. In addition, as seen by SEM, the faceted tubular shape of the particles (Figure 3, lower) did not change during the amine treatment but the particles were swollen and exhibited a high density of cracks, most likely due to the stress generated by the expansion process.

Several experimental parameters may be adjusted to fine tune the pore size and volume of MCM-41 silica through postsynthesis treatment with DMDA or other appropriate amines.^[7] First, since the degree of silica condensation in the starting material is likely to depend on the synthesis temperature, therefore, under otherwise identical DMDA treatment conditions, materials prepared at higher temperature are expected to expand to a lesser extent. For example, a MCM-41 silica prepared at 100°C had a pore size of 3.3 nm and a pore volume of $1.17 \text{ cm}^3 \text{ g}^{-1}$, which, upon amine treatment under the same conditions as above, the pore size increased to only 14 nm and the pore volume to $2.62 \text{ cm}^3 \text{ g}^{-1}$. This is in contrast to the the MCM-41 silica prepared at 80°C , in which values of 25 nm and $3.31 \text{ cm}^3 \text{ g}^{-1}$, respectively, were obtained. The amine to silica ratio is also important. For example, treatment of the same amount of MCM-41 silica (0.8 g) prepared at 100°C for 48 h at 120°C for two days in the presence of 0.3 or 0.5 g of DMDA, instead of 1 g, increased the pore size to only 6.9 or 9.4 nm, respectively, while the pore volume increased to 1.64 or $2.18 \text{ cm}^3 \text{ g}^{-1}$. Additional parameters for adjusting the pore size and volume are the temperature and duration of the postsynthetic treatment.

A tentative representation of the pore size expansion process is shown schematically in Scheme 1. Although the neutral amine may self-assemble into inverse cylindrical micelles due to hydrophobic interactions between the carbon chains, no direct proof of this effect on the pore size expansion



Scheme 1. Representation of the pore size expansion process using postsynthetic hydrothermal treatment in the presence of DMDA.

can be provided at this time. Work devoted to this issue is being carried out using labeled DMDA.

To our knowledge, the pore volume of the current MCM-41AT material ($3.31 \text{ cm}^3 \text{ g}^{-1}$) is the highest ever reported. Materials such as aerogels,^[8] mesocellular silica foams,^[9] and some hexagonal mesoporous silica prepared in the presence of amphiphilic triblock copolymers ("SBA-15")^[10] exhibit pore volumes of up to $2.5 \text{ cm}^3 \text{ g}^{-1}$, which is more than 30 % less than that of the current MCM-41AT. Because of its particularly narrow pore size distribution and very high surface area, this material has potential applications in separation or catalysis involving large molecules. Several important applications of such materials ranging from adsorption to catalysis and stabilization of nanoparticles are being developed in our laboratory. In addition, similar pore size expansion procedures using cubic MCM-48 silicas as starting materials are in progress.

Experimental Section

MCM-41 silica was prepared as follows: 3.848 g of tetramethylammonium hydroxide (TMAOH; 25 %) was diluted with water (37.1 g) before adding cetyl trimethyl ammonium bromide (CTAB; 5.466 g) under vigorous stirring. After 15 min, silica (Cab-O-Sil; 2 g) was added. The overall mixture composition was $1.0 \text{ SiO}_2:0.317 \text{ TMAOH}:0.45 \text{ CTAB}:67 \text{ H}_2\text{O}$. The gel obtained after stirring for an additional 30 min was transferred into a Teflon-lined autoclave and heated statically under autogenous pressure at 80°C for 40 h. The obtained materials were filtered, washed extensively, dried, and calcined at 540°C , first in flowing nitrogen then in air. For post-synthetic pore size expansion, 0.8 g of the as-prepared sample was added to an emulsion of DMDA (1 g) and water (30 g) at RT. After about 1 h of stirring, the mixture was heated at 120°C for 2 days under autogenous pressure. Further separation and calcination were carried out as described above.

Adsorption measurements were performed using a Coulter Ominorp 100 gas analyzer. Pore size distributions were calculated using the Kruk-Jaroniec-Sayari method.^[11] XRD spectra were obtained on a Siemens D5000 diffractometer using $\text{CuK}\alpha$ radiation ($\lambda = 0.15418 \text{ nm}$). SEM images were recorded on a JEOL 840A microscope operated at an accelerating voltage of 10–20 kV. TEM images were obtained using a Philips 430 instrument operated at 100 kV. The specimen were embedded in an epoxy resin and ultrathin sections (approximately 60 nm) were cut and examined.

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Template Synthesis of the First 1,4,7-Triphosphacyclononane Derivatives**

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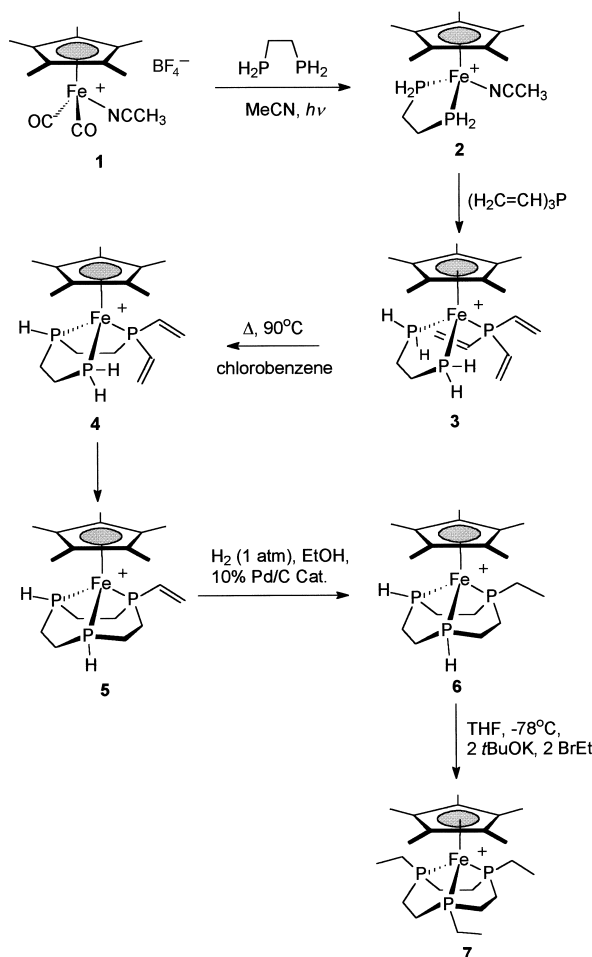
The small-ring tridentate macrocyclic ligands 1,4,7-triazacyclononane (tacn) and 1,4,7-trithiacyclononane have occupied a central role in the development of macrocyclic coordination chemistry.^[1] Surprisingly, the related homoleptic phosphorus compound, 1,4,7-triphosphacyclononane is unknown in the literature and prior to our present study, the smallest triphosphorus macrocyclic ring system known is 11-membered and was prepared by a nonstereoselective, high-dilution method.^[2] To date, the smallest (and indeed only) triphosphorus macrocycle prepared stereoselectively is based upon the 1,5,9-triphosphacyclododecane ([12]-ane-1,5,9-P₃) core by Norman and co-workers who developed a $\text{Mo}(\text{CO})_3$ template-assisted synthesis^[3] which has led to a number of derivatives and in the free ligand being released subsequently by us.^[4] This method has not allowed formation of smaller ring systems;^[5] we have consequently chosen to investigate alternative templates that may provide stereospecific routes to smaller facially capping triphosphorus macrocycles.

In this respect, we have studied reactions of cyclopentadienyliron "piano-stool" complexes which appear ideally suited to the formation of *fac*-triphosphane derivatives. In addition, the sequential selective incorporation of diphosphanes followed by monophosphanes (which is difficult to control in the $\text{M}(\text{CO})_3$ templates) is well established;^[6] this should give rise to far greater flexibility in the synthetic methodology. Herein we report on the synthesis of the first triphosphacyclononane (9aneP₃) derivatives by an iron(II)-mediated intramolecular cyclization of 1,2-bis(phosphanyl)ethane and trivinylphosphane.

The new compounds and synthetic methodologies reported herein are summarized in Scheme 1. The precursor complex $[\text{Fe}(\text{CH}_3\text{CN})(\text{CO})_2(\eta^5\text{-Me}_5\text{C}_5)]\text{BF}_4$ (**1**) was prepared by the oxidative cleavage of the dimer $[\{\text{Fe}(\text{CO})_2(\eta^5\text{-Me}_5\text{C}_5)\}_2]$ by $\text{Cp}_2\text{Fe}^+ \text{BF}_4^-$ as described by Astruc and Catheline.^[7] UV photolysis of a 1:1 mixture of **1** and 1,2-bis(phosphanyl)ethane gives the diprimary phosphane acetonitrile cation **2** as the tetrafluoroborate salt. The $^{31}\text{P}\{^1\text{H}\}$ spectrum of **2** shows the expected singlet at $\delta = 7$, which appears as a triplet ($^1J_{\text{PH}} = 343 \text{ Hz}$) in the proton-coupled spectrum. Heating **2** with an equimolar quantity of trivinylphosphane in 1,2-dichloroethane or chlorobenzene at $70\text{--}80^\circ\text{C}$ over 2 h gave the diprimary monotertiary phosphane complex **3**. The latter was identified by the presence of a doublet at $\delta = 15.5$ and a triplet at $\delta = 49.5$ ($^2J_{\text{PP}} = 47 \text{ Hz}$) in the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum. When **3** was heated at 80°C in 1,2-dichloroethane or chlorobenzene

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Scheme 1. Synthesis of the fully ethylated complex **7** from the precursor **1**.

for 24 h, the characteristic doublet and triplet in the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum were gradually replaced by a new set of peaks consistent with an AMX spin system at $\delta_{\text{A}} = 18.8$ (dd), $\delta_{\text{M}} = 85.0$ (dd), and $\delta_{\text{X}} = 95.2$ (t) (where $J_{\text{A,M}} = 36$, $J_{\text{A,X}} = J_{\text{M,X}} = 22$ Hz) and which correspond to the primary, tertiary, and secondary phosphanes, respectively, of the intermediate complex **4**. The assignments were confirmed by ^{31}P NMR spectroscopy, where the high- and low-field signals appear as a triplet and doublet, respectively ($^1J_{\text{P(A),H}} = 344$ Hz, $^1J_{\text{P(M),H}} = 348$ Hz). Continued heating led to the gradual disappearance of these resonance signals and the growth of two signals at $\delta = 108.2$ (d, $J_{\text{A,X}} = 5$ Hz, secondary phosphanes) and $\delta = 117.0$ (t, $J_{\text{A,X}} = 5$ Hz, tertiary phosphane) representing the expected A_2X spectrum of the fully cyclized product **5**. These low-field shifts are characteristic of coordinated phosphanes in fused five-membered chelate rings.^[8] When complex **2** is heated with a 50 % molar excess of trivinylphosphane in chlorohydrocarbons, complexes **4** and **5** form more rapidly suggesting an intramolecular base-catalyzed Michael-type addition of primary phosphane to the coordinated vinylphosphane adduct. This is further supported by the observation that the addition of triethylamine (≤ 1 molar equivalent) leads to complete conversion to **5** within 12 h.

Complex **5** is isolated as its air- and moisture-stable tetrafluoroborate salt in 50 % yield. Catalytic hydrogenation of the vinyl group in **5** occurs readily at room temperature and

1 atm pressure with a Pd/C catalyst to give **6** in high yield (85 %). Treatment of **6** with 2 molar equivalents of potassium *tert*-butoxide and excess bromoethane gives the fully ethylated complex $[\text{Fe}(\eta^5\text{-Me}_5\text{C}_5)(9\text{aneP}_3\text{-Et}_3)]^+$ (**7**), which was isolated as a mixed bromide/tetrafluoroborate salt in quantitative yield (Scheme 1), the bromide counterion originating from the bromoethane.

The structure of the $[\text{Fe}(\eta^5\text{-Me}_5\text{C}_5)(9\text{aneP}_3\text{-Et}_3)]^+$ ion is shown in Figure 1.^[9] The structure clearly shows the nine-membered triphosphamacrocycle coordinating in a facial

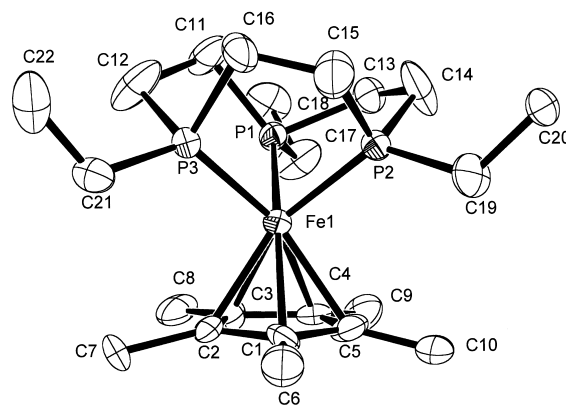


Figure 1. Structure of the cation **7** (Ortep representation^[15]). Selected bond lengths [Å] and angles [°]: Fe-P1 2.1912(12), Fe-P2 2.195(2), Fe-P3 2.195(2); P1-Fe-P2 85.02(8), P1-Fe-P3 84.15(7), P2-Fe-P3 86.41(5), Fe-P1-C11 110.6(2), Fe-P1-C13 109.3(2), Fe-P1-C17 122.9(7), Fe-P2-C14 109.9(3), Fe-P2-C15 110.2(4), Fe-P2-C19 127.7(3), Fe-P3-C12 109.5(3), Fe-P3-C16 110.8(3), Fe-P3-C21 125.8(3).

fashion to the Fe^{II} center. The Fe-P bond lengths (av 2.194(2) Å) are very similar to those in related cyclopentadienyl complexes of acyclic phosphanes (e.g. in $[(1,2\text{-C}_6\text{H}_4\text{-PMePh})_2](\text{PMePh})(\eta^5\text{-Cp})\text{Fe}]^+$, Fe-P av 2.179(2) Å^[7a]) and the P-Fe-P angles (av 85.2(1)°) are somewhat compressed in comparison to those observed in related Mo^0 complexes of the 12-membered triphosphane analogue.^[4b] The cyclopentadienyl methyl groups are displaced out of the C_5 ring plane away from the macrocycle indicating steric repulsion between the two rings; this is also manifested in the Fe-P-C(ethyl) bond angles (av 125.5(7)°).

In conclusion, the metal-templated cyclization of 1,2-bis(phosphanyl)ethane with trivinylphosphane at an Fe^{II} center has produced coordinated 1-vinyl-1,4,7-triphosphacyclononane. The vinyl substituent is readily hydrogenated to an ethyl group and this resulting disubstituted phosphane macrocycle is then cleanly converted to the C_3 -symmetrical triphosphorus macrocycle. This is the first reported synthesis of triphosphacyclononanes; the further chemistry of this and related compounds is being investigated. Preliminary studies indicate that this template system is indeed very versatile; details of variations in macrocyclic structure and substituents, and of the chemistry of derived complexes will appear in the near future.

Experimental Section

2: A solution of **1** (1.0 g, 2.42 mmol) and 1,2-bis(phosphanyl)ethane (2.30 mL of a 10 % wt/v solution in toluene, 2.42 mmol) was irradiated for

12 h with a Hanovia 125 W UV lamp. The solvent was subsequently removed and the resultant residue crystallized from hot tetrahydrofuran to give complex **2** as large red crystals. Yield = 0.9 g (82 %). $^{31}\text{P}\{^1\text{H}\}$ NMR (CDCl_3 , 36.23 MHz): δ = 7.0; ^1H NMR (CDCl_3 , 400 MHz): δ = 4.72 (d br., 4H, J_{PH} = 334 Hz; PH_2), 2.46 (s, 3H; CH_3CN), 2.0–1.7 (m, 4H; CH_2), 1.56 (s, 15H; CpCH_3); ^{13}C NMR (CDCl_3 , 100 MHz): δ = 130.2 (s; CH_3CN), 85.8 (s, C; Cp), 15.3 (m; CH_2), 8.6 (s; CpCH_3), 3.7 (s; CH_3CN); elemental analysis calcd for $\text{C}_{14}\text{H}_{26}\text{NP}_2\text{BF}_4\text{Fe}$ (%): C 40.71, H 6.36, N 3.39; found: C 40.5, H 6.4, N 3.2; IR (KBr): $\tilde{\nu}$ = 2315 m (ν_{PH}), 2241 m (ν_{CN}).

5: A solution of $[\text{Fe}(\eta^5\text{-Me}_5\text{C}_5)(\text{CH}_3\text{CN})(1,2\text{-bis}(\text{phosphanyl})\text{ethane})]\text{BF}_4$ (1.0 g, 2.42 mmol) and trivinylphosphane (0.28 mL, 2.50 mmol) in 1,2-dichloroethane (50 mL) was heated at 80 °C for 2 h. After cooling, the mixture was filtered and the volatiles removed in vacuo to give **3** as a yellow-orange solid. $^{31}\text{P}\{^1\text{H}\}$ NMR (CDCl_3 , 36.23 MHz): δ = 49.5 (t, J = 47 Hz), 15.5 (d, J = 47 Hz). The solid was dissolved in chlorobenzene (50 mL) containing triethylamine (0.1 mL, 0.72 mmol) and the solution heated at 80 °C for 12 h. After the mixture had been filtered, the solvent was removed in vacuo and the orange-yellow residue extracted into ethanol (150 mL). Removal of solvent gave **5** as a yellow solid which was crystallized from dichloromethane/diethyl ether at 4 °C to give bright yellow plates. Yield = 600 mg (50 %). $^{31}\text{P}\{^1\text{H}\}$ NMR (CDCl_3 , 36.23 MHz): δ = 115.2 (t, J = 5 Hz), 106.2 (d, J = 5 Hz); ^1H NMR (CDCl_3 , 400 MHz): δ = 6.38 (m, 1H; PCH:CH_2), 6.04 (dd, 1H, J = 12 and 33 Hz; PCH:CH_2), 5.70 (t, J = 18 Hz, 1H; PCH:CH_2), 5.55 (d br., 2H, J = 343 Hz; PH), 2.1–1.5 (m, 12H; CH_2), 1.66 (s, 15H; CpCH_3); ^{13}C NMR (CDCl_3 , DEPT, 100 MHz): δ = 134.0 (d, 30 Hz; PCH:CH_2), 128.2 (d, J = 5 Hz; PCH:CH_2), 89.0 (s; Cp), 28.9 (dd, J = 30 and 5 Hz; CH_2), 23.3 (m; CH_2), 22.0 (dd, J = 29 and 5 Hz; CH_2), 10.9 (s; CpCH_3); IR (KBr): $\tilde{\nu}$ = 2335 m (ν_{PH}); MS (APCI): m/z : 397 (100) $[(\eta^5\text{-Me}_5\text{C}_5)\text{FeL}]^+$; elemental analysis calcd for $\text{C}_{18}\text{H}_{32}\text{BF}_4\text{P}_3\text{Fe}$ (%): C 44.66, H 6.68; found: C 44.7, H 6.6.

6: Hydrogen was bubbled slowly through a solution of **5** (0.30 g, 6.2×10^{-4} mol) in 1 % aqueous ethanol containing 10 % palladium on carbon for five days. The catalyst was filtered off with the aid of celite and the solvent removed in vacuo to give a yellow solid. Yield = 0.30 g (quant.); $^{31}\text{P}\{^1\text{H}\}$ NMR (CDCl_3 , 36.23 MHz): δ = 124.6 (t, J = 7 Hz), 108.7 (d, J = 7 Hz); ^1H NMR (CDCl_3 , 400 MHz): δ = 5.54 (d br., 2H, J = 343 Hz; PH), 1.95 (m, 2H; CH_2CH_3), 1.9–1.0 (m, 12H; CH_2), 1.70 (s, 15H; CpCH_3), 1.20 (dt, J = 14 and 7 Hz, 3H; CH_2CH_3); ^{13}C NMR (CDCl_3 , DEPT, 100 MHz): δ = 89.3 (s; Cp), 27.4 (m; CH_2), 23.8 (m; CH_2), 22.7 (ddd, J = 31, 14, and 3 Hz; CH_2), 21.1 (d, J = 18 Hz; CH_2), 11.4 (s; CpCH_3), 9.6 (d, J = 7 Hz; CH_3); IR (KBr): $\tilde{\nu}$ = 2350 m (ν_{PH}); MS (APCI): m/z : 399 (100) $[(\eta^5\text{-Me}_5\text{C}_5)\text{FeL}]^+$; elemental analysis calcd for $\text{C}_{18}\text{H}_{34}\text{BF}_4\text{P}_3\text{Fe}$ (%): C 44.47, H 7.06; found: C 44.4, H 7.0.

7: To a solution of **6** (100 mg, 2.1×10^{-4} mol) in THF (25 mL) at -78°C was added potassium *tert*-butoxide (60 mg, 5.4×10^{-4} mol) and the mixture stirred for five minutes at this temperature before warming to 0 °C. The mixture was cooled to -78°C and ethyl bromide (0.2 mL, 2.68 mmol) added thereto. The mixture was stirred at -78°C for 30 min then at room temperature overnight. After the mixture had been filtered, the solvent was removed in vacuo to give a yellow solid. Yield = 110 mg (97 %); $^{31}\text{P}\{^1\text{H}\}$ NMR (CDCl_3 , 36.23 MHz): δ = 124.9 (s); ^1H NMR (CDCl_3 , 400 MHz): δ = 2.04 (br., 6H; CH_2CH_3), 1.85–1.45 (m, 12H; CH_2), 1.72 (s, 15H; CpCH_3), 1.22 (br., 9H; CH_2CH_3); ^{13}C NMR (CDCl_3 , DEPT, 100 MHz): δ = 87.2 (s; Cp), 25.7 (m; CH_2), 20.2 (m; CH_2), 11.3 (s; CpCH_3), 8.4 (s; CH_3); MS (APCI): m/z : 455 (100) $[(\eta^5\text{-Me}_5\text{C}_5)\text{FeL}]^+$; elemental analysis calcd for $\text{C}_{22}\text{H}_{42}\text{BrO}_3\text{BrO}_3\text{F}_2\text{P}_3\text{Fe}$ (%): C 49.04, H 7.87; found: C 49.0, H 7.8.

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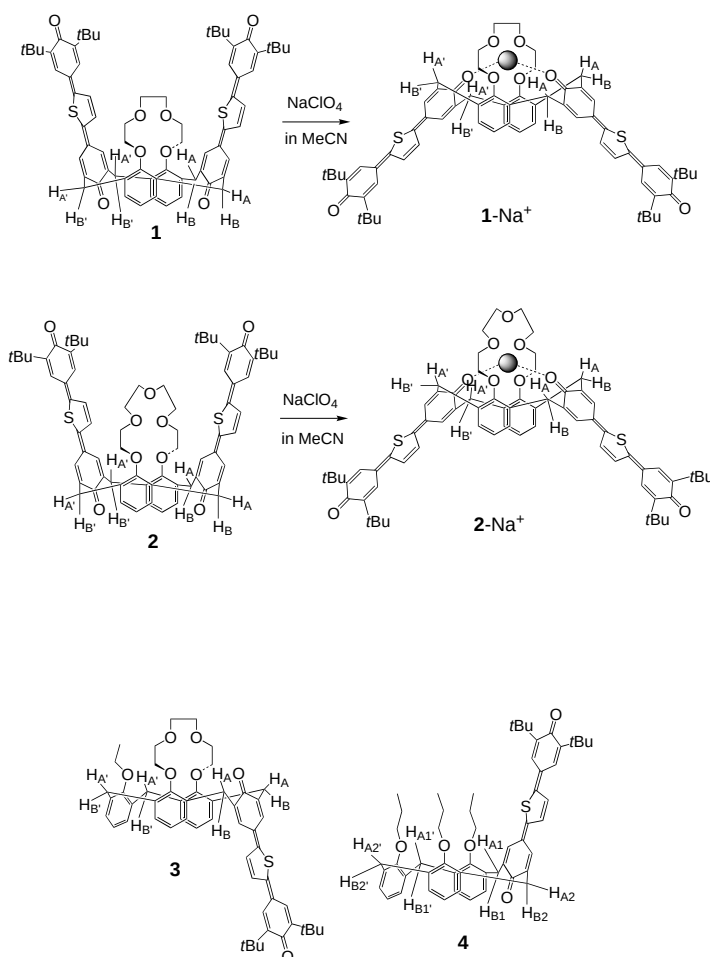
Note added in proof: During publication of this manuscript we also reported on a similar iron template mediated macrocyclization of 1,2-arylene biphosphane precursors (“A New Nine-Membered Triphosphorus Macrocyclic”, *Organometallics* **2000**, 19, 2652); this paper dealt with the formation of a rigid ring system related to the larger eleven-membered macrocycles previously described by Kyba and co-workers (see, for example: E. P. Kyba, R. E. Davies, S.-T. Liu, K. A. Hassett, S. B. Larsen, *Inorg. Chem.* **1985**, 24, 4629). In view of the rigidity of the chelating 1,2-arylene biphosphane functionality in these macrocycles, they may be expected to offer substantially different coordination properties than the parent aliphatic ring system reported for the first time herein; they would also be expected to be electronically distinct. Indeed, the versatility of the new template methodology described is emphasized by the opportunity to introduce stereochemical control, which in turn enables synthetic routes to alternative macrocyclic structures and supports unprecedented ring-closure reactions. These observations are exemplified in our recent discussion of the formation of chiral, intermediate ring sizes (*Angew. Chem.* **2000**, 112, 2834; *Angew. Chem. Int. Ed.* **2000**, 39, 2722). Our oversight in not cross-referencing the three articles is partly corrected through this note, added in proof upon the request of the editor.

Through-Space Exciton Coupling and Multimodal Na⁺/K⁺ Sensing Properties of Calix[4]arenecrowns with the Thienylene Analogue of *para*-Terphenylquinone as Chromophore**

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Dominique Guillaumont, Fabio Pichierri, and
Shinichiro Nakamura

Calix[4]arenes are today utilized as excellent platforms or shaping units for creating attractive host molecules with ion-sensing properties.^[1] In the nineties, chromogenic recognition by chromophore-derived calix[4]arenes^[2] and electrochemical recognition by quinone-derived calix[4]arenes^[3] of alkali metal cations were reported. However, as far as we know, there are no reports on through-space interactions between two chromophores incorporated in a calixarene skeleton, although a paper dealing with intramolecular electron transfer in calix[4]arenequinone derivatives appeared quite recently,^[4] and multifunctional ion-sensing calixarenes that combine chromogenic and redox-active properties in one molecule are unknown. The combination of these properties could offer a promising route towards novel sensing materials.

We have now designed and synthesized the novel calix[4]arenecrowns **1** and **2**, together with their reference compounds **3** and **4**, since the thienylene analogue of *p*-terphenylquinone as chromophore shows a strong absorption maximum in the visible region and affords a very stable radical anion and radical cation in electrochemical reactions.^[5] We found that the two diametrically arranged chromophores of **1** and **2** undergo through-space exciton coupling on absorption of visible light. The transition dipole moment in the exciton coupling^[6] is strongly influenced by a conformational change of the two interacting chromophores. A remarkable conformational change indeed occurred in **1** and **2** upon complexation of Na⁺ or K⁺ ions (Scheme 1), which resulted in a significant color change. Moreover, **1** and **2** can also recognize electrochemically Na⁺ and K⁺ ions.^[7] Thus, **1** and **2** are the first

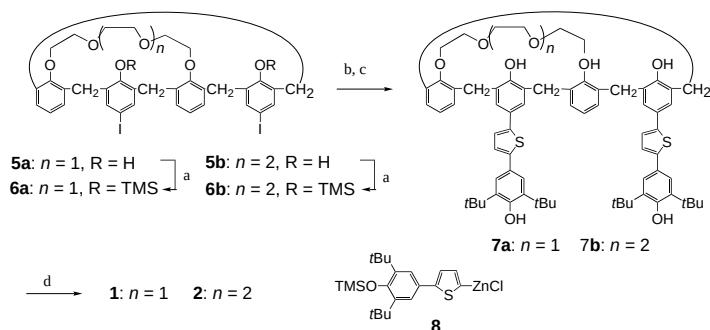


Scheme 1. The calix[4]arenecrowns **1** and **2** and the reference compounds **3** and **4**. Formation of **1-Na⁺** and **2-Na⁺**.

ion-sensing host molecules that exhibit both chromogenic and electrochemical recognition properties.

The synthesis of **1** and **2** (Scheme 2) started from the diiodo compounds **5a**^[8] and **5b**,^[8] respectively. The reference monoheteroquinones **3** and **4** were synthesized by similar routes.^[9]

Compound **4** adopts a partial cone conformation both in the solid state and in solution, which was confirmed by X-ray crystal structure analysis^[10] and by the ¹H NMR spectrum,^[9]



Scheme 2. Synthesis of the calix[4]arenecrowns **1** and **2**. a) BSA, MeCN, CHCl₃, reflux, **5a**: 50%, **5b**: 50%; b) **8**, cat. Pd⁰, THF, 25 °C; c) TBAF, THF, 25 °C, **7a**: 31%, **7b**: 28%; d) PbO₂, CH₂Cl₂, 25 °C, **1**: 92%, **2**: quantitative. BSA = *N,O*-bis(trimethylsilyl)acetamide, TMS = trimethylsilyl, TBAF = tetrabutylammonium fluoride.

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which gave the differences in calix methylene proton chemical shifts: $\Delta\delta(\text{H}_{\text{B1}}, \text{H}_{\text{A1}}) = 0.33 - 0.26$, $\Delta\delta(\text{H}_{\text{B2}}, \text{H}_{\text{A2}}) = 0.38 - 0.31$, and $\Delta\delta(\text{H}_{\text{B1}'}, \text{H}_{\text{A1}'}) \approx \Delta\delta(\text{H}_{\text{B2}'}, \text{H}_{\text{A2}'}) = 1.00 - 0.98$. Compound **3** adopts a cone conformation in solution, since the chemical shift differences $\Delta\delta(\text{H}_{\text{B}}, \text{H}_{\text{A}}) = 1.54 - 1.48$ and $\Delta\delta(\text{H}_{\text{B}'}, \text{H}_{\text{A}'}) = 1.22 - 1.21$ are large. In contrast, **1** exists in a 1,3-alternate conformation in solution, since the chemical shift differences $\Delta\delta(\text{H}_{\text{B}}, \text{H}_{\text{A}}) \approx \Delta\delta(\text{H}_{\text{B}'}, \text{H}_{\text{A}'}) = 0.29 - 0.18$ are very small. Compound **2** also exists in a 1,3-alternate conformation, as inferred from the small $\Delta\delta(\text{H}_{\text{B}}, \text{H}_{\text{A}})$ and $\Delta\delta(\text{H}_{\text{B}'}, \text{H}_{\text{A}'})$ values of 0.64–0.45.^[11]

Compounds **1–3** form 1:1 complexes with Na^+ ions under saturation conditions. The most stable conformation of **1**- Na^+ is a cone structure, since the $\Delta\delta(\text{H}_{\text{B}}, \text{H}_{\text{A}}) \approx \Delta\delta(\text{H}_{\text{B}'}, \text{H}_{\text{A}'}) = 1.07 - 1.03$ values are large. The complex **2**- Na^+ also exists in a cone conformation, as inferred from the ^1H NMR spectrum ($\Delta\delta(\text{H}_{\text{B}}, \text{H}_{\text{A}}) \approx \Delta\delta(\text{H}_{\text{B}'}, \text{H}_{\text{A}'}) = 1.13 - 1.10$). Hence, **1** and **2** have the 1,3-alternate conformation and undergo ring inversion to the cone conformations upon complexation. Interestingly, the bulky thienylene analogues of *p*-terphenylene rotate from above to below the calixarene macrocyclic plane.

The structures of **1** and **1**- Na^+ were optimized by molecular orbital (MO) and molecular mechanics (MM) methods. Optimization at the HF/3-21G level (Gaussian 94^[12]) was first performed for the free form and then for the Na^+ complex of 1,3-bis(*p*-benzoquinonemethide)-derived calix[4]-arene-crown-4. Then, by retaining the MO-optimized structure and replacing the methide groups by 2-(3,5-di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-2,5-dihydrothiophene-5-ylidene groups, the MM optimization was executed.^[13] The resulting 1,3-alternate conformation of **1** and the highly flattened cone conformation of **1**- Na^+ are shown in Figure 1. In the optimized cone **1**- Na^+ , the Na^+ ion lies at the center of the crown ether bridge, 2.3 Å from the four crown oxygen atoms and 2.2 Å from the quinone oxygen atoms.

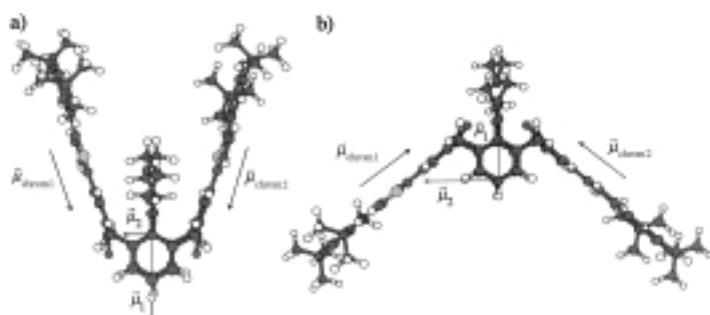


Figure 1. Side views of the molecular structures of a) **1** and b) **1**- Na^+ , indicating the transition dipole moments.

Whereas the monoheteroquinone **3** exhibits only one intense absorption band at 568 nm in the electronic spectrum (Figure 2a), the bis-heteroquinone **1** exhibits two absorption

bands in the visible region at 540 and 579 nm, of which the band at shorter wavelength has a higher intensity than that at longer wavelength (Figure 2b).^[9] Upon addition of NaClO_4 to an acetonitrile solution of **1**, the intensity of the longer

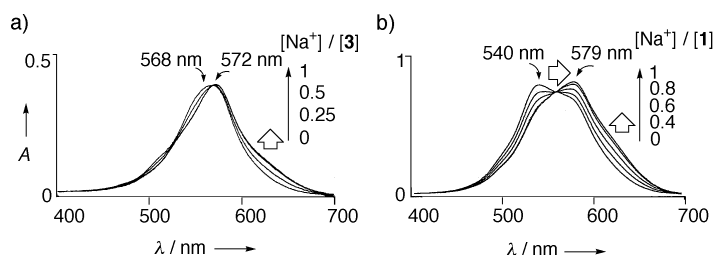


Figure 2. Spectroscopic changes upon addition of NaClO_4 to a solution of a) **3** and b) **1** in MeCN. *A* = absorbance.

wavelength band increased, and that of the shorter one decreased, accompanied by the appearance of a new broad and weak band at around 630 nm (Figure 2b). In consequence, the color of the solution turned from purple to blue. This spectroscopic feature is excellently reproduced by INDO/s calculations^[14] on **1** and **1**- Na^+ based on the MO-MM optimized structures when through-space exciton coupling between the two diametrically arranged chromophores is taken into account. As shown in Table 1, there is a fairly good agreement between experimental and theoretical values of $\lambda_{1\text{max}}$ and $\lambda_{2\text{max}}$, as well as for the reversal of the absorption intensity upon complexation.^[15] In exciton coupling systems,^[6] the excited state splits into two energy levels due to the

Table 1. Observed and calculated^[a] electronic spectral data of the calix[4]arenequinones **1** and **3**, and their Na^+ complexes.

Compound	Calculated				Observed (in MeCN)			
	$\lambda_{1\text{max}}$ [nm]	<i>f</i>	$\lambda_{2\text{max}}$ [nm]	<i>f</i>	$\lambda_{1\text{max}}$ [nm]	ϵ	$\lambda_{2\text{max}}$ [nm]	ϵ
1	555	0.68	493	4.78	579	105 758	540	118 856
1 - Na^+	575	3.12	515	1.99	579	124 546	540	97 396
3	λ_{max} [nm]	<i>f</i>			λ_{max} [nm]	ϵ		
3 - Na^+	529	2.56			568	81 127		
	549	2.54			572	83 129		

[a] INDO/s calculation.

interaction between the two transition dipole moments (out-of-phase (α state) and in-phase (β state) combinations of the transition dipole moment vectors), which is indicated by a splitting of the absorption band. According to the INDO/s results the splitting of the absorption band does indeed result from the combination of the two individual transition dipole moments μ_{chrom1} and μ_{chrom2} , that is, exciton coupling takes place. The two individual dipole moments almost lie in the molecular planes of the two chromophores. Hence, the angle between the two chromophores determines the relative intensity of the two absorption bands. The angle between μ_{chrom1} and μ_{chrom2} for the Na^+ -free form of **1** in the 1,3-alternate conformation is estimated to be about 43°. According to the exciton coupling theory, if the angle is less than 90°, as in this case, the α state combination becomes energetically more stable than the β state combination. The transition to the β state at shorter wavelengths gives rise to a more intense

band than that to the α state at longer wavelengths, because the intensity is proportional to the square of the sum of the vectors. In contrast, the angle between μ_{chrom1} and μ_{chrom2} is estimated to be about 106° for the cone **1**-Na⁺, that is larger than 90° . In this case, the β state becomes more stable than the α state, and the longer wavelength band has the higher intensity. This explains the reversal of the intensity of the two maxima on going from **1** to **1**-Na⁺ (Figure 2b).

The crown-5 bridged bis-heteroquinone **2** also exhibits two absorption maxima (at 542 and 577 nm; Figure 3). Although the reversal of the intensities on going from **2** to **2**-Na⁺ is not so pronounced as in the case of **1**, the occurrence of exciton coupling is strongly supported by the electronic spectral feature shown in Figure 3.

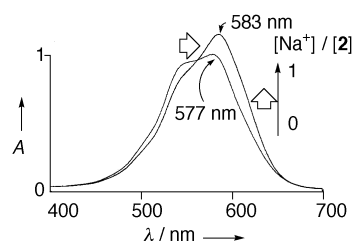


Figure 3. Spectroscopic changes upon addition of NaClO₄ to a solution of **2** in MeCN. A = absorbance.

The INDO/s calculations on the monoquinones **3** and **3**-Na⁺^[16] gave only one absorption maximum and reproduced well the experimental values (Table 1). The observation of exciton coupling for **1** and **2** reveals that the conformational fixation in these compounds appears to be fairly good even in solution. This is the first observation of through-space dipole interactions between two chromophores in calixarene systems.

Significant changes in the spectra of **1** and **3** were not observed even after 18 equivalents of KPF₆ were added. However, a spectral change similar to that shown in Figure 3 was clearly observed on addition of one equivalent of KPF₆ to **2**. Indeed, crown-4-bridged **1** and **3** showed 100- to 1000-fold higher selectivity for Na⁺ versus K⁺ than crown-5-bridged **2** with its larger ionophoric cavity (Table 2).

As shown in Table 3, the first half-wave reduction potential E_{red}^1 of **1**, **3**, and **2** shifted anodically (stabilization shift) by $\Delta E = 250$, 250, and 170 mV, respectively, on addition of NaPF₆. Interestingly, a similar anodic shift of $\Delta E = 200$ mV was also observed for **4**, although the association constant for the complexation of the non-crown-bridged **4** with Na⁺ is more than 10^4 times smaller than that of the crown-4-bridged **3**. These facts indicate that a significant Na⁺ binding enhance-

Table 2. Association constants $K^{\text{[a]}}$ for the 1:1 complexes of **1**–**3** with Na⁺ and K⁺, determined by UV/Vis titration in MeCN at room temperature.

Compound	$K(\text{Na}^+)$	$K(\text{K}^+)$
3	$> 1.2 \times 10^6$	7.0×10^2
1	$> 1.2 \times 10^6$	4.7×10^3
2	$> 1.2 \times 10^6$	$> 1.2 \times 10^6$

[a] Obtained by processing UV/Vis data using a nonlinear least square curve fitting computer program.

Table 3. Redox potentials E in V^[a] of the calix[4]arenes **1**–**4**, and the anodic shifts ΔE in mV in the presence of five equivalents of NaPF₆ or KPF₆ in PhCN.

Compound		E_1^{red}	E_2^{red}	$E_{\text{ox}}^{\text{pl}}$
4	$E(\text{free})$	−0.51	−0.76	+1.23
4	$\Delta E(\text{Na}^+)^{\text{[b]}}$	200	240	10
3	$E(\text{free})$	−0.50	−0.70	+1.21
3	$\Delta E(\text{Na}^+)^{\text{[b]}}$	250	170	160
1	$E(\text{free})$	−0.52	−0.68	+1.21
1	$\Delta E(\text{Na}^+)^{\text{[b]}}$	250	170	160
1	$\Delta E(\text{K}^+)^{\text{[c]}}$	150	100	130
2	$E(\text{free})$	−0.51	−0.68	+1.30
2	$\Delta E(\text{Na}^+)^{\text{[b]}}$	170	100	30
2	$\Delta E(\text{K}^+)^{\text{[c]}}$	140	90	20

[a] 0.1M TBAP, vs. SCE, scan rate 50 mV s^{−1}. E_1^{red} and E_2^{red} are the first and second half-wave reduction potentials, respectively. $E_{\text{ox}}^{\text{pl}}$ is the first oxidation peak potential. [b] In the presence of five equivalents of NaPF₆. [c] In the presence of five equivalents of KPF₆ in PhCN:MeCN = 5:1 v/v.

ment occurs when the chromophores are reduced to the corresponding radical anions, that is, the complexes with the alkali metal ions are thermodynamically much more stable in the radical anion state than in the neutral state of the heteroquinone moieties. Thus, **4** can act as a redox-switching Na⁺ transporting mediator due to the significant difference in the binding capability between the neutral state and the radical anion. In terms of the ΔE values of the reduction potentials, the electrochemical Na⁺/K⁺ selectivity of **1** is still higher than that of **2**.

If there is an unfavorable interaction between an encapsulated positively charged Na⁺ or K⁺ ion and the heteroquinone moieties in radical cation form, the alkali metal ion binding is anticipated to become much more difficult when the heteroquinone chromophores are oxidized. However, as shown in Table 3, large anodic shifts (destabilization shifts) of the oxidation potentials $E_{\text{ox}}^{\text{pl}}$ by $\Delta E = 160$ mV are observed for **3** and **1** on binding an Na⁺ ion, and this indicates that the binding capabilities of the crown-4 bridges of **3** and **1** for Na⁺ ions are so strong that the unfavorable interaction can easily be overcome. The relatively small anodic shifts in $E_{\text{ox}}^{\text{pl}}$ of **2** can be ascribed to a weak destabilizing interaction owing to the loose contact between the encapsulated Na⁺ or K⁺ and the carbonyl oxygen atoms of the radical cation of the heteroquinone moieties of **2**. Thus crown-4-bridged **1** is an especially novel host molecule that senses Na⁺/K⁺ by the induction of pronounced electrochemical anodic shifts not only in the reduction potential but also in the oxidation potential.

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- [10] Single crystals of **4**: monoclinic, space group *P*₂₁/*n*, *Z* = 4, *a* = 15.684(4), *b* = 10.589(4), *c* = 29.278(4) Å, β = 96.52(2)°, *V* = 4830(2) Å³ (*R* = 0.114 and *R*_w = 0.140 for 2393 observed reflections with *I* > 3.00σ(*I*)). Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-137583. 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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Baker, J. P. Stewart, M. Head-Gordon, C. Gonzalez, J. A. Pople, Gaussian, Inc., Pittsburgh, PA, **1995**.

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- [15] In addition to the calculated absorption bands listed in Table 1, a new, forbidden transition, assignable to the weak absorption band at around 630 nm (Figure 2), resulted for **1**-Na⁺ and **3**-Na⁺ only when the Na⁺ ion was taken into account in the INDO/s calculation. Therefore, this weak band may originate from the ion–dipole interaction between the encapsulated alkali metal cation and the carbonyl groups of the heteroquinones.
- [16] Stable cone conformations were optimized for both **3** and **3**-Na⁺ with the same calculation method as for **1** and **1**-Na⁺.

In Situ ¹H MAS NMR Spectroscopic Observation of Proton Species on a Mo-Modified HZSM-5 Zeolite Catalyst for the Dehydroaromatization of Methane**

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Zeolites and oxide-modified zeolites have been widely used in light-paraffin aromatization reactions.^[1,2] It has been generally accepted that the acidic sites, together with the dehydrogenation centers of the metal oxide components, play key roles in paraffin activation and the subsequent aromatization. In 1993, we were the first to report that methane, an ultra stable molecular, could undergo aromatization on molybdenum modified HZSM-5 catalysts under nonoxidative conditions.^[3] Subsequent investigations^[4] demonstrated that the Brønsted acidity and the channel structure of the zeolites, as well as the oxidation states of the Mo species, are crucial factors that affect the performance of the catalysts. However, most of the discussions concerning the nature of the active species, for instance the location of the Mo species on or in the zeolite and the variation of the zeolitic acidity, are based on data obtained from off-line characterizations using “ideal” catalysts under conditions far from those in the real catalytic processes.^[4a–c]

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Recently, the development of in-situ NMR spectroscopic methods has opened up a realistic way to investigate in situ heterogeneous catalytic reactions. Although several novel techniques, such as high temperature MAS-rotor,^[5] flowing MAS experiments,^[6] and different experimental methods^[7, 8] have been reported to be powerful tools for the characterization of steady state processes in catalytic reactions, none of these methods has been shown to be effective for high temperature reactions, especially at temperatures greater than 800 K. Following the idea of the CAVERN device^[8] from Haw's group, we have developed a method applicable to the NMR analysis of high-temperature catalytic reactions. Using a specially designed apparatus (Figure 1) a real fixed-bed catalytic reaction can be conducted in the temperature range of 300–1123 K. After the reaction the catalyst can be transferred to a conventional MAS-rotor and sealed in situ. Herein, we present the in situ ¹H MAS NMR spectroscopic results of the dynamic variation of the proton signals in a Mo modified ZSM-5 catalyst for the catalytic methane aromatization reaction. This reaction was carried out under conditions identical to those in the real catalytic processes.

Figure 2 shows the ¹H MAS NMR spectra of a Mo/HZSM-5 catalyst (6% Mo) treated with a methane flow (1500 mL g⁻¹ h⁻¹) for 1 h at 573, 673, and 873 K. In addition to a very sharp resonance ($\delta \approx -0.1$) due to residual methane remaining in the crystal channels of the zeolite, the signals of zeolitic hydroxyl species are clearly resolved. This result demonstrated that treatment with a flow of methane for only 1 h at 573 K can efficiently remove the water molecules adsorbed onto the zeolite surface, whereas a complete dehydration of such samples by conventional methods can only be achieved by degassing at 673 K and 10⁻² Pa for more

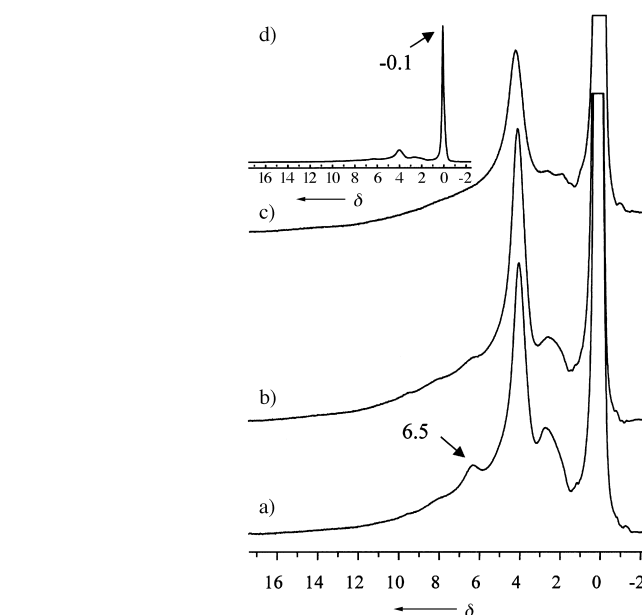


Figure 2. ¹H MAS NMR spectra of a Mo/HZSM-5 catalyst (6% Mo) treated with a methane flow (1500 mL g⁻¹ h⁻¹, 1 atm) for 1 h at a) 573 K, b) 673 K, and c) 873 K. Spectrum d) is the whole spectrum of (c), the resonance at about $\delta = -0.1$ is from the remaining methane inside the zeolite channels.

than 18 h.^[9] The signal at $\delta = 4.1$ is assigned to the Brønsted acid sites, while the signals at $\delta = 1.7$ and 2.4 are due to the silanol groups at framework defects or at the external surface of the zeolite, and to OH groups at nonframework aluminum species, respectively.^[9] Moreover, the partially obscured small and broad signals at about $\delta = 6.5$ can be, depending on the shape of the spectral line,^[10] attributed to water molecules

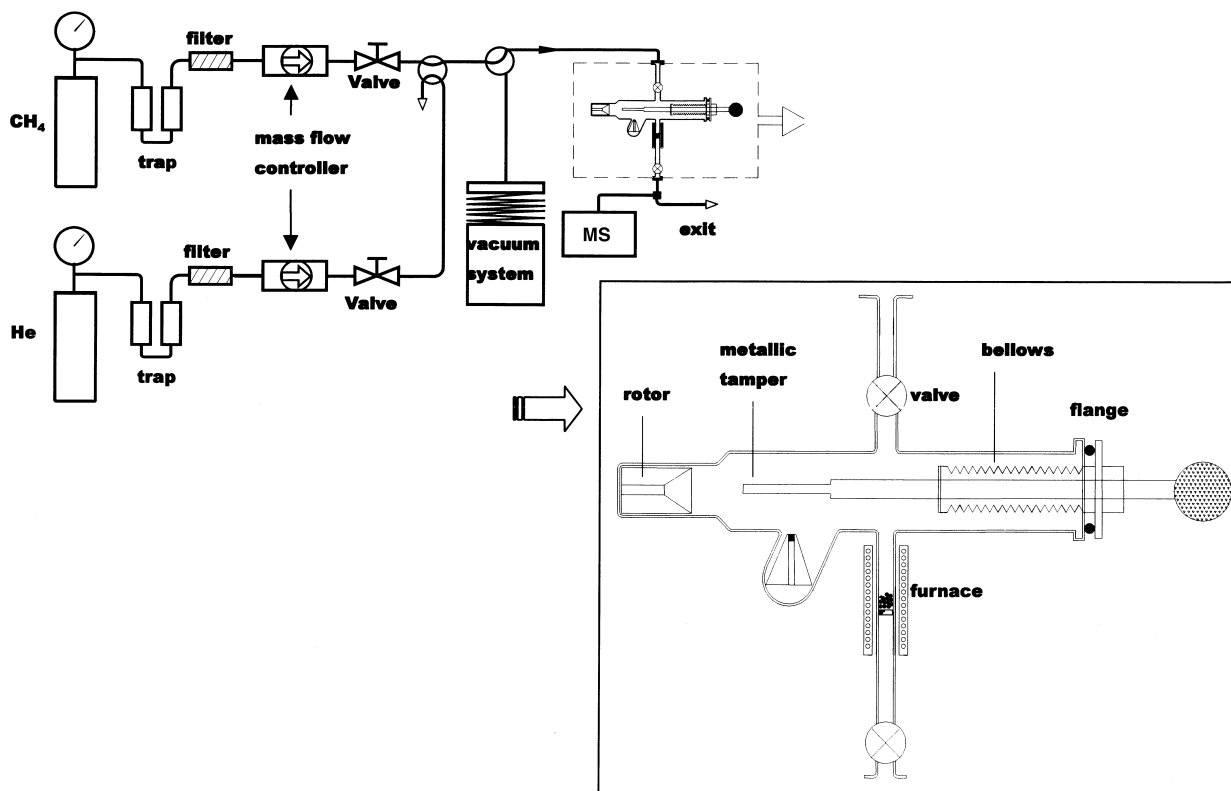


Figure 1. Sketch of the experimental set up for in situ MAS NMR study; the reaction and sample preparation section detail is enlarged (bottom right).

adsorbed onto Lewis acid sites or another kind of Brønsted acid sites.

During treatment at higher temperatures the narrow resonance for the adsorbed water gradually disappeared (Figure 2). The amount of zeolitic hydroxyl species appeared to diminish, particularly in the temperature range of 673–873 K. However, no other significant variations were observed in the proton MAS spectra. This observation indicates that the water molecules, including those strongly adsorbed onto Lewis sites of the zeolite, desorb during the temperature-raising process before the reaction starts. At the same time, apart from a small decrease of the Brønsted acid sites as a result of temperature effects, no reaction products or by-products were observed at or below 873 K.

On increasing the temperature to 973 K for 10 min the color of the catalyst changed from blue-white to black which is the typical color of molybdenum carbide. In conjunction with this observation a distinct change in the proton NMR signals occurred (Figure 3a and b). The Brønsted acid signal at $\delta = 4.1$ decreased dramatically, and two new resonances at $\delta = 6.8$

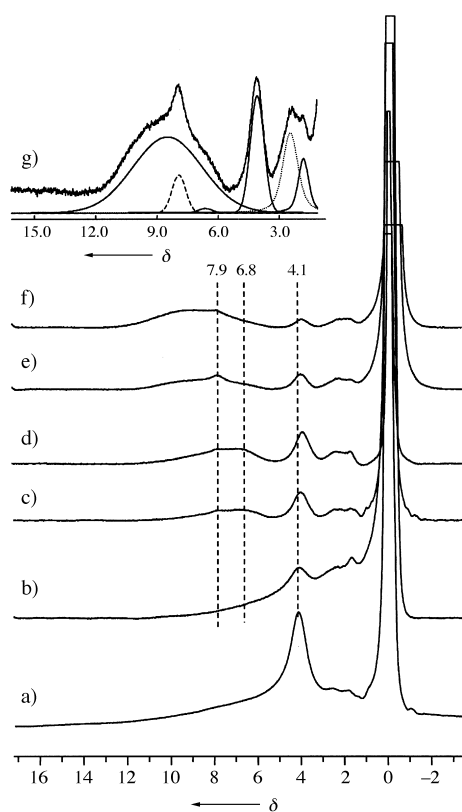
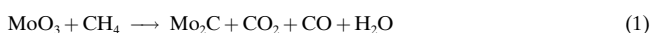


Figure 3. ^1H MAS NMR spectra of Mo/HZSM-5 (6%) treated with a) methane flow at 873 K for 1 h. b)–f) after the methane dehydroaromatization reaction (973 K, $1500\text{ mL g}^{-1}\text{ h}^{-1}$, 1 atm) reaction times: b) 10 min, c) 30 min, d) 1 h, e) 3 h, f) 6 h. Spectrum (g) is an expansion of spectrum (e), with six lines simulating the original spectrum.

and 7.9 emerged clearly when the reaction time was prolonged (Figure 3c). The first of these two new signals can be assigned to the water molecules formed and adsorbed during the reaction [Eq. (1); the formula is nonstoichiometric].



During their diffusion over the surface or through the gas phase the water molecules can reabsorb on the nearby Lewis acid sites. The signal at $\delta = 7.9$ is assigned to the hydrogen atoms of the aromatic species that are produced on the aromatization centers—the Brønsted acid sites^[4a]—and are then adsorbed on the catalyst. At the same time the intensity of the Brønsted acid signal decreases further, an indication that Brønsted acid sites take part in the aromatization or hydrogen transfer process of the reaction. Choudary et al.^[11] and Buckles et al.^[2] suggested that in the aromatization of propane or of a mixture of propane/propene and methane, Brønsted protons participated in the paraffin activation and the subsequent hydrogen transfer reaction. Direct observation of the variations in the proton signal during the methane dehydroaromatization provides strong support for an acid-induced aromatization mechanism. As we previously reported no benzene formation is observed when the Na form of the ZSM-5 zeolite is used instead of a proton form.^[4a] Thus, we could conclude that Brønsted protons are essential to the methane dehydroaromatization reaction.

From TPSR experiments it has been observed that the water formation began at 973 K and that at the same temperature benzene started to appear 15 min later.^[12] This is in good agreement with our present proton MAS NMR results. Moreover, a signal corresponding to a methylene species at about $\delta = 1.2$,^[13] which in ref. [4g] is suggested as the intermediate species and as being responsible for the formation of the possible primary product of ethane,^[4b] could not be observed. Even if this species exists, it may overlap with the rather broad line of the nonframework Al-OH species.

The reaction process was stable up to 1 h (Figure 3d). After a further 2 h reaction time a new signal with similar chemical shift to the signal at $\delta = 7.9$ but with a much broader form appeared, while the amount of adsorbed water ($\delta = 6.7$) is greatly reduced (Figure 3e). A clearer ^1H MAS NMR profile of Figure 3e can be found in Figure 3g in which an enlarged spectrum and the simulated lines are presented. The broad signal can be attributed to the more condensed aromatic materials, that is aromatic carbonaceous deposits on the catalyst surface. The broad line width reveals its mixed composition and complicated nature. In fact, this signal can be observed even at the beginning of the reaction (Figure 3c), though it is very weak at this time. It is difficult to determine the exact ratio of the two overlapping signals at about 8 ppm, but in Figure 3c–f we can find that the amount of the aromatic carbonaceous deposits greatly increased with the reaction time. This is in an agreement with the thermogravimetric measurements and with carbon number balance calculation based on GC results of the residue.^[4e]

For the first time the variation of the amount of Brønsted acid sites during a real high-temperature reaction (973 K), as well as the formation of water, benzene, and aromatic carbonaceous deposits in the reaction process have been directly observed. This gives a more detailed description of what has happened on the catalyst surface during the reaction than data from the gas phase analysis (mass spectrometer).

Experimental Section

A series of Mo/HZSM-5 catalysts were prepared as described in ref. [4b]. HZSM-5 powders were impregnated with aqueous solutions containing a given amount of ammonia heptamolybdate (AHM), then dried at RT and 373 K for 12 and 8 h, respectively. After calcined at 773 K for 5 h, the catalysts were crushed and sieved to yield granules of 20–60 mesh.

A specially designed device was built for the MAS NMR study. It contains a gas flow section, a reaction and sample preparation section, and an on-line mass spectrometer (Figure 1). After the reaction (the temperature can be as high as 1123 K), the reaction and sample preparation section can be taken off and turned through 90°. Then, in the reaction atmosphere the sample can be transferred into a NMR-rotor with the aid of a tamper (to pack the sample into the tube) that is mounted in a bellows attached to the upper side of the flange. The rotor is then sealed with a cap in a rack with the aid of tamper. A spin rate as high as 12 kHz can be reached following this sample filling method. In the present case, the samples were treated with a methane flow ($SV = 1500 \text{ mL g}^{-1} \text{ h}^{-1}$, 1 atm) for 1 h at 573, 673, and 873 K. Subsequently, the catalysts were subjected to reactions of 10 min, 0.5 h, 1 h, 3 h, and 6 h. In all the cases, the temperature was raised at a rate of $15^\circ \text{C min}^{-1}$.

Proton MAS NMR spectra were recorded in 4 mm ZrO_2 rotors at 400.13 MHz on a Bruker DRX-400 spectrometer fitted with a MAS probe. For each spectrum, a $\pi/10$ pulse and a 4 s relaxation delay were used, and 400 scans were accumulated. Samples were spun at 8 kHz, and the DSS (DSS = sodium 4,4-dimethyl-4-silapentane sulfonate) signal was taken as the reference for the ^1H chemical shifts. The deconvolution of the spectra was conducted using WINNMR, supplied by the spectrometer manufacturer.

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to be produced when the temperature reached 973 K, with a maximum at about 13 min after the reaction began. Two minutes later (15 min after the start of the reaction) the start of benzene formation is observed. The formation of water stopped about 1 h later at the same temperature, but that of benzene increases slowly in the first hour, and then appears to be constant. This indicates that the water formation is a stoichiometric (not catalytic) reaction [see Eq. (1)], where as benzene formation is a catalytic reaction.

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First Crystal Structure of a Medicinally Relevant Gold Protein Complex: Unexpected Binding of $[\text{Au}(\text{PET}_3)]^+$ to Histidine**

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Antiartihritic gold(I) compounds such as the injectable drugs aurothiomalate^[1] (Myochrysine) and aurothioglucose (Solganol),^[2] and the oral drug^[3] $[\text{Au}^{\text{I}}(\text{PET}_3)(2,3,4,6\text{-tetra-}O\text{-acetyl-}\beta\text{-D-thioglucose-5'})]$ (auranofin) are “prodrugs”, which undergo facile ligand displacement reactions.^[4, 5] The critical target sites are thought to be thiolate sulfurs (cysteine residues) in proteins and enzymes,^[5] Au^{I} being a very “soft” (class ‘b’) metal ion.^[6, 7] However, there is a lack of structural data on adducts of antiartihritic Au^{I} complexes with proteins.^[8] We report here investigations of reactions between the enzyme cyclophilin and the antiartihritic complex^[9, 10] $[\text{Au}(\text{PET}_3)\text{Cl}]$ (**1**), and the first X-ray crystal structure of a protein adduct with a Au^{I} phosphane complex. Unexpectedly, in cyclophilin crystals, Au^{I} binds to the nitrogen atom of an active site His residue, despite the presence of four Cys thiol groups. The results have implications for understanding the biological chemistry of gold antiartihritic complexes. Additional interest in this work arises from the need for site-specific heavy-atom reagents for X-ray crystallography.^[8]

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Cyclophilins are ubiquitous proteins involved in protein folding, transport, and assembly, and are linked to general cellular stress responses.^[11] They are peptidyl prolyl isomerase (PPIase) enzymes, and cyclophilin A (an immunophilin) is the major intracellular receptor for the immunosuppressive drug, cyclosporin A (CsA). We chose cyclophilin-3 (Cyp-3, one of the most abundantly expressed cyclophilin isoforms in the free living nematode *Caenorhabditis elegans*) for study, since it has a known X-ray structure^[12] and possesses four free Cys thiol groups which are potential Au^I target sites.

First the reaction between Cyp-3 and **1** was studied in solution by mass spectrometry. For a molar ratio of Cyp-3:**1** of 1:3.5, the sample after one day revealed three new peaks with masses of 18 612.0 u (calcd 18 615.5 u), 18 736.8 u (calcd 18 733.6 u), and 18 932.9 u (calcd 18 930.6 u), corresponding to {Cyp-3 + Au}, {Cyp-3 + [AuPEt₃]}, and {Cyp-3 + Au + [AuPEt₃]}, respectively (Figure 1). For a molar ratio Cyp-3:**1** of 1:7, the sample after five days revealed that the peaks for

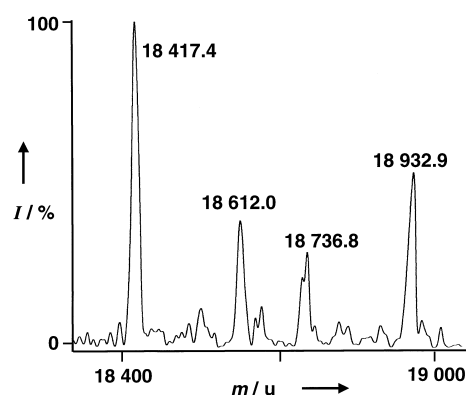


Figure 1. LC-ESI-MS of Cyp-3 (13.5 μ M) after incubation with **1** (47.3 μ M) for 24 h at 291 K. The peaks correspond to Cyp-3 (18 417.4 u, theoretical mass: 18 419.2 u); {Cyp-3 + Au} (18 612.0 u, theoretical mass: 18 615.5 u); {Cyp-3 + [Au(PEt₃)]} (18 736.8 u, theoretical mass: 18 733.6 u); {Cyp-3 + Au + [Au(PEt₃)]} (18 932.9 u, theoretical mass: 18 930.6 u).

Cyp-3 and {Cyp-3 + [AuPEt₃]} had disappeared, peaks for {Cyp-3 + Au} (18 618.8 u), and {Cyp-3 + Au + [AuPEt₃]} (18 930.8 u, major peak) were still detectable, and new peaks appeared at 19 242.5 u corresponding to {Cyp-3 + Au + 2[AuPEt₃]} (calcd 19 245.7 u) and 18 812.8 u corresponding to {Cyp-3 + 2Au} (calcd 18 812.4 u). These results appeared to be consistent with chloride displacement and [AuPEt₃]⁺ binding to the protein, followed by PEt₃ displacement (and oxidation to OPEt₃). Such a displacement might be facile in the region of Cys40 and Cys168 (highly conserved in all divergent loop cyclophilins) since their sulfur atoms are within 5.5 Å and could readily provide linear S-Au-S coordination for Au^I.

Reactions of Cyp-3 with the disulfide 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) also suggested that all four Cys thiol groups are accessible. By UV/Vis spectroscopy, the average thiol content of Cyp-3 was determined to be 4.2 mol thiol per mol Cyp-3. The reaction was also investigated by LC-ESI-MS. The chromatographic peak for Cyp-3 alone corresponded to a mass of 18 418.5 u (mean value, theoretical mass 18 419.2 u).^[13] This peak disappeared after reaction of Cyp-3

with DTNB, and the major new chromatographic peak had a mass of 19 210.6 u (Figure 2), corresponding to {Cyp-3 + 2DTNB} (i.e. $4 \times 1/2$ DTNB, calcd: 19 211.2 u). This implies that all four Cys thiol groups form mixed disulfides with DTNB and are therefore accessible.

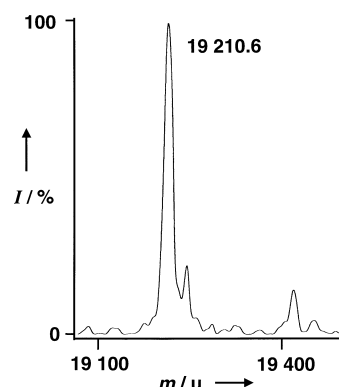


Figure 2. LC-ESI-MS of Cyp-3 after reaction with excess DTNB. The major peak (19 210.6 u) corresponds to four half DTNB species bound to Cyp-3, that is all four Cys residues blocked as mixed disulfides (theoretical mass 19 211.2 u).

To identify the gold coordination sites, we treated single crystals of Cyp-3 with complex **1** for periods of 0.42, 2, 4, 6 h, and two weeks. The crystals were then frozen and examined by X-ray diffraction. All crystals soaked with **1** changed their appearance. They became cracked and lost their sharply defined edges. A crystal structure determined after 0.42 h soaking did not show the presence of gold in the calculated electron density map, and after soaking for 6 h did not diffract at all. After samples had been soaked for two weeks, cracking was severe. The electron density map from crystals soaked for 2 or 4 h contained a large peak corresponding to a single gold site (Figure 3). The structure was solved by using isomorphous replacement since that of unliganded Cyp-3 is known.^[12] In the first difference map, Au was clearly seen together with additional electron density in the region expected for a ligand *trans* to His133 N ϵ 2. This was interpretable as PEt₃ as indicated by the shape of the electron density. The structure was refined with SHELXL97^[14] using 20 154 unique data (5 % for the calculation of R_{free}) and initial constraints on the Au–N and Au–P bond lengths of 2.05 and 2.27 Å, respectively, values similar to those in crystalline [Au(2-isopropylimidazole)(Pcy₃)]^[15] and [Au(phthalimido)(PEt₃)]^[16]. The final R factor was 18.48 % and R_{free} 24.68 %.

Surprisingly, the gold ion was not bonded to a Cys sulfur atom, but instead to the N ϵ 2 nitrogen atom of His133 (Au–N 2.06 Å) and to PEt₃ (Au–P 2.27 Å). The linear geometry (N–Au–P angle 167.7°) is characteristic of Au^I. The temperature factors for the complex (N ϵ 2 13.86, Au 43.77, P 79.45, C(av) 74.96) suggest that the PEt₃ group is undergoing rotational motion.

Of the four His residues in Cyp-3, the imidazole rings of only His54 and His133 are solvent accessible, both in a solvent channel running between two Cyp-3 molecules related by a twofold screw axis parallel to the *b* axis. Cys168 also appears to be readily accessible and the sulfur atom of Cys163

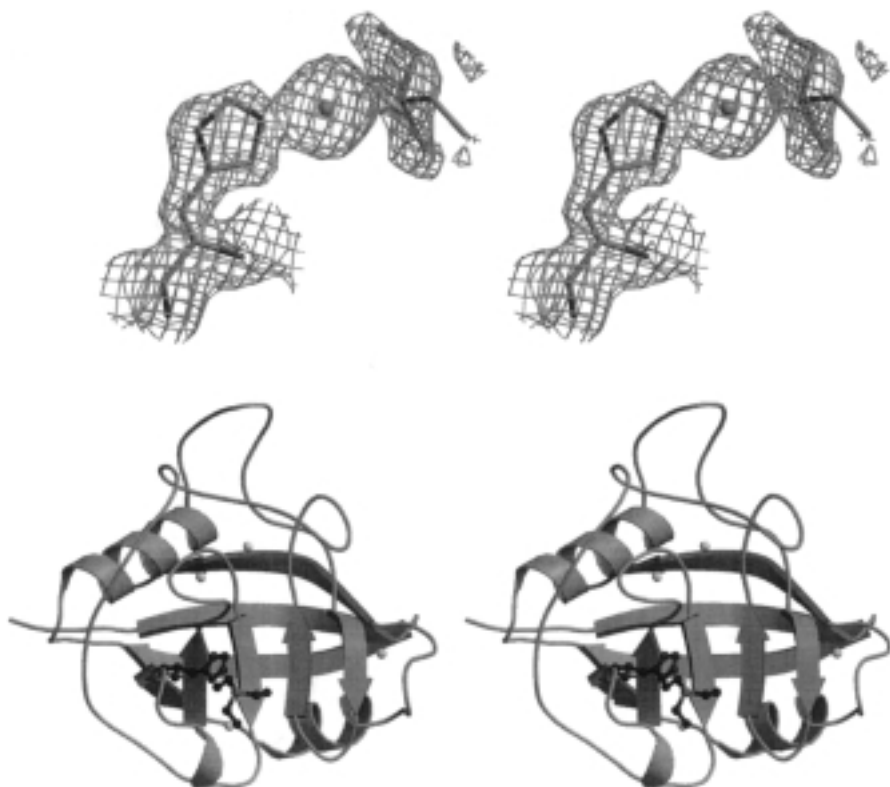


Figure 3. Top: Refined structure of the gold binding site in Cyp-3, showing His133 bound to PET_3 . The $2F_o - F_c$ electron density map is contoured at 1.75σ . The lack of definition of the PET_3 ligand is probably due to rotational disorder. Bottom: Stereoview of Cyp-3 after reaction with **1** showing the S atoms of the four Cys residues (light spheres: Cys40 (top left), Cys168 (top right), Cys122 (bottom left), Cys163 (bottom right)) and $[\text{AuPET}_3]^+$ bound to the $\text{N}\epsilon_2$ center of His133. Drawn using Bobscript,^[26] MolScript,^[27] and Raster3D.^[28]

is visible from the protein surface, but these sites may not be able to accommodate $[\text{AuPET}_3]^+$ without severe structural distortions and disruption of the crystal.

Gold binding at His133 should inhibit Cyp-3 since this is an active-site residue, and this was found to be the case. A chymotrypsin-coupled assay^[17] of the isomerization of the tetrapeptide substrate Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide (Bachem) showed that the concentration of **1** required to inhibit 50% of Cyp-3 PPIase activity was 14 nM (Figure 4), a value comparable with that for inhibition of human CypA by CsA.^[18]

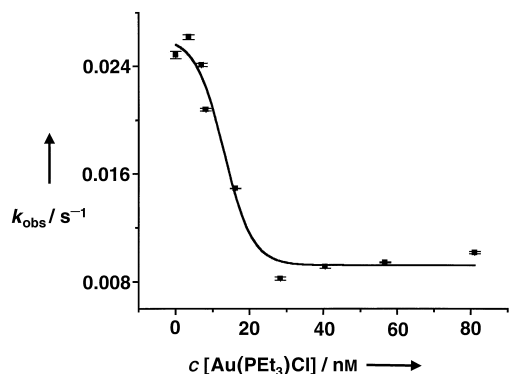


Figure 4. Variation of the observed rate constant for PPIase activity of Cyp-3 with increasing concentration of **1**. An IC_{50} value of 14 nM was calculated from this curve.

Au^{I} has a moderate affinity for N ligands in low molecular mass complexes,^[19] but a much higher affinity for thiolate groups. Hence a Cys-S atom is much more effective than an imidazole-N atom in displacing the Cl^- ion from complex **1** in aqueous solution.^[20] Complex **1** forms adducts with His residues of serum albumin only after the thiolate S atom at Cys34 has been saturated with Au^{I} , first as the mono-adduct Cys34-S- (AuPET_3) , and then as a bisadduct Cys34-S- $(\text{AuPET}_3)_2$.^[2, 4, 21, 22] Such a bisadduct could also form with Cyp-3 in the presence of excess **1**.

This appears to be the first X-ray structural characterization of an adduct of a gold-phosphane complex with a His residue in a protein.^[8] Previously it was suggested^[23] that Au^{I} -His interactions are responsible for the ability of complex **1** to induce low-spin- to high-spin-state transitions of heme proteins such as cytochrome c, but direct structural evidence for this was not obtainable. The present results confirm that His imidazole rings can indeed be target sites for $[\text{AuPET}_3]^+$ binding in proteins.

Experimental Section

$[\text{Au}(\text{PET}_3)\text{Cl}]$ (**1**) was purchased from Strem Chemicals and Alfa-Johnson Matthey plc. Cyclophilin-3 (Cyp-3) was expressed and purified as described previously.^[12]

ESI-HPLC-MS: Cyp-3 in Tris buffer (10 mM; pH 7.3), or in HEPES buffer (20 mM; pH 7.0), was treated with complex **1** (3.5 or 7 molar equivalents of a 2.5 mM stock solution in ethanol or methanol, diluted to 150 mM with buffer before use), and incubated for 1–5 days at 291 K. Prior to ESI-MS, the protein was desalted, separated from free gold complex on a Phenomenex (Jupiter) C_{18} reverse-phase column, and eluted with H_2O /acetonitrile/TFA (0.05% v/v). Positive-ion electrospray mass spectrometry was performed on a Platform II instrument (Micromass), with source temperature of 413 K and data acquisition in continuum mode, scan rate 900 u s^{-1} . The data were processed with a Mass Lynx (version 2.3) Windows NT PC data system using a maximum entropy software algorithm. Mass accuracy was within 0.1 m/z unit.

Determination of free SH groups: Cyp-3 (14.0 μM , concentration determined by Bradford assay) was incubated with DTNB (5 mM) in HEPES buffer (20 mM; pH 7.0) for 30 min at 291 K, and the absorption was measured at 412 nm.^[24, 25]

Crystallization, Au^{I} soaking, and data collection: Tetragonal crystals of Cyp-3 were grown using the hanging-drop vapour diffusion method: Cyp-3 (16 mg mL^{-1}) in sodium citrate (50 mM; pH 5.6), 16–17.5% wt/v methoxy-polyethylene glycol 5000 (MPEG 5000) (4 μL) suspended over sodium citrate (100 mM), 32–35% MPEG 5000 at 291 K. Average crystal dimensions: $0.5 \times 0.4 \times 0.4 \text{ mm}$. For crystal soaking, an aliquot of a stock solution of **1** in DMSO was diluted with sodium citrate (100 mM) containing 33–35% MPEG 5000, pH 5.6, giving a final Au^{I} concentration of 3.5 mM with 5% DMSO present. The stock solution of **1** in DMSO gave rise to only one

$^{31}\text{P}\{^1\text{H}\}$ NMR peak at $\delta = 34.5$ when monitored over a period of one month, showing that the Au–P bond is stable under these conditions. Crystals were transferred from hanging drops with 0.4–0.5 mm cryoloops into cryoprotectant solution (3 μL , mother liquor plus 20% glycerol), and 5 s after removal from cryoprotectant were mounted in a cryoloop (Hampton Research) and flash frozen in liquid N_2 . X-ray data were collected at 100 K (Oxford Cryosystems) using MAR image plates at the Daresbury synchrotron source, and processed using DENZO. The data set consists of 185 907 measured reflections and provides a unique data set of 20 154 reflections with an $R_{\text{merge}} = 0.068$ and an overall completeness to 1.85 Å of 0.983. R_{merge} in the 1.88–1.85 Å resolution shell is 0.253.

PPIase assay: Cyp-3 was incubated with **1** at 277 K at molar ratios of **1**: Cyp-3: of 0, 0.43, 0.86, 1.0, 2.0, 3.5, 5.0, 7.0, and 10.0, for 48 h in HEPES buffer (50 mM) containing NaCl (86 mM), pH 8.0, and 4 μL aliquots were then assayed at 275 K following the procedure previously described.^[12]

ESI-MS spectra of Cyp-3 after reaction with Et_3PAuCl (**1**) for five days, a colour version of Figure 3, and plots of absorbance versus time for assay of the PPIase activity of Cyp-3 are available in the Supporting Information. The atomic coordinates (code 1E3B) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (<http://www.rcsb.org/>).

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DeNO_x of Exhaust Gas from Lean-Burn Engines through Reversible Adsorption of N₂O₃ in Alkali Metal Cation Exchanged Faujasite-Type Zeolites**

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Nitrogen oxide (NO_x) adsorbents are key control components for the tailpipe emissions of transport vehicles powered with lean-burn engines. Current adsorbents are basic oxides which adsorb NO_x as nitrates, but suffer from poisoning by sulfur oxides. We discovered that NO and NO_2 can be selectively trapped as dinitrogen trioxide in alkali metal exchanged faujasite zeolites above 200 °C. The trapped N_2O_3 molecules compete with water molecules on a specific adsorption site and can be displaced by a change in partial pressure. Dinitrogen trioxide adsorption is not affected by the presence of sulfur oxides.

Modern lean-burn internal combustion engines operated with excess air show reduced fuel consumption and carbon dioxide emission but produce excessive amounts of nitrogen oxides (NO_x). The emission of NO_x at the tailpipe is the major source of pollution from transport vehicles powered by hydrocarbon fuels.^[1] Since there is a trade-off between NO_x and particulate carbon formation in the lean-burn engine, an efficient NO_x -elimination system for the exhaust can offer a solution to the particulate carbon emissions at the same time.^[2] In the emerging technologies that focus on reducing the NO_x emission from lean-burn engines, temporary storage

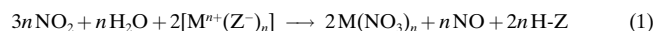
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of NO_x molecules on an adsorbent is a key step. In a NO_x -storage-and-reduction catalytic system,^[3] an oxidation catalyst that converts nitric oxide into nitrates is combined with basic oxides that are responsible for nitrate chemisorption. Periodically the nitrates are reduced in the rich exhaust gas produced by the engine. In the selective- NO_x -recirculation technique,^[4] trapped NO_x is periodically desorbed and reinjected into the engine. The drawback of nitrate adsorbents is that they are gradually deactivated by the accumulation of the more stable sulfates that originate from the combustion of fuel containing organic sulfur.

Dinitrogen trioxide can be formed in *dry* zeolites upon exposure to nitric oxide,^[5] to nitric oxide and oxygen,^[6, 7] or to mixtures of nitrogen dioxide and nitric oxide.^[6] In *dry* zeolites the dinitrogen trioxide molecule undergoes heterolytic splitting into a nitrosonium cation and a nitrite and/or nitrate anion,^[7, 8] or reacts with Brønsted acid sites to form a nitrosonium cation and water, as evidenced by IR spectroscopy.^[9] With real exhaust gas, which always contains water, the NO_x chemistry is different.^[5, 9, 10] In alkali or alkaline earth metal exchanged zeolite Y, nitrogen dioxide reacts with water at 80 °C to form nitrate with release of nitric oxide [Eq. (1); M^{n+} represents the valent charge compensating cation, and Z^- the negatively charged zeolite lattice]. The zeolite shows no affinity for NO at this temperature in the presence of water vapour.^[10]



A representative mixture of oxidized exhaust gas composed of helium with 500 ppm NO, 500 ppm NO_2 , 5% water, 10% O_2 , and 5% CO_2 was conducted over a bed of Na-Y zeolite at 150 °C (Figure 1A). The change in the NO and NO_2 concentrations between the inlet and outlet (Figure 1A) reveal a temporary complete adsorption of the NO_2 and a simultaneous formation of nitric oxide, the quantity of which corresponds to about one third of the adsorbed NO_2 [Eq. (1)].

A similar experiment performed at 250 °C (Figure 1B) reveals totally different adsorption chemistry. At this temperature NO and NO_2 are simultaneously adsorbed for a few minutes. The formation of N_2O_3 in the zeolite under these conditions was revealed by a Rietveld refinement in combination with Fourier analysis of the X-ray diffraction powder pattern (Figure 2). N_2O_3 molecules were shown to be localized at a unique position in the supercage of zeolite Y (Figure 2A and B). Under these conditions the supercages are filled with networks of water molecules that link sodium ions at the SIII positions with sodium ions SII and SV.^[11] The refined geometry of the N_2O_3 molecule (Figure 2C) is very close to the most stable asymmetric conformer.^[12] By assuming this position in the zeolite Na-Y the three oxygen atoms of an N_2O_3 molecule substitute three oxygen atoms of water molecules in the sodium–water net. Oxygen atom On1 bridges an SIII and an SV sodium ion, while the two On2 oxygen atoms are coordinated to the same SIII sodium ion and an SII sodium ion (Figure 2C).

If a homogeneous distribution of the sodium ions and water molecules over the hydrated Na-Y crystals prior to NO_x

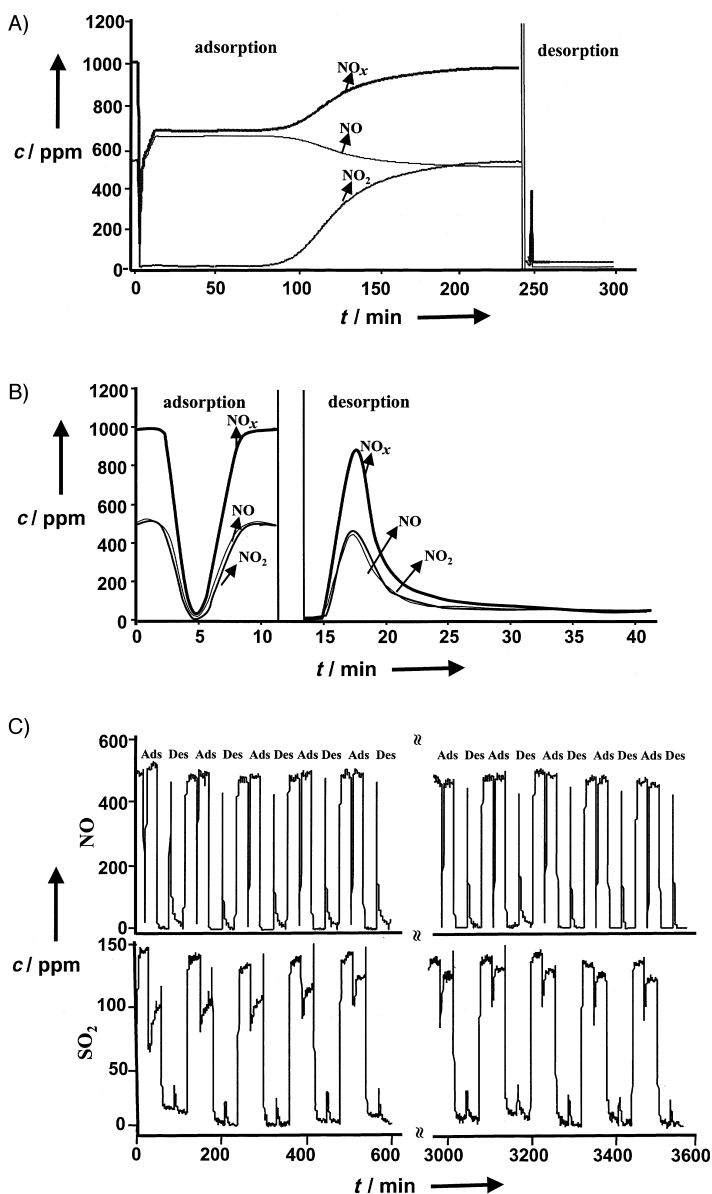
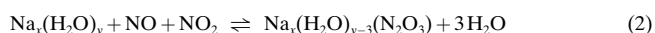


Figure 1. NO_x , NO_2 , NO, and SO_2 concentration changes in the outlet of the Na-Y adsorbent bed during NO_x adsorption and desorption at 150 °C (A) and 250 °C without and with SO_2 (B and C, respectively). Adsorption conditions: helium with 500 ppm NO, 500 ppm NO_2 , 5% CO_2 , 5% H_2O , 10% O_2 , and 150 ppm SO_2 (in C); VHSV = 30 000 h^{-1} . Desorption conditions: helium containing 5% H_2O ; VHSV = 15 000 h^{-1} .

adsorption is assumed, then each supercage contains at least one SV sodium cation together with SII and SIII sodium ions, and offers at least one adsorption site for N_2O_3 . The fraction of supercages holding an N_2O_3 molecule in the saturated Na-Y sample was 0.15 according to the refined occupancy factors (see the supporting information) and 0.12 according to the NO and NO_2 uptake derived from the inlet and outlet concentration profiles (Figure 1B). The partial saturation of the adsorption sites reflects the competition between the adsorption of N_2O_3 molecules and three water molecules [Eq. (2)].



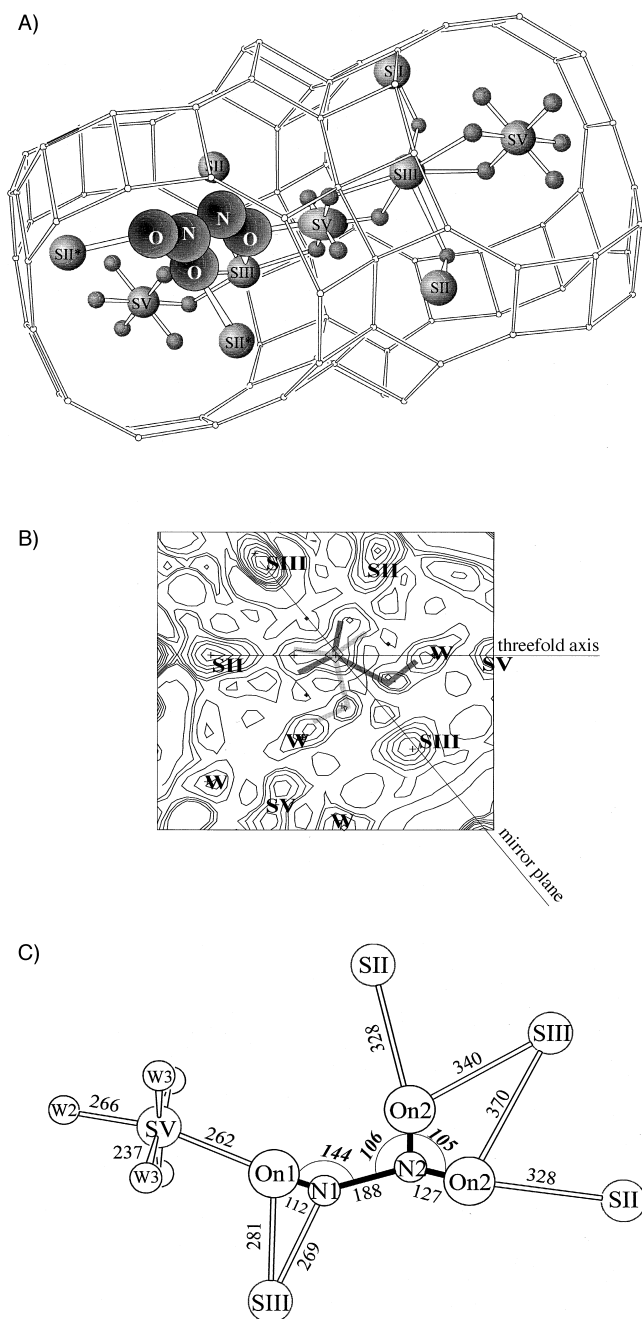


Figure 2. A) Rietveld-refined position of N₂O₃ (largest spheres), water (small spheres), and sodium cations (medium size spheres) in zeolite Na-Y (the lines in the ball and stick representation of the zeolite show the TO₂ tetrahedra of the framework; oxygen atoms are found between the Si or Al atoms). B) Observed electron density map revealing two symmetrically equivalent positions for the N₂O₃ molecule. C) Detailed representation of the N₂O₃ molecule and adjacent sodium ions. Bond lengths [pm] and angles [°] are indicated.

N₂O₃ can be desorbed as NO and NO₂ by displacing the equilibrium in Equation (2) by flushing the adsorbent bed with water-containing gas (Figure 1B). In contrast to this, the nitrate-saturated zeolite Na-Y can not be regenerated with hydrated helium gas (Figure 1A).

The formation of dinitrogen trioxide at 260 °C was monitored by FT-IR spectroscopy (Figure 3A). IR absorption bands were observed around 1270, 1590, and 1905 cm⁻¹ in an

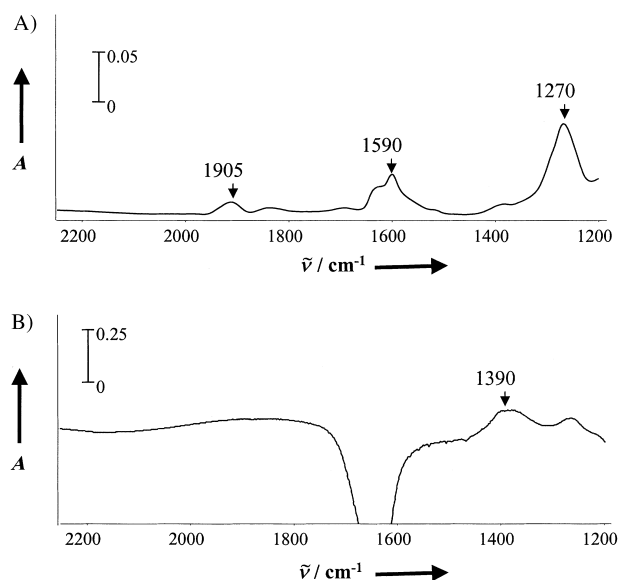


Figure 3. FT-IR difference spectra A) of Na-Y saturated with N₂O₃ at 260 °C and after evacuation of the cell with wet helium and B) of Na-Y saturated with nitrate obtained by cooling the N₂O₃ saturated Na-Y sample 40 °C with fresh hydrated Na-Y sample. A = absorption.

N₂O₃-saturated sample, which were assigned to the nitro symmetric stretch, nitro asymmetric stretch, and nitroso stretch, respectively, of the asymmetric N₂O₃ conformer.^[7, 9, 12–14] The signals disappear upon evacuation of the sample with hydrated helium gas. Cooling the N₂O₃-saturated sample to 40 °C leads to an IR absorption band around 1390 cm⁻¹ (Figure 3B), which is assigned to nitrate.^[10]

The temperature dependence of the nitrate and dinitrogen trioxide adsorption capacity, derived from the NO and NO₂ uptake curves, is shown in Figure 4. Nitrate formation diminishes with increasing temperature and vanishes above 300 °C. The N₂O₃ adsorption capacity peaks at 250 °C.

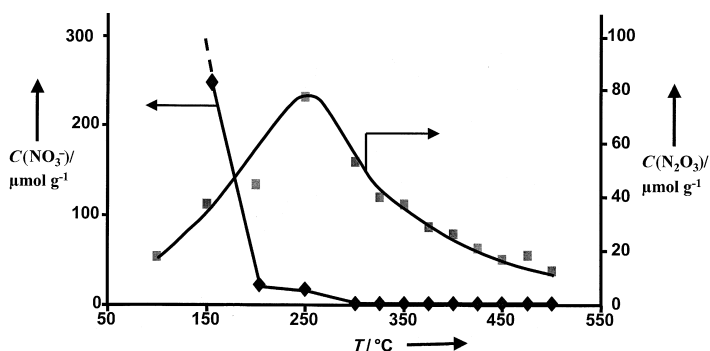


Figure 4. Temperature dependence of the nitrate and dinitrogen trioxide adsorption capacity C of zeolite Na-Y.

Experiments carried out at 250 °C with synthetic exhaust gas containing more NO₂ than NO showed that NO₂ can be adsorbed in molar quantities exceeding those of NO. Inspection of the adsorption site of N₂O₃ (Figure 2A and B) reveals that the adsorption of N₂O₄ can occur on the same site as N₂O₃. The preferential formation of N₂O₃ from equimolar mixtures of NO and NO₂ is explained by the higher stability of

N_2O_3 relative to N_2O_4 ^[15] and the much faster association kinetics.^[16]

An Na-Y sample was subjected to successive isothermal adsorption–desorption cycles at 250 °C for three days using synthetic exhaust gas spiked with 150 ppm SO_2 . Regeneration of the sample was achieved with hydrated helium gas (Figure 1C). In the 30 cycles made the N_2O_3 adsorption capacity remained constant at $(77 \pm 6) \mu\text{mol g}^{-1}$, which corresponds to the capacity measured in absence of SO_2 . The low uptake of SO_2 in the first cycles decreased with operation time (Figure 1C). In experiments to test for the control of NO_x emission, this N_2O_3 adsorbent shows high sulfur resistance. Furthermore, it allows the use of regeneration through a change of partial pressure in a water-containing gas such as hydrated air and does not necessitate heating.

Zeolites with the faujasite (FAU) framework topology can exist in different chemical compositions. For reasons of charge balance, the molar sodium content of the zeolite must equal the molar aluminum content in the framework. The formation of the water–sodium clusters that are active in N_2O_3 adsorption requires specific occupations of SII, SIII, and SV sites with sodium (Figure 2) and depends on the Si/Al atomic ratio of the faujasite framework. If the occupancies of these sites in Na-X (Si/Al = 1.2) and Na-Y (Si/Al = 1.7) zeolites are too high, nitrate adsorption is favored irrespective of temperature. The highest adsorption capacity for N_2O_3 was encountered in commercial Na-Y samples with Si/Al atomic ratios ranging from 2.4 to 2.6. Siliceous Na-Y zeolites such as EMC-1 (Si/Al = 3.6)^[17] have a decreased N_2O_3 capacity of 47 μmol of N_2O_3 per gram at 250 °C. This result is a direct consequence of the lower number of sodium ions available to form SII–SIII–SV water nets. Moreover, the screening of zeolite samples showed that N_2O_3 adsorption is unique to Y zeolites with FAU topology. Even the closely related EMC-2 zeolite (Si/Al = 3.7; EMT topology)^[17] did not show appreciable N_2O_3 adsorption.

Experimental Section

The NO and NO_2 concentrations in the NO_x adsorption experiments were monitored with a chemiluminescence detector (Eco Physics, CLD 700 EL ht). N_2O was analyzed on a ND-IR spectrophotometer (Maihak, Unor 610). A bed of compressed zeolite powder with particle size of 0.3–0.50 mm was mounted in a quartz tube of 0.9 mm internal diameter that fitted in a tubular furnace. He , O_2 , CO_2 , SO_2 , NO , and NO_2 were fed from gas cylinders using mass flow controllers. A stream of water-saturated helium gas was added after mixing all the other gases. The experiments depicted in Figure 1 were performed on 400 mg of Na-Y zeolite (Si/Al atomic ratio = 2.6, Zeolyst), which represents a bed volume of 0.6 mL. The adsorber was first by-passed for a few minutes to verify the inlet NO_x levels. The gas was then allowed to flow through the adsorbent bed at 300 mL min^{-1} (standard conditions), which corresponds to a volumetric hourly space velocity (VHSV) of 30 000 h^{-1} . After saturation of the adsorbent, identified by a NO_x level reaching the inlet value, the adsorber was closed, the lines flushed with the desorption gas, and the adsorbent bed evacuated while monitoring the NO_x concentration.

The FT-IR spectra were recorded using a Nicolet 730 instrument equipped with a cell with CaF_2 windows holding a Na-Y zeolite wafer with a density of 5 mg cm^{-2} . The spectral resolution was 2 cm^{-1} and the number of scans 500. The sample was heated to 260 °C in a wet helium atmosphere, exposed to helium gas containing 500 ppm NO , 500 ppm NO_2 , and 5 % water, and evacuated with hydrated helium gas (Figure 3A). In another experiment (Figure 3B) the sample was cooled to 40 °C after exposure to the NO_x -containing gas at 260 °C.

The position and nature of the adsorbed NO_x species were determined from a comparative Rietveld study of the X-ray powder patterns of the Na-Y sample contacted with hydrated helium gas (5 % water) and with a gas mixture composed of 500 ppm NO , 500 ppm NO_2 , 5 % CO_2 , 5 % H_2O , and 10 % O_2 at 250 °C. XRD patterns of samples in sealed capillaries (internal diameter: 0.5 mm) were recorded at room temperature in a Debye Scherrer geometry on a Stadi P diffractometer with $\text{CuK}\alpha_1$ radiation. Both samples were also measured at 250 °C between $2\theta = 4$ –35° and no changes in the intensity of the Bragg reflections at room temperature and 250 °C were detected. The arrangement of water molecules and sodium ions in the host are not dependent on temperature, but on the amount of water present in the cavities.^[11] Refinement was conducted on data sets between $2\theta = 4$ –70°. The low angle region was simultaneously refined with a different set of profile parameters. The GSAS software package was used to analyze the data. Structural parameters were refined starting with the data for a sample hydrated at 200 °C.^[11] This cation–water arrangement described the sample used for NO_x adsorption correctly. Only small changes in the powder pattern were observed after the adsorption of NO_x . An analysis of the observed and difference electron density allowed the NO_x molecules positions to be determined, which were identified by comparison with the asymmetric N_2O_3 conformer (Figure 2). Initially the molecules were introduced as rigid bodies into the Rietveld refinement. Towards the end of the refinement the rigid-body constraints were dropped. The occupancy factors within the molecule were constricted to represent the molecular formula. Thermal parameters of the molecule were kept fixed. The refined powder pattern and the refined parameters of Na-Y holding N_2O_3 are given in the Supporting Information.

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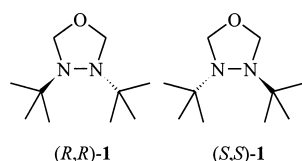
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Pronounced Steric Hindrance for Nitrogen Inversion in 1,3,4-Oxadiazolidines**

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In tertiary amines^[1] and aziridines^[2] the barrier for nitrogen inversion decreases as the size of the nitrogen substituents increases; this occurs because the pyramidal ground state is destabilized by the steric interaction of the substituents. Therefore, triisopropylamine^[3a,b] and diisopropyl-3-pentylamine^[3c] are characterized by an almost planar configuration of the nitrogen atom in the ground state, a geometry that corresponds to the transition state of the inversion process. In contrast to this, the barrier for double nitrogen inversion in cyclic hydrazines rises with increasing the size of the nitrogen substituents^[4] due to steric hindrance of the transition state.^[5] If inversion occurs via a biplanar transition state in sterically hindered systems, for example double nitrogen inversion in diaziridines,^[6] the transition state should be additionally destabilized by electronic interaction between the lone electron pairs of adjacent nitrogen atoms.

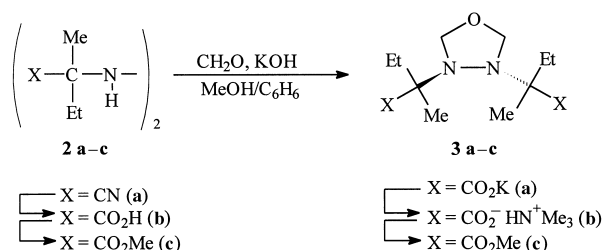
Indeed, as previously reported, high barriers for nitrogen inversion were found for compounds with bulky nitrogen substituents, for example, for 1,2-di-*tert*-butyldiazetidine the barrier for nitrogen inversion has been determined to be $\Delta G^\ddagger = 91.3 \text{ kJ mol}^{-1}$ at 155°C .^[5] For 3,4-di-*tert*-butyl-1,3,4-oxadiazolidine (**1**)^[5b,c,7] only a lower limit of $\Delta G^\ddagger > 99.6 \text{ kJ mol}^{-1}$ at 130°C could be deduced by NMR studies.^[8]



The true barrier for nitrogen inversion for 1,3,4-oxadiazolidine derivatives has now been determined through indirect evidence (by the introduction of chiral substituents on the

nitrogen atoms and monitoring diastereomerization by NMR spectroscopy) and by direct evidence (enantiomerization by the stopped-flow multidimensional gas chromatographic method (sfMDGC)).

3,4-Bis(1'-methoxycarbonyl-1'-methylpropyl)-1,3,4-oxadiazolidine (**3c**) was synthesized as shown in Scheme 1. Compound **3c** contains four stereogenic centers (two at carbon



Scheme 1. Synthesis of **3c**.

atoms and two at nitrogen atoms) however, only 6 of the 16 possible stereoisomers are observed, because of the presence of constitutionally equivalent chiral substituents on the nitrogen atoms and the absence of *cis* isomers (Figure 1).

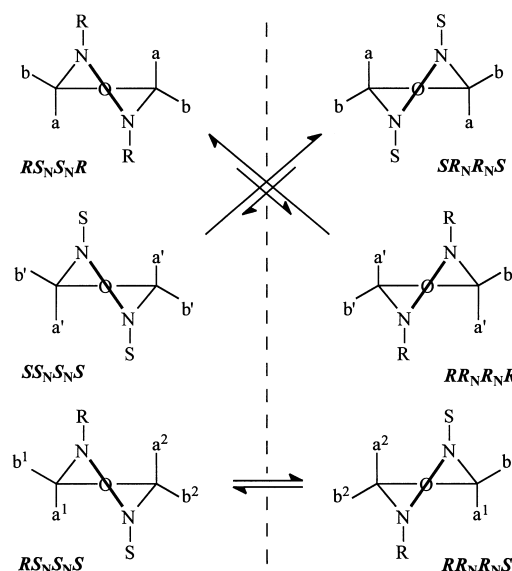


Figure 1. Stereoisomers of **3c**; substituents: R: (*R*)-C(Et)(Me)COOMe; S: (*S*)-C(Et)(Me)COOMe.

Two enantiomeric pairs exist for the major and minor diastereomers (*RR,RN,R*)-**3c**/(*SS,SN,S*)-**3c** and (*RS,SN,R*)-**3c**/(*SR,RN,S*)-**3c**, which are interconverted by nitrogen inversion until thermodynamic equilibrium is reached; for the third diastereomer there is one enantiomeric pair (*RS,SN,S*)-**3c**/(*SR,RN,R*)-**3c** which represents a quasi-*meso* form^[9] (with respect to configurationally opposite N substituents) undergoing a degenerated inversion. Therefore, only three sets of signals for each of the three diastereomers are observed in the ¹H and ¹³C NMR spectra of the synthetic mixture (Figure 2); in all the cases the signals from the protons and the carbon atoms of the ring do not overlap.

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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.

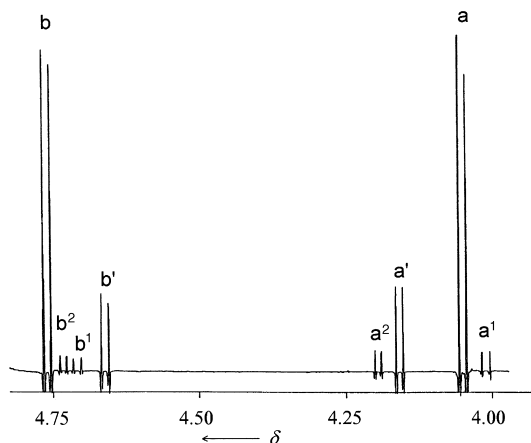


Figure 2. 400 MHz ^1H NMR spectrum of **3c** in CD_3OD showing the signals of the NCH_2O protons of the major, minor, and quasi-*meso* diastereomers of **3c** (for assignments see Figure 1).

In the quasi-*meso* diastereomer all four protons are non-equivalent and two AB systems with different ^2J spin–spin coupling constants are observed, whereas only one AB system is found for each of the major and minor diastereomers (Figure 2).

The kinetics of the diastereomerization process (in $[\text{D}_6]$ benzene at 56.5°C) for the major diastereomer of **3c** (Figure 3) gave the following parameters: $k_1^{\text{inv}} = 1.8 \times 10^{-5} \text{ s}^{-1}$, $k_{-1}^{\text{inv}} = 6.6 \times 10^{-5} \text{ s}^{-1}$, $\Delta G_1^\ddagger = 112.6 \pm 1.7 \text{ kJ mol}^{-1}$, $\Delta G_{-1}^\ddagger = 107.2 \pm 1.7 \text{ kJ mol}^{-1}$, which represents a very high barrier for nitrogen inversion for 1,3,4-oxadiazolidines.

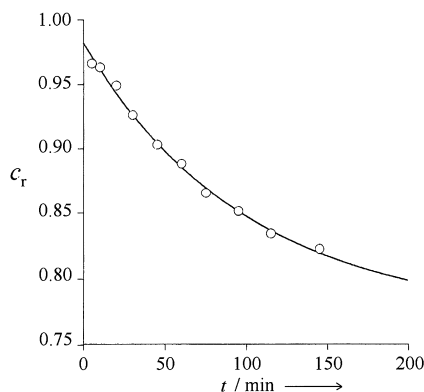


Figure 3. Kinetics of the diastereomerization process of the major diastereomer of **3c** in $[\text{D}_6]$ benzene at 329.5 K , initial concentration $c_0 = 0.1 \text{ mol L}^{-1}$ (the relative concentration $c_r = c_{\text{major}}/c_0$ versus time t is plotted). The regression curve is calculated with the following equation: $c = c_\infty + (c_0 - c_\infty)e^{-2kt}$ ($k = 8.5 \times 10^{-5} \text{ s}^{-1}$ where k is the sum of the rate constants of forward and back reaction calculated for one nitrogen atom).

An even higher barrier for nitrogen inversion $\Delta G^\ddagger$ was anticipated for 3,4-di-*tert*-butyl-1,3,4-oxadiazolidine^[8b] (**1**) because the conformational energy of the methyl group is higher than that of CO_2Me (7.12 and 5.32 kJ mol^{-1} , respectively).^[10] Thus, **1** was prepared and the enantiomers were separated by gas chromatography on a Chirasil- β -Dex column^[11] ($20 \text{ m} \times 250 \mu\text{m}$ internal diameter, film thickness 400 nm , 85°C , 0.45 bar H_2 , separation factor $\alpha = 1.17$).

To quantify the barrier for nitrogen inversion of **1** in the inert gas phase, the stopped-flow multidimensional gas chromatographic method (sfMDGC),^[12] which was recently developed for the determination of very high inversion (enantiomerization) barriers, was applied (see Experimental Section). The sfMDGC experiment can easily be carried out at different temperatures (Table 1), which allows the activation parameters $\Delta H_{\text{gas}}^\ddagger$ and $\Delta S_{\text{gas}}^\ddagger$ to be determined. The barrier for nitrogen inversion ($\Delta G_{\text{gas}}^\ddagger = 131.8 \text{ kJ mol}^{-1}$ at 56.5°C compared to $\Delta G_{\text{gas}}^\ddagger = 112.6 \text{ kJ mol}^{-1}$ for **3c**) for a pyramidal nitrogen atom in **1** is the highest value so far for a five-membered cyclic compound.

Table 1. Results of the sfMDGC experiment for **1**.

$T [^\circ\text{C}]$	$t [\text{min}]$	$A [\%]^{\text{[a]}}$	$k_{\text{gas}} [\text{s}^{-1}]$	$\Delta G_{\text{gas}}^\ddagger [\text{kJ mol}^{-1}]$
126.2	20.8	98.6	1.12×10^{-5}	136.7 ± 0.8
139.2	28.4	95.7	2.60×10^{-5}	138.4 ± 0.8
150.8	21.0	91.7	7.23×10^{-5}	138.8 ± 0.9
160.2	24.3	81.0	1.65×10^{-4}	139.0 ± 1.3
170.0	22.5	79.7	1.94×10^{-4}	141.5 ± 0.1

[a] A = Major peak area.

The mean values of $\ln(k/T)$ were plotted as a function of T^{-1} in an Eyring diagram. The activation parameters of **1** were found by using a linear fit ($r = 0.98$), and are $\Delta H_{\text{gas}}^\ddagger = 101.1 \pm 0.2 \text{ kJ mol}^{-1}$ and $\Delta S_{\text{gas}}^\ddagger = -93 \text{ J K}^{-1} \text{ mol}^{-1}$.

The experimental value of $\Delta H^\ddagger$ for **1** is considerably lower than that calculated for nitrogen inversion,^[1a] and the high negative value of $\Delta S^\ddagger$ can be considered as evidence for a dissociative mechanism for the inversion process. Such a dissociative inversion mechanism has been proposed for several heterocycles: for *N*-chlorooxaziridines (calculated: $\Delta H^\ddagger = 185 \text{ kJ mol}^{-1}$, experimental: $\Delta H^\ddagger = 64.1 \text{ kJ mol}^{-1}$, $\Delta S^\ddagger = -182 \text{ J K}^{-1} \text{ mol}^{-1}$),^[13] *N*-chlorodiaziridines (calculated: $\Delta H^\ddagger = 143 \text{ kJ mol}^{-1}$, experimental: $\Delta H^\ddagger = 46.9 \text{ kJ mol}^{-1}$, $\Delta S^\ddagger = -176 \text{ J K}^{-1} \text{ mol}^{-1}$),^[14] and Tröger's Base (experimental: $\Delta H^\ddagger = 62.7 \text{ kJ mol}^{-1}$, $\Delta S^\ddagger = -168 \text{ J K}^{-1} \text{ mol}^{-1}$).^[12b] The increase in the negative entropy is characteristic for charge separation in heterolytic processes,^[15] but not for homolytic processes.^[16] Thus, we assume that nitrogen inversion in **1** proceeds via dissociation with the breaking of a $\text{CH}_2\text{--O}$ bond and formation of a methyleneiminium ion. It is known that 1,3,4-oxadiazolidines react with amines as aminomethylating reagents with the breaking of this particular bond.^[17]

Experimental Section

Synthetic procedures and spectroscopic data can be found in the Supporting Information.

The stopped-flow multidimensional gas chromatography (sfMDGC) was performed on a Siemens Sichromat 2 gas chromatograph equipped with two ovens, a pneumatically controlled six-port valve (Valco), a cooling trap for use with liquid nitrogen in oven 2, two flame-ionization detectors and a C-R 6A integrator (Shimadzu). The whole process is monitored by a control computer.

For the separation of enantiomers of **1** a fused silica column coated with Chirasil- β -Dex^[11] ($12.5 \text{ m} \times 0.25 \text{ mm}$ internal diameter, $0.4 \mu\text{m}$ film thickness, 110°C) in oven 1 was employed. Either the first or second eluted (pure) enantiomer was trapped on the reactor column, which was a

deactivated fused silica column coated with dimethylpolysiloxane (1 m × 0.25 mm internal diameter, 0.002 µm film thickness). The reactor column was quickly heated to the temperature T and enantiomerization commenced. After the reaction time t the reactor column was rapidly cooled with liquid nitrogen and the new enantiomeric mixture was transferred onto the second separation column coated with Chirasil-β-Dex (12.5 m × 0.25 mm internal diameter, 0.4 µm film thickness, 110 °C), where the enantiomers were separated. The carrier gas used was helium. The experiment was repeated three times at each temperature. The rate constant k for inversion was calculated according to Equation (1) using the observed enantiomeric ratio (% *er*) of the major peak area, the temperature T , and the reaction time t .

$$k = \frac{1}{2t} \ln \frac{er + 1}{er - 1} \quad (1)$$

The mean values of $\ln(k/T)$ were plotted as a function of T^{-1} according to the Eyring equation. The activation parameters $\Delta H_{\text{gas}}^\ddagger$ and $\Delta S_{\text{gas}}^\ddagger$ were obtained from the gradient and the y intercept, respectively, of a linear fit. A statistical factor κ of 0.5 for a reversible inversion process was applied.

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Stable and Selective Hybridization of Oligonucleotides with Unnatural Hydrophobic Bases**

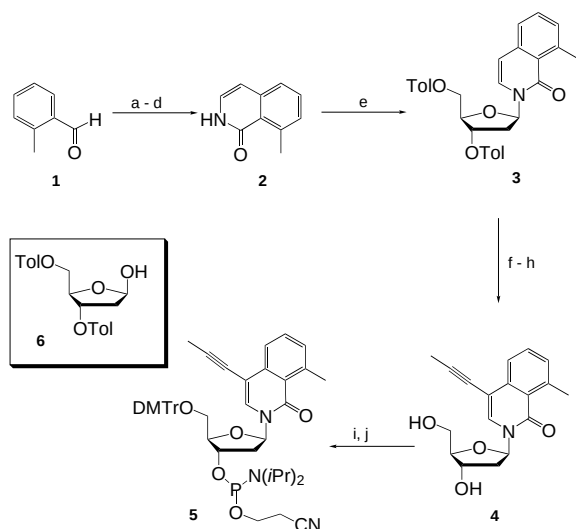
Markus Berger, Anthony K. Ogawa,
Dustin L. McMinn, Yiqin Wu, Peter G. Schultz,* and
Floyd E. Romesberg*

DNA duplex stability and sequence specificity are based on the complementary Watson–Crick hydrogen-bonding (H-bonding) patterns of adenine with thymine (dA:dT base pair) and cytosine with guanine (dC:dG base pair). Additional, stable and selective base pairs would facilitate hybridization or encoding experiments in cases where natural sequences cross hybridize, or where increased information storage is desirable.^[1–4] Moreover, the characterization of DNA containing such base pairs may provide increased insight into DNA structure and function.^[5–15] We recently reported the synthesis and characterization of several unnatural nucleosides that possess hydrophobic groups instead of a purine or pyrimidine base.^[2,3] Among these, hydrophobic nucleosides containing the isocarbostyryl core were particularly interesting due to their ability to form stable self-pairs in duplex DNA (Isocarbostyryl = 1-hydroxyisoquinoline).^[3] We now describe a series of isocarbostyryl derivatives that culminate in a self-pair which is both more stable and more selective than either a dA:dT or a dC:dG pair in the same sequence context.

The isocarbostyryl core was derivatized with a propynyl group at C7, a substitution known to impart increased stability to both natural^[9,16] and unnatural DNA,^[2,3] as well as with a C3 methyl group, which is expected to pack in the hydrophobic base interface. The 7-propynyl-3-methylisocarbostyryl nucleoside (**PIM**) was synthesized and converted to phosphoramidites **5** as shown in Scheme 1.^[17] The free base **2** was generated according to the procedure of Hirato et al.,^[18] and

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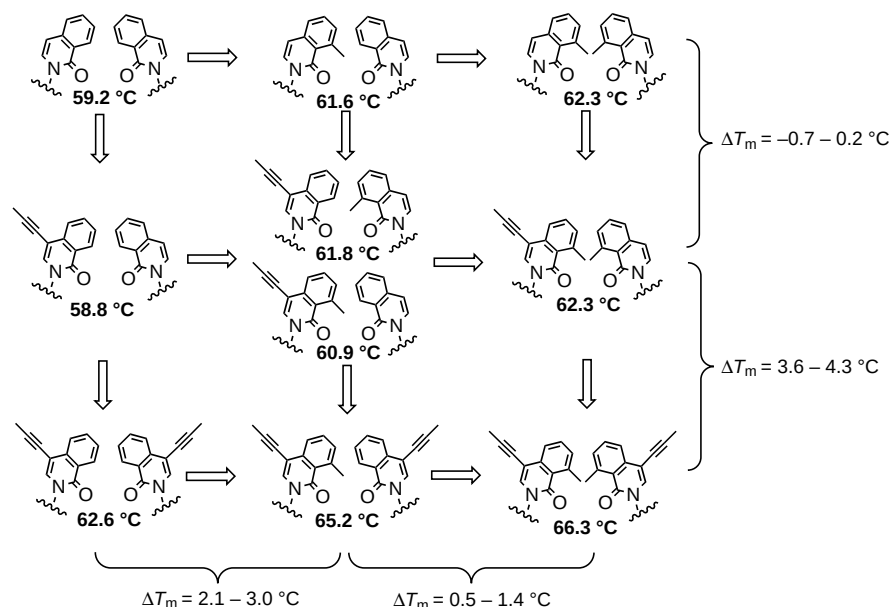
Scheme 1. a) $(\text{MeO})_2\text{CHCH}_2\text{NH}_2$, benzene; b) $\text{Cl}_3\text{CCO}_2\text{Et}$, THF, $\text{P}(\text{OMe})_3$, TiCl_4 , CH_2Cl_2 ; c) H_2O_2 , AcOH ; d) Ac_2O , then NaOH ; e) NaH , CH_3CN , **6**; f) ICl , CH_2Cl_2 ; g) $\text{HC}\equiv\text{CCH}_3$, CuI , $[\text{PdCl}_2(\text{PPh}_3)_2]$, NEt_3 ; h) NaOMe , MeOH ; i) DMTrCl , pyridine; j) $(i\text{Pr})_2\text{N}-\text{P}(\text{ClOCH}_2\text{CH}_2\text{CN})_2$, DIPEA , CH_2Cl_2 . Tol = tolyl, DMTr = 4,4-dimethoxytriphenylmethyl, DIPEA = diisopropylethylamine.

the propynyl group was introduced by an iodination and a Sonogashira-coupling^[19, 20] with the protected nucleoside **3**.^[21] The isocarbostyryl (**ICS**), 7-propynylisocarbostyryl (**PICS**), and 3-methylisocarbostyryl (**MICS**) nucleosides and phosphoramidites were synthesized as described previously.^[2, 3] The thermal stability of the unnatural base pairs was evaluated by determining the melting temperature (T_m) of duplex **7** with X and Y = dA, dT, dG, dC, **ICS**, **PICS**, **MICS**, or **PIM** (10 mM 1,4-piperazinediethanesulfonic acid (PIPES), 100 mM NaCl, pH 7). The stabilities of native and unnatural base pairs are reported in Table 1.

GCGTACXCATGCG

CGCATGYGTACGC

7



Scheme 2. The relationship between structural modification and thermal stability.

Table 1. Denaturation temperatures (T_m) for oligonucleotide **7** containing a unnatural and natural base pairs (X:Y).^[a]

X = Y =	ICS	PICS	MICS	PIM	A	T	G	C
ICS	59.3							
PICS	58.8	62.6						
MICS	61.6	61.8	62.3					
PIM	60.9	65.2	62.3	66.3				
A	55.1	55.5	53.6	55.1	52.7	58.7	52.3	48.8
T	53.0	53.7	55.1	54.7	59.2	49.8	52.8	47.2
G	52.2	51.4	54.8	56.2	55.4	53.3	50.5	61.8
C	51.0	54.5	54.8	53.4	48.4	45.0	60.7	44.8

[a] The melting temperature experiments were carried out on a Cary 300 - Bio UV/Vis spectrophotometer. The heating rates were $0.5^\circ\text{C min}^{-1}$. Conditions: 3 μM DNA, 10 mM PIPES, 10 mM MgCl_2 , 100 mM NaCl, pH 7.

In the sequence context of duplex **7**, the least stable unnatural self-pair, **ICS:ICS** is as stable as a dA:dT pair (59.3°C and 59.2°C for **ICS:ICS** and dA:dT, respectively), as reported previously.^[2] The relationship between structural modifications and thermal stability is illustrated in Scheme 2. Addition of a C3 methyl group to a single nucleobase, or to both nucleobases of a pair stabilizes the resulting base pair by $2.1\text{--}3.0^\circ\text{C}$ and $3.1\text{--}3.7^\circ\text{C}$, respectively. The addition of a propynyl group to one nucleobase of the unnatural pair results in either little effect (for example, **MICS:MICS** versus **PIM:MICS**: $\Delta T_m = 0.2^\circ\text{C}$) or a destabilizing effect on duplex formation (as in **ICS:ICS** versus **PICS:ICS** and **MICS:ICS** versus **PIM:ICS**: $\Delta T_m = -0.7^\circ\text{C}$). However, the addition of a propynyl group to the both nucleobases of a pair results in a significant increase in duplex stability by $3.4\text{--}4.0^\circ\text{C}$.

The additive effects of both methyl and propynyl substitution result in a remarkably stable **PIM:PIM** self-pair. The **PIM:PIM** self-pair is significantly more stable than a dC:dG base pair ($T_m = 66.3^\circ\text{C}$ and 61.8°C for **PIM:PIM** and dC:dG, respectively) in this sequence context. Furthermore, the combinations of methyl and propynyl substitution allow

nucleobase combinations which result in base pairs with a range of stabilities, from the stability of a dA:dT pair to significantly more stable than a dC:dG pair.

Minimization of cross-hybridization, which results from mispairing within hybridizing oligonucleotides, is of great practical importance.^[15, 22–27] To minimize nonselective hybridization with an expanded set of DNA bases, the unnatural bases must selectively pair with each other relative to mispairing with native bases. The selectivities of the unnatural bases were determined by synthesizing and characterizing a series of oligonucleotides containing mispairs between unnatural and native bases. The results are reported in Table 1. Relative to the most stable mispair (**PIM:dC**), the **PIM:PIM** self-pair has a melting temperature difference of 10.1°C and

is, therefore, very selective against mispairing. For comparison, in the same sequence context, the thermal selectivities for the native dA:dT and dC:dG base pairs are only 3.8 °C and 6.4 °C.

In order for the unnatural base pairs to be of practical value, oligonucleotides containing multiple unnatural bases should maintain high stability and specificity. To examine this we determined the melting temperatures of duplex DNA containing multiple **PICS** self-pairs. The measured T_m values, displayed in Table 2, reveal that two **PICS** self-pairs result in

Table 2. Melting temperatures T_m of duplex DNA with multiple **PICS** self-pairs.

Duplex ^[a]	T_m [°C]
⁵ GCGTAXXCATGCG ³	51.8
³ CGCATXXGTACGC ⁵	
⁵ GCGTACXCXTGCG ³	56.9
³ CGCATGXGXACGC ⁵	
⁵ GCGTACXCA ³ XGCG ³	63.2
³ CGCATGXGT ³ XC GC ⁵	
⁵ GCGTACXCATGCG ³	62.6
³ CGCATGXGTACGC ⁵	
⁵ GCGXACTCA ³ XGCG ³	62.0
³ CGCXTGAGT ³ XC GC ⁵	
⁵ GCGTACTCA ³ XGCG ³	61.2
³ CGCATGAGT ³ XC GC ⁵	
⁵ GCGTAXXCATGCG ³	49.1
³ CGCATGXGTACGC ⁵	
⁵ GCGTACXCXTGCG ³	51.4
³ CGCATGXGTACGC ⁵	
⁵ GCGTACXCA ³ XGCG ³	53.5
³ CGCATGAGT ³ XC GC ⁵	
⁵ GCGTACACATGCG ³	59.2
³ CGCATGTGTACGC ⁵	

[a] X = **PICS**.

additional duplex stability if they are separated by native pairs. Despite the decrease in duplex stability when two **PICS** self-pairs are adjacent, selectivity against mispairing with native bases remains reasonably high. Therefore, oligonucleotides containing at least two **PICS**, in any sequence context examined, are strongly selective for correct hybridization and should make it possible to increase the stringency and information content of hybridization or encoding experiments.

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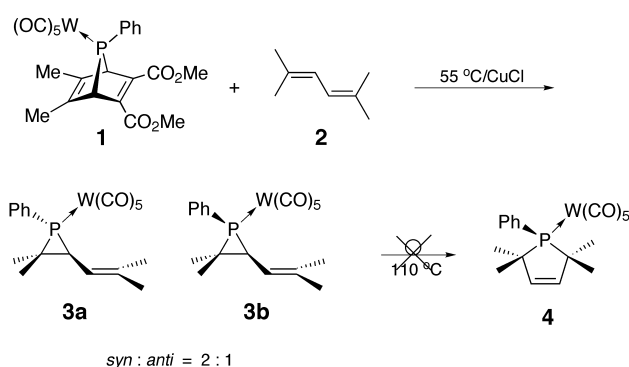
Synthesis of a Complexed 2,2-Bisphosphirane**

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and Koop Lammertsma*

The past two decades saw an explosive growth in phosphorus-containing heterocycles of which the three-membered ring structure occupies a prominent place.^[1] Even spiro-condensed systems containing this structural element have been synthesized.^[2] Surprisingly absent in the plethora of heterocyclic organophosphorus structures are, however, the simple C–C-connected phosphiranes, in sharp contrast to the related bisoxiranes,^[3] bisaziridines,^[4] bissiliranenes,^[5] and bis-thiiranes.^[6] Even bisphosphirenes were described recently.^[7] Here we report on the synthesis of a saturated analogue.

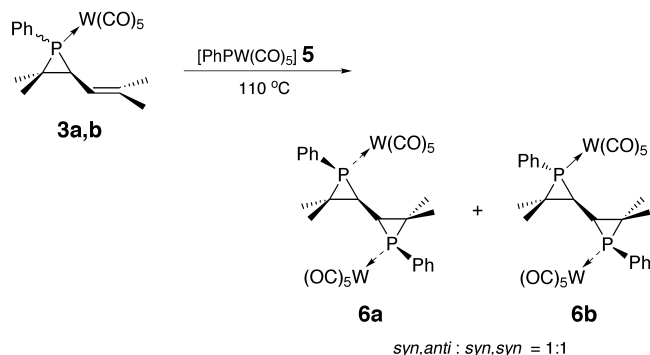
A convenient route to the three-membered phosphiranes is provided by the addition of in-situ-generated terminal-complexed phosphinidenes to olefins.^[1b] As unencumbered reactive intermediates, phosphinidenes, such as [PhPW(CO)₅] (**5**), are considered to react as electrophilic singlet carbene-like species.^[1b, 8] Double addition to conjugated dienes appears an obvious path to bisphosphiranes. However, after formation of the initial vinylphosphirane no second addition has yet been observed. Instead and in analogy to their hydrocarbon analogues, vinylphosphiranes with a *s-cisoid* conformation are subject both to a 1,3-sigmatropic shift yielding phospholenes and to epimerization at the phosphorus center.^[1b, 9] Steric factors may contribute to the inability to add a second [RPW(CO)₅] unit in the systems studied thus far. We therefore decided to explore the double addition on a transoid noncyclic diene or more precisely the addition to a *s-trans* vinylphosphirane. A transoid conformation is preferred for 2,5-dimethyl-2,4-hexadiene (**2**)^[10] and its methyl groups are considered small enough not to interfere with the second phosphinidene addition.

Reaction of [PhPW(CO)₅] (**5**), generated from the 7-phosphanorbornadiene complex **1**, with diene **2** at 55 °C in the presence of CuCl affords the *syn*- and *anti*-vinylphosphiranes **3a** and **3b**, respectively, in a 2:1 ratio in 76 % yield. In this case *syn* and *anti* refer to the orientation of the [W(CO)₅] with respect to the vinyl group. These isomers were distinguished based on their ³¹P and ¹H NMR data. The major product isolated by crystallization is the *syn* isomer **3a**, whose olefinic proton is 0.25 ppm more deshielded ($\delta_{\text{H}} = 4.97$; no P-phenyl shielding), whose phosphorus atom is 18.2 ppm more de-



shielded ($\delta_{\text{P}} = -118.3$), and whose ¹J_{PW} coupling is 7.8 Hz larger (256.2 Hz). All of these findings are in agreement with earlier assignments for cyclic *syn*-vinylphosphiranes.^[11] The *cis/trans* and *E/Z* assignments of the methyl substituents are based on a combination of COSY and NOE NMR measurements. For example, for **3a** NOE interactions were observed between the phosphirane ring proton and the *trans*-methyl group and between the olefinic proton and the *E*-CH₃ group.

To explore whether the vinyl groups of **3a, b** are susceptible to a second phosphinidene addition they were treated with an additional equivalent of [PhPW(CO)₅] at 110 °C (without CuCl). Monitoring the reaction mixture by ³¹P NMR spectroscopy showed indeed the formation in about 50 % yield of the diastereomeric bisphosphirane complexes (**6a**) (*syn,anti* isomer) and **6b** (*syn,syn* isomer) in a 1:1 ratio. The *syn,anti* isomer is easily recognized by its set of doublets ($\delta = -125.7$ and -135.0 , *J*_{PP} 31.7 Hz) and the *syn,syn* form by its



characteristic singlet ($\delta = -126.1$) in the ³¹P NMR spectrum. Both products were isolated in 25 % yield and fully characterized by mass spectrometry, and ³¹P, ¹³C, and ¹H NMR spectroscopy. Interestingly, the ¹³C NMR spectrum of the *syn,syn* isomer displays an AA'X system (A,A' are P atoms) with a second-order line pattern (5 + 3) for the spiro-carbon atom (X).^[12] The *anti,anti* isomer, if present, could not be detected.

Heating *syn*-vinylphosphirane **3a** in toluene (both with and without CuCl) at 110 °C for 1 h does not give detectable amounts of phospholene **4**, as expected, but instead leads to P-epimerization (yielding *anti* **3b**) and formation of bisphosphiranes **6a** and **6b** in a 1:1 ratio. Reaction of **3a** with [PhPW(CO)₅] at 110 °C also gives a 1:1 mixture of **6a** and **6b**.

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We attribute the equal formation of the *syn,anti*- and *anti,anti*-bisphosphiranes (and no *anti,anti*) from both the reaction of **3a** and the 2:1 mixture of **3a** and **3b** to occur thermodynamically as a result of P-epimerization and intermolecular transfer of the phosphinidene complex.

The *syn,syn* conformation of the C_2 -symmetrical complex **6b** was established unequivocally by a single-crystal X-ray structure determination,^[13] which revealed the expected geometrical features (Figure 1). The P–C2 and P–C3 bond

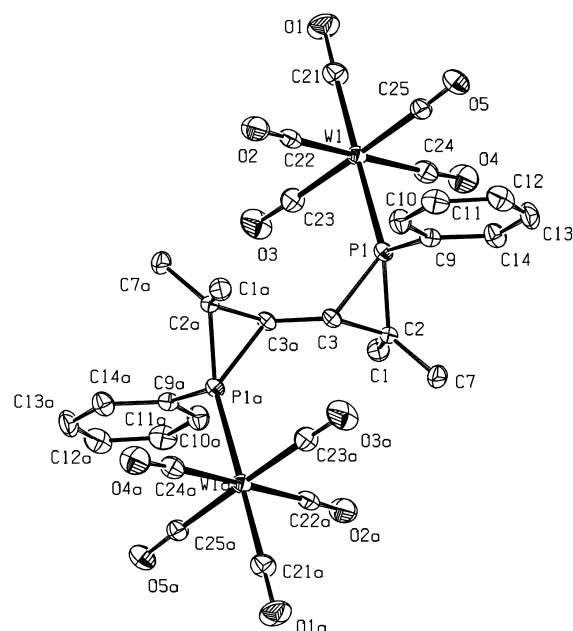


Figure 1. Structure of **6b**. Hydrogen atoms are omitted for clarity. Selected distances [Å] and angles [°]: P1–C2 1.848(5), P1–C3 1.842(5), C2–C3 1.537(7), C3–C3a 1.501(7), P1–W1 2.5031(13), P1–C9 1.809(5); W1–P1–C9 114.98(16), C2–P1–C3 49.2(2), P1–C3–C2 65.6(3), P1–C3–C3a 121.0(3), P1–C3–C3a–P1a 180.0(3).

lengths of 1.848(5) and 1.842(5) Å, respectively, and the C–P–C bond angle of 49.2(2)° are typical for $[W(CO)_5]$ -complexed phosphiranes. The connecting C3–C3a bond of 1.501(7) Å appears relatively short but its length is similar to that in bicyclopropane (1.517 Å).^[14] The ab initio MP2/6-31G*-optimized geometry of uncomplexed *syn,syn*-bisphosphirane **7** (devoid of substituents, Figure 2) illustrates the stabilizing effect of the $[W(CO)_5]$ group and shows the interaction between the two rings to be minimal with a C–C bond length of 1.499 Å. Energy calculations at the G2MP2 level (to obtain ΔH_f values) in conjunction with an elaborate set of isodesmic

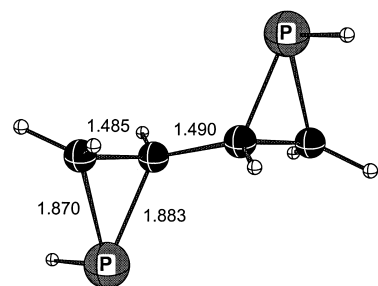


Figure 2. MP2/6-31G* structure of **7**.

reactions do not indicate an increment in strain energy of the phosphirane ring. Thus, even though the computed strain energy for **7** of 39.59 kcal mol^{−1} is slightly less than twice the value of 21.27 kcal mol^{−1} reported for C₂PH₅,^[2d] the energy difference of 2.95 kcal mol^{−1} can be accounted for by the substituent effect. For example, the strain energy of the phosphirane already reduces by 1.05 kcal mol^{−1} (also at the G2MP2 level) on C-substituting the ring with a methyl group.

Experimental Section

NMR spectra were recorded on Bruker AC 200, MSL 400 (¹H, ¹³C), and WM 250 (³¹P) spectrometers using SiMe₄ (¹H, ¹³C) or 85% H₃PO₄ (³¹P) as external standards. High-resolution mass spectra (HR-MS) were recorded on a Finnigan MAT 90 and FT-IR spectra on a Mattson 630 Galaxy spectrophotometer. Elemental analysis was performed by Micro-analytischer Labor Pascher, Remagen-Bandorf (Germany).

3a, b: Complex **1** (0.51 g, 0.77 mmol) and 2,5-dimethyl-2,4-hexadiene (85 mg, 0.77 mmol) were heated at 60 °C for 3 h in toluene with CuCl (56 mg, 56 mmol). After evaporation of the solvent, the residue was chromatographed over silica with pentane/toluene (4/1) to yield a 2:1 isomeric mixture of **3a** and **3b** (0.32 g; 76%) as determined by ³¹P NMR spectroscopy. **3a**: m.p. 70–71 °C (pentane); ³¹P NMR (CDCl₃): δ = −118.3 (¹J(P,W) = 256.2 Hz); ¹³C NMR (CDCl₃): δ = 198.0 (d, ²J(C,P) = 29.9 Hz; *cis*-CO), 195.6 (d, ²J(C,P) = 8.1, ¹J(C,W) = 125.7 Hz; *trans*-CO), 138.3 (d, ³J(C,P) = 8.9 Hz; C=C(CH₃)₂), 135.7 (d, ¹J(C,P) = 24.6 Hz; *ipso*-Ph), 132.1 (d, ³J(C,P) = 10.2 Hz; *m*-Ph), 129.7 (d, ⁴J(C,P) = 2.0 Hz; *p*-Ph), 128.6 (d, ²J(C,P) = 9.1 Hz; *o*-Ph), 118.0 (d, ²J(C,P) = 3.3 Hz; C=C(CH₃)₂), 35.5 (d, ¹J(C,P) = 15.4 Hz; PCH), 30.4 (d, ¹J(C,P) = 17.2 Hz; PC(CH₃)₂), 25.6 (d, ²J(C,P) = 2.7 Hz; *trans*-PC(CH₃)₂), 25.5 (d, ⁴J(C,P) = 3.1 Hz; (*E*)-C=C(CH₃)₂), 21.7 (d, ²J(C,P) = 9.1 Hz; *cis*-PC(CH₃)₂), 19.1 (d, ⁴J(C,P) = 2.6 Hz; (Z)-C=C(CH₃)₂); ¹H NMR (CDCl₃): δ = 7.44–7.18 (m, 5H, Ph), 4.97 (m, ³J(H,P) = 6.76, ³J(H,H) = 8.10, ⁴J(H,H) = 1.4 Hz, 1H; CH=C), 2.49 (pseudo-t, ²J(H,P) = 8.10, ³J(H,H) = 8.10 Hz, 1H; CHP), 1.86 (d, ⁵J(H,P) = 3.68 Hz, 3H; (Z)-C=C(CH₃)), 1.74 (d, ⁵J(H,P) = 5.53 Hz, 3H; (*E*)-C=C(CH₃)), 1.34 (d, ³J(H,P) = 18.29 Hz, 3H; *cis*-CH₃), 1.13 (d, ³J(H,P) = 12.19 Hz, 3H; *trans*-CH₃); HR-MS: calcd for C₁₉H₁₉O₅PW (%): 542.04800, found: 542.04806. **3b**: ³¹P NMR (toluene): δ = −136.5 (¹J(P,W) = 248.4 Hz); ¹³C NMR (CDCl₃): δ = 198.4 (d, ²J(C,P) = 29.7 Hz; *cis*-CO), 196.0 (d, ²J(C,P) = 8.1, ¹J(C,W) = 125.7 Hz; *trans*-CO), 136.6 (d, ³J(C,P) = 6.0 Hz; C=C(CH₃)₂), 133.3 (d, ³J(C,P) = 9.9 Hz; *m*-Ph), 129.7 (d, ⁴J(C,P) = 2.0 Hz; *p*-Ph), 128.4 (d, ³J(C,P) = 9.1 Hz; *o*-Ph), 117.3 (d, ²J(C,P) = 7.2 Hz; C=C(CH₃)₂), 36.7 (d, ¹J(C,P) = 16.7 Hz; PCH), 29.3 (d, ¹J(C,P) = 15.5 Hz; PC(CH₃)₂), 28.1 (d, ²J(C,P) = 6.5 Hz; *cis*-PC(CH₃)₂), 25.7 (d, ⁴J(C,P) = 2.4 Hz; (*E*)-C=C(CH₃)₂), 19.7 (d, ²J(C,P) = 2.4 Hz; *trans*-PC(CH₃)₂), 18.7 (d, ⁴J(C,P) = 1.9 Hz; (Z)-C=C(CH₃)₂); ¹H NMR (CDCl₃): δ = 7.12–7.54 (m, 5H, Ph), 4.72 (m, CH=C), 2.32 (d, ³J(H,H) = 8.90 Hz, 1H; CHP), 1.94 (m, 3H, (Z)-C=C(CH₃)), 1.77 (d, ⁵J(H,P) = 4.02 Hz, 3H; (*E*)-C=C(CH₃)), 1.49 (d, ³J(H,P) = 19.15 Hz, 3H; *cis*-CH₃), 1.29 (d, ³J(H,P) = 11.54 Hz, 3H; *trans*-CH₃).

6a, b: Complex **1** (0.88 g, 1.3 mmol) was heated at 110 °C for 20 h with a 2:1 mixture of *syn,anti* isomers of vinylphosphirane (**3**) (0.98 g, 1.8 mmol) in toluene (5 mL). The solution was concentrated to a volume of 3 mL and pentane (6 mL) was added. Complex **6b** precipitated as a white solid (0.33 g, 25%). The extract was evaporated to dryness and the residue was chromatographed over silica, starting with pentane as eluent, and slowly converting to toluene to give complex **6a** (0.29 g, 22%). **6a** (*syn, anti* isomer): m.p.: 179–180 °C (decomp); ³¹P NMR (CDCl₃): δ = −125.7 (d, ¹J(W,P) = 256.9, ³J(P,P) = 31.7 Hz), −135.0 (d, ¹J(W,P) = 256.0, ³J(P,P) = 31.5 Hz); ¹³C-NMR: δ = 197.9 (d, ²J(P,C) = 30.3 Hz; *trans*-CO), 197.1 (d, ²J(P,C) = 27.1 Hz; *trans*-CO), 195.6 (d, ²J(P,C) = 8.0 Hz; *cis*-CO), 195.5 (d, ²J(P,C) = 7.8 Hz; *cis*-CO), 135.5 (¹J(P,C) = 26.7 Hz; *ipso*-Ph), 133.3 (d, ³J(P,C) = 9.7 Hz, *m*-Ph), 131.8 (¹J(P,C) = 27.5 Hz; *ipso*-Ph), 131.8 (d, ³J(P,C) = 10.4 Hz; *m*-Ph), 130.4 (d, ⁴J(P,C) = 1.8 Hz; *p*-Ph), 130.1 (d, ⁴J(P,C) = 1.0 Hz; *p*-Ph), 129.1 (d, ²J(P,C) = 9.0 Hz; *o*-Ph), 128.9 (d, ²J(P,C) = 9.3 Hz; *o*-Ph), 36.0 (dd, ¹J(P,C) = 19.3, ³J(P,C) = 3.6 Hz; *anti*-PCH), 35.2 (dd, ¹J(P,C) = 18.4, ²J(P,C) = 4.5 Hz; *syn*-PCH), 28.8 (d, ¹J(P,C) = 15.4 Hz; *syn*-PC(CH₃)₂), 28.4 (dd, ¹J(P,C) = 13.3, ³J(P,C) = 1.4 Hz; *anti*-PC(CH₃)₂), 28.2 (d, ²J(P,C) = 6.7 Hz; *cis*(*anti*)-PC(CH₃)₂),

26.4 (d, $^2J(\text{P,C}) = 2.4$ Hz; *trans(syn)*-PC(CH₃)₂), 21.7 (d, $^2J(\text{P,C}) = 8.6$ Hz; *cis(syn)*-PC(CH₃)₂), 20.0 (d, $^2J(\text{P,C}) = 2.7$ Hz; *trans(anti)*-PC(CH₃)₂); ^1H NMR (CDCl₃): $\delta = 7.2$ – 7.4 (m, 10H, Ph), 1.72 (d, $^3J(\text{H,P}) = 17.45$ Hz, 3H; *cis(syn)*-PC(CH₃)₂), 1.66 (d, $^3J(\text{H,P}) = 19.23$ Hz, 3H; *cis(anti)*-PC(CH₃)₂), 1.64 (d, $^3J(\text{H,P}) = 10.65$ Hz, 3H; *trans(anti)*-PC(CH₃)₂), 1.39 (m, 2H; PCH), 1.27 (d, $^3J(\text{H,P}) = 12.01$ Hz, 3H; *trans(syn)*-PC(CH₃)₂). **6b** (*syn,syn* isomer): m.p. 179–180 °C (decomp); ^{31}P NMR: (CDCl₃): $\delta = -126.1$ ($^1J(\text{P,W}) = 217.2$ Hz); ^{13}C NMR: $\delta = 197.1$ (m, $^{2+5}J = 29.6$ Hz; *trans*-CO), 195.6 (m, $^{2+5}J(\text{P,C}) = 7.6$ Hz; *cis*-CO), 134.9 (m, $^{1+4}J = 27.7$ Hz; *ipso*-Ph), 131.8 (m, $^{3+6}J = 11.6$ Hz; *m*-Ph), 130.2 (s, *p*-Ph), 128.8 (m, $^{2+5}J = 9.4$ Hz; *o*-Ph), 34.5 (m, $^{1+2}J = 13.2$ Hz; PCH), 29.2 (m, $^{1+3}J = 14.0$ Hz; PC(CH₃)₂), 25.5 (s, *trans*-PC(CH₃)₂), 21.5 (m, $^{2+4}J = 8.4$ Hz; *cis*-PC(CH₃)₂); ^1H NMR (CDCl₃): $\delta = 7.2$ – 7.4 (m, 10H, Ph), 1.78 (s, 2H, CHP), 1.69 (m, $^3J(\text{H,P}) = 17.50$ Hz, 6H; *cis*-CH₃), (m, $^3J(\text{H,P}) = 12.15$ Hz, 6H; *trans*-CH₃); HR-MS: calcd for C₃₀H₂₄O₁₀P₂W₂ (%): 973.98645; found: 973.98637; IR (KBr): $\bar{\nu} = 2073, 1950$ cm⁻¹ (CO).

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- [13] Crystal structure determination of C₃₀H₂₄O₁₀P₂W₂, $M_r = 974.11$, colorless, space group $P\bar{1}$ (no. 2), $a = 8.9501(7)$, $b = 12.4181(6)$, $c = 15.7582(10)$ Å, $\alpha = 88.342(4)$, $\beta = 83.656(6)$, $\gamma = 71.097(5)^\circ$, $V = 1646.8(2)$ Å³, $Z = 2$, $\rho = 1.964$ g cm⁻³, $\mu(\text{MoK}\alpha) = 7.13$ mm⁻¹. A total of 10787 reflections were measured at 150 K on a CAD4T/rotating

anode diffractometer ($\lambda = 0.71073$ Å) and averaged in a unique set of 7569 reflections ($R_w = 0.036$). The structure was solved by Patterson methods (DIRDIF-96) and refined on F^2 (SHELXL97) to a final $R1 = 0.0312$ (6350 reflections with $I > 2\sigma(I)$; $wR2 = 0.072$; $S = 1.045$). The data were corrected for absorption using PLATON/DELABS. Hydrogen atoms were taken into account at calculated positions. 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-138219. 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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Micellization versus Cyclodextrin–Surfactant Complexation**

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Upon addition of cyclodextrin (CD) to a surfactant solution, a considerable change in the physicochemical properties can be observed, that is, the surface tension of the surfactant solution usually increases.^[1] When a concentration of cyclodextrin well in excess of the surfactant concentration is reached, the surface tension approaches that of pure water, indicating that neither surfactant–cyclodextrin complexes nor cyclodextrins themselves are surface active. Other properties such as cloud point, molar conductance, sodium ion activity, ultrasonic properties, spectral behavior, and hydrophobicity are modified by the addition of CD's. It has traditionally been assumed that the cyclodextrin molecules complex the monomers of the surfactant in such a way that the process of micellization will only begin once the surfactant monomers have saturated the cyclodextrin capacity for complexation. The strength of CD–hydrocarbon chain interactions increases for the common water-soluble ionic surfactants as the length of the alkyl chains increases.^[2] The large diameter of β -cyclodextrin allows the hydrocarbon chain to coil inside the cavity.^[3]

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Association constants for interaction between surfactants and CDs have been determined using various techniques such as conductance, conductance stopped-flow, speed of sound, fluorescence, surfactant-selective electrode, and surface tension. However, the data in the literature show an amazingly large scattering in the values of the association constants as well as the association mode. For example, the constant K_1 for 1:1 complex formation between sodium dodecyl sulfate (SDS) and β -CD has been reported to be 300, 356, 1380–7230, 3630, 6600, 8360, 21000, and 25600 M^{-1} .^[4] For the interaction between SDS and α -CD, some researchers determined only the 1:1 complex formation constants (K_1) while others determined 1:1 and 1:2 complex formation constants (K_1 and K_2) or their product ($K_1 K_2$). A similar situation has been observed in the case of cationic surfactants.

Recently, we developed a kinetic model based on the micellar pseudophase formalism to study the effect of β -CD in micellar systems. The model was successfully applied to reactions that are catalyzed by β -CD^[5, 6] and those that are not.^[7] In both cases we observed a significant quantity of uncomplexed β -CD in equilibrium with the micellar system and the absence of interactions between β -CD and the micelles once these have been formed. Here we present the results of a study on the basic hydrolysis of *m*-nitrophenylacetate (*m*-NPA) in micelle- β -CD mixed systems of dodecyltrimethylammonium bromide (LTABr), trimethyltetradecylammonium bromide (TTABr), and trimethyloctadecylammonium chloride (OTACl). Contrary to expectations^[8] the concentration of the uncomplexed β -CD in equilibrium with the micellar system increases with the length of the hydrocarbon chain of the surfactant. The main implications of this result are of crucial importance for using CDs as transfer agents for hydrophobic substances^[1] and for determining the stoichiometry of the surfactant-CD complexes.

The experimental procedure for the preparation of *m*-NPA and for the study of its basic hydrolysis in mixed micelle- β -CD systems has recently been described.^[5] Figure 1 shows the variation of the pseudo-first-order rate constant k_{obs} for the

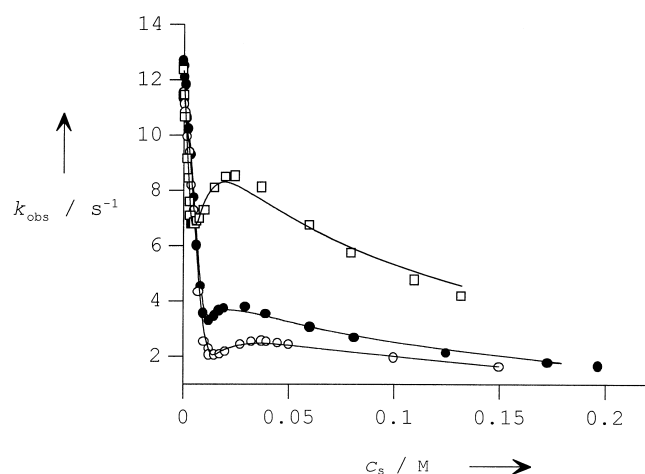


Figure 1. The influence of surfactant concentration c_s on k_{obs} for the alkaline hydrolysis of *m*-NPA in the presence of β -CD ($c_{\text{CD}} = 1.00 \times 10^{-2}\text{ M}$). $\circ$ LTABr- β -CD systems, $\bullet$ TTABr- β -CD systems, $\square$ OTACl- β -CD systems. $c_{\text{OH}^-} = 0.175\text{ M}$, $T = 25^\circ\text{C}$. Curves were obtained by fitting Equation (1) to the experimental results (see Table 1).

basic hydrolysis of *m*-NPA with the surfactant concentration. The experiments were carried out under conditions which allowed the influence of the surfactant concentration on k_{obs} to be studied, while ensuring that the total concentration of β -CD remained constant. The surfactant concentration at the minimum of the curve obtained by plotting k_{obs} versus this concentration is taken as the onset of micellization and equal to the critical micellar concentration (cmc_{app}).^[9] The inhibitory effect of added surfactant below the concentration where micellization starts is attributed to complexation of the surfactant monomers by β -CD and release of *m*-NPA into the bulk aqueous medium. This decreases k_{obs} by reducing the concentration of the *m*-NPA- β -CD complexes. The observed behavior of k_{obs} after micellization is typical of micellar catalyzed reactions.^[10]

A more detailed analysis showed that the surfactant concentration at the point of micellization (cmc_{app})^[9] shifts to lower values when the length of the hydrocarbon chain increases (Figure 1): $\text{cmc}_{\text{app}} = 1.25 \times 10^{-2}$, 1.00×10^{-2} , and $4.50 \times 10^{-3}\text{ M}$ for LTABr, TTABr, and OTACl respectively in the presence of a constant concentration of β -CD (see Table 1). This decrease in cmc_{app} and in particular the fact that these values are lower than $c_{\beta\text{-CD,tot}}$ has normally been attributed to the existence of surfactant-CD complexes with stoichiometry 1:2.

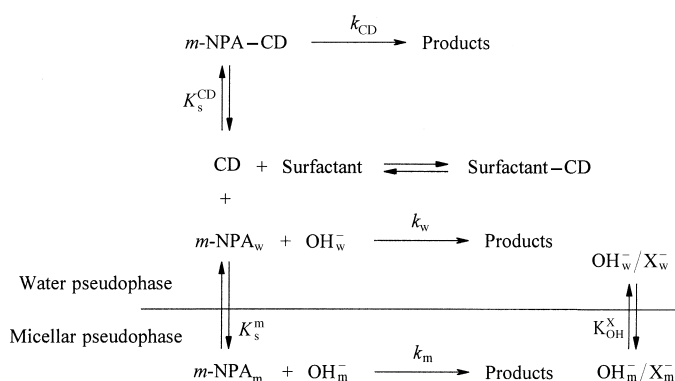
Micellization in mixed surfactant-CD systems has traditionally been believed to occur only after the complexation capacity of the cyclodextrins has been saturated. Therefore, the concentration of surfactant at cmc_{app} will be $\text{cmc}_{\text{app}} = c_{\text{CD,tot}} + c_{\text{surfactant monomers}}$. For surfactants with a long hydrocarbon chain the concentration of surfactant monomers in equilibrium with the micellar system is very small. Thus, when experiments are carried out with a high $c_{\text{CD,tot}}$ it can be observed that $\text{cmc}_{\text{app}} = c_{\text{CD,tot}}$. At the same time, the supposition that the complexation capacity of the CD must be saturated before micellization begins would indicate that $c_{\text{CD,tot}} \approx c_{\text{surfactant-CD}}$. Therefore the quotient $c_{\text{CD,tot}}/\text{cmc}_{\text{app}}$ will provide us with the stoichiometry of the surfactant-CD complex. As we will show, this traditional notion should be rejected. Likewise, the minimum value of the curve obtained by plotting k_{obs} versus c_s shifts to give greater values of k_{obs} as the length of the hydrocarbon chain of the surfactant increases.

To explain the experimental behavior we applied our kinetic model based on the micellar pseudophase formalism (Scheme 1). Three simultaneous pathways were included: reaction of the free substrate with OH^- in aqueous medium, catalyzed reaction of the CD-complexed substrate, and reaction of the substrate with OH^- at the Stern layer of the micelles.

The rate equation (1) for k_{obs} was derived based on Scheme 1, where k_w , k_{CD} , and k_m are the rate constants for the reaction of the substrate in water, in the substrate-CD

$$k_{\text{obs}} = \frac{k_w c_{\text{OH}^-} + (k_m K_s^m - k_w) m_{\text{OH}} c_{\text{D}_n} + k_{\text{CD}} K_s^{\text{CD}} c_{\beta\text{-CD},f}}{1 + K_s^m c_{\text{D}_n} + K_s^{\text{CD}} c_{\beta\text{-CD},f}} \quad (1)$$

complex, and at the Stern layer of the micelles, respectively; K_s^m and K_s^{CD} are the binding constants of the substrate to the



Scheme 1. The micellar pseudophase formalism used in the kinetic model.

micelles and cyclodextrin, and m_{OH} and c_{D_n} are the interfacial concentration of OH^- and the concentration of micellized surfactant. Details on the calculation of c_{D_n} and m_{OH} have recently been described for β -CD–micelle mixed systems.^[5, 6]

For the application of Equation (1) it is necessary to determine the concentration of uncomplexed β -CD ($c_{\beta-CD,f}$) for each surfactant concentration. It is difficult to evaluate $c_{\beta-CD,f}$ due to the great disparity of the equilibrium constants for complexation between different surfactants and β -CD. In previous studies we have obtained $c_{\beta-CD,f}$ by means of simulation, supposing that the complex formed between the surfactant and β -CD presents a stoichiometric ratio of 1:1. The cmc_{app} values presented earlier seem to indicate the existence of complexes with ratios of 1:1 and 1:2, which would suggest that the simulation needs to take two complexation constants into account, the values of which should be closely linked. Hence, we calculated $c_{\beta-CD,f}$ on the basis of k_{obs} values for surfactant concentrations smaller than cmc_{app} . The experimental values of k_{obs} , obtained from a study of the influence of the β -CD concentration on k_{obs} in the basic hydrolysis of m -NPA in the absence of surfactants, lead to a kinetic behavior of saturation [Eq. (2)]. Equation (2) can be rearranged [Eq. (3)] to obtain values for $c_{\beta-CD,f}$ from experiments carried out in the presence of surfactants through the use of $k_w = (9.0 \pm 0.1) M^{-1} s^{-1}$, $k_{CD} = (31 \pm 2) s^{-1}$, and $K_s^{CD} = (54 \pm 5) M^{-1}$ determined previously.^[5]

$$k_{obs} = \frac{k_w c_{OH^-} + k_{CD} K_s^{CD} c_{\beta-CD,f}}{1 + K_s^{CD} c_{\beta-CD,f}} \quad (2)$$

$$c_{\beta-CD,f} = \frac{k_w c_{OH^-} - k_{obs}}{k_{obs} K_s^{CD} - k_{CD} K_s^{CD}} \quad (3)$$

As opposed to other methods used in the past, this method of calculating $c_{\beta-CD,f}$ has the advantage of not presupposing any stoichiometry for the surfactant–CD complex. The kinetic model which has been developed considers that $c_{\beta-CD,f}$ remains constant once micelles have been formed and that there is no interaction of any kind between the β -CD and the micellar system once the latter has formed.^[5, 6] The curves described in Figure 1 correspond with the fit of Equation (1) to the

experimental k_{obs} values. Table 1 shows the values obtained for k_m and K_s^m in simple micellar systems and in mixed micelle– β -CD systems. Likewise the values of the $c_{\beta-CD,f}$ obtained at the point of micellization are shown.

The concentration $c_{\beta-CD,f}$ at the point of micellization increases with the length of the hydrocarbon chain of the surfactant. This increase in $c_{\beta-CD,f}$ is the reason why the values of k_{obs} at the minimum of the k_{obs} versus c_s curves increase with the length of the hydrocarbon chain. It would be reasonable to suppose that the concentration of uncomplexed β -CD in equilibrium with the micellar system would decrease when the length of the hydrocarbon chain of the surfactant increases, due to the enhanced hydrophobicity. However, it is

 Table 1. Results obtained by fitting Equation (1) to the experimental data for the alkaline hydrolysis of m -NPA in micelle– β -CD mixed systems.^[a]

Surfactant	$c_{\beta-CD,tot}$ [M]	cmc_{app} [M]	$c_{\beta-CD,f}$ [M]	cmc_{real} [M]	K_s^m [M ⁻¹]	k_m [s ⁻¹]
LTABr	–	–	–	1.00×10^{-2}	25 ± 3	12.9 ± 0.4
	1.00×10^{-2}	1.50×10^{-2}	2.34×10^{-4}	5.23×10^{-3}	29 ± 3	13.6 ± 0.2
TTABr	–	–	–	1.20×10^{-3}	63 ± 4	10.0 ± 0.2
	1.03×10^{-2}	1.00×10^{-2}	1.06×10^{-3}	7.60×10^{-4}	44 ± 4	18.6 ± 0.3
OTACl	–	–	–	5.00×10^{-4}	130 ± 10	16.1 ± 0.5
	1.00×10^{-2}	5.50×10^{-3}	3.35×10^{-3}	–	73 ± 9	25.3 ± 0.5

[a] See text for details. For each entry the first line of data is for the simple micellar systems, while the second line is for the mixed micelle– β -CD systems.

necessary to consider the mixed micelle–CD as a system in which the surfactant monomers display a competition between autoassociation, micellization, and complexation with the CD. This therefore contradicts the traditional notion which suggests that the complexation capacity of the CD must be saturated before the micellization process can begin. An increase in the hydrophobicity of the surfactant gives rise to an increase in its affinity for complexation with the CD, but at the same time implies an increase in its tendency to micellize. The critical micellar concentration of a surfactant can be considered as a measure of its tendency towards autoassociation. Usually the more surface active the amphiphilic monomer,^[11] the higher the tendency for micellization and, hence, the lower the critical micellar concentration. Accordingly, the longer the total carbon chain length of the monomeric surfactant, the lower the critical micellar concentration.

Studies of the literature that deal with the variation of the surfactant–CD complexation constants indicate that this variation increases with the length of the hydrocarbon chain of the surfactant. Beyond a certain length, which depends on the type of surfactant used, deviations are produced from this linear relationship, indicating that the association constants of surfactant–CD complexes reach a limiting value.^[12] The balance between micellization and CD complexation causes $c_{\beta-CD,f}$ to increase with the length of the hydrocarbon chain in the sequence LTABr < TTABr < OTACl.

The implications of this concentration $c_{\beta-CD,f}$ in the investigation of the mixed micelle– β -CD systems are very important, particularly for studying the stoichiometry of the surfactant– β -CD complexes as the existence of a significant concentration of uncomplexed CD clearly questions the

traditional methodology. In fact, the results shown in Table 1 indicate that the surfactant- β -CD complexes fundamentally display a stoichiometric ratio of 1:1. From data in Table 1 we can derive the concentration of complexed β -CD in the presence of OTACI as $c_{\text{surfactant-}\beta\text{-CD}} = 6.65 \times 10^{-3} \text{ M}$ ($c_{\beta\text{-CD, tot}} = c_{\beta\text{-CD, f}} + c_{\text{surfactant-}\beta\text{-CD}}$). Because cmc_{app} is smaller than $c_{\text{surfactant-}\beta\text{-CD}}$ we can suggest that there are OTACI- β -CD complexes with stoichiometries of both 1:1 and 1:2. Therefore one of the main conclusions to be drawn from this study is the necessity to revise the stoichiometries of the existing surfactant-CD complexes referred to in the library, derived from the values of cmc_{app} .

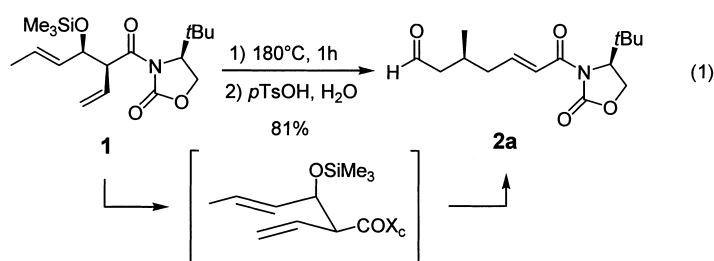
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Domino Michael Aldol and Domino Michael Mannich Reactions: Highly Stereoselective Synthesis of Functionalized Cyclohexanes**

Christoph Schneider* and Oliver Reese

Efficiency and elegance are valued characteristics of domino transformations.^[1] Even more appealing are those domino processes which form carbon-carbon bonds and thereby generate new chiral centers stereoselectively. We report here on domino Michael aldol and domino Michael Mannich reactions which in a single step give rise to highly substituted and functionalized cyclohexanes with very high stereocontrol.^[2] The 7-oxo-2-enimides **2** used as substrates for the domino reactions were easily obtained in good yields and stereoselectivity by a thermal [3.3]-sigmatropic rearrangement of silylated *syn*-aldols **1** [Eq. (1)].^[3] Compounds **2** have been successfully employed by us in syntheses of enantiopure tetrahydropyrans,^[4] piperidines,^[5] terpenols,^[6] and polyol structures.^[7]



We have already been able to show that chemoselective nucleophilic additions to the aldehyde moiety in **2a** are feasible and lead to oxygen and nitrogen heterocycles by intramolecular hetero Michael additions.^[4, 5] We have now found that this sequence can be reversed when organocopper and -aluminum reagents are employed. Thus, the Lewis acid assisted addition of monoorganocuprates (Yamamoto cuprates)^[8] to **2a** gave rise to the cyclohexanol **3a**^[9] in moderate yield but complete stereocontrol (Table 1). The reaction is assumed to proceed by an initial, highly stereoselective Michael addition^[10] of the cuprate reagent to the aluminum chelate complex **A**^[11] and then formation of an imide enolate which is trapped intramolecularly by the aldehyde. The homogenous *syn*-stereochemistry of the aldol reaction results from the intramolecular transposition of the metal ion from the enolate oxygen atom to the aldehyde oxygen atom in the presumed transition structure **B**. As principal side product we

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Table 1. Domino Michael aldol reactions.^[a]

Compound	R ² M	Product (R ²)	Yield [%]
2a	<i>n</i> BuCu/LiI	3a (<i>n</i> Bu)	41
2b	<i>n</i> BuCu/LiI	3b (<i>n</i> Bu)	83
2b	EtCu/MgBr ₂	3c (Et)	71
2b	allylCu/MgBr ₂	3d (allyl)	81
2a	Me ₂ AlSPh ^[b]	3e (SPh)	77
2a	Me ₂ AlSEt ^[b]	3f (SEt)	57
2b	Me ₂ AlSPh ^[b]	3g (SPh)	76
2b	Me ₂ AlNC ₅ H ₁₀ ^[b]	3h (piperidinyl)	40

[a] The stereoselectivity in all cases was >20:1. This value was determined by ¹H NMR (300 MHz) spectroscopy, and means that no other signal of an additional stereoisomer was detected in the ¹H NMR spectrum. X_c = chiral auxiliary. [b] Without additional Lewis acid Me₂AlCl.

isolated the product of the double addition of the organocuprate to the aldehyde and the conjugate double bond in 15–20% yield. The chiral auxiliary was cleaved with lithium benzylate.^[12]

When the reactivity of the carbonyl group was attenuated through conversion to the ketone **2b**,^[13] the yields of the domino Michael aldol reactions increased substantially. A competing nucleophilic addition to the ketone did not occur here and the tertiary cyclohexanols **3b–d** were obtained in good to very good yields and high stereoselectivity (Table 1). The use of Grignard reagents as cuprate precursors broadened the scope of the method considerably.^[14]

Hetero-substituted cyclohexanols **3e–h** were prepared easily by using aluminum thiolates and amides. In particular, the aluminum thiolates Me₂AlSPh and Me₂AlSEt introduced by Nozaki et al.^[15] furnished sulfur-substituted cyclohexanols **3e–g** in good yields as single stereoisomers. Considering the high oxophilicity of aluminum these reagents presumably attack the aldehyde moiety of **2a** reversibly giving rise to aluminum *S,O*-hemiacetals which are known to be good in situ protecting groups for aldehydes.^[16]

In an extension of this work we envisioned the synthesis of amino-substituted cyclohexanols by reaction of **2a** with aluminum amides. Instead of the expected cyclohexanols we obtained diamino cyclohexanes **4a–d** in good yields as single stereoisomers (Table 2). Evidently, aluminum *N,O*-hemiacetals^[16] were initially formed, and these collapsed to the iminium salts **C** which after the Michael addition of a second equivalent Me₂AlNR₂ were trapped by the imide enolate in a Mannich reaction.^[17] A complete reversal of stereoselectivity was observed compared to the aldol reaction. Because the iminium nitrogen atom cannot coordinate to the aluminum ion due to the lack of vacant coordination sites, the intra-

Table 2. Domino Michael Mannich reactions.^[a]

Product	Amine	Yield [%]
4a	morpholine	69
4b ^[b]	piperidine	57
4c	(Bn) ₂ NH	65
4d	Et ₂ NH	46

[a] The stereoselectivity in all cases was >20:1. This value was determined by ¹H NMR (300 MHz) spectroscopy, and means that no other signal of an additional stereoisomer was detected in the ¹H NMR spectrum. X_c = chiral auxiliary. [b] The 5-phenyl-substituted 7-oxo-2-enimide was used as substrate.

molecular transposition of the metal ion as in **B** is prohibited here. Thus, the large iminium group occupies the sterically more favorable proequatorial position in the transition structure **D**, which eventually results in the *anti* configuration of the Mannich products.

We have reported two very efficient and highly stereoselective domino transformations for the synthesis of functionalized cyclohexanes. Just three synthetic operations (aldol reaction, Cope rearrangement, domino reaction) are necessary to prepare the enantiopure carbocycles from simple precursors. This substantiates the synthetic value of the silyloxy-Cope rearrangement of chiral *syn*-aldols for organic synthesis.

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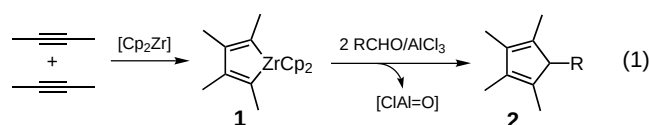
Deoxygenative Cycloaddition of Aldehydes with Alkynes Mediated by AlCl_3 and Zirconium: Formation of Cyclopentadiene Derivatives**

Zhenfeng Xi* and Pixu Li

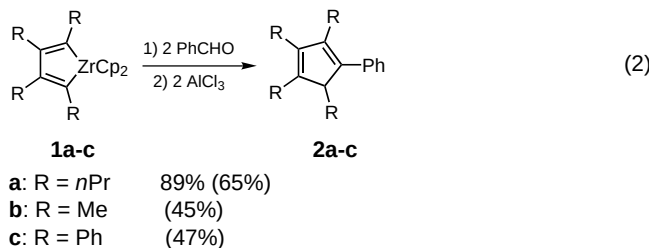
Cleavage or deoxygenation of C–O double bonds in carbonyl compounds is of significant general interest for organic transformations.^[1–4] Metal-promoted cycloaddition of unsaturated organic substrates has attracted much attention,

because such reactions provide a straightforward route to useful cyclic compounds.^[5] Although novel methods for constructing cyclic compounds could be developed by cycloaddition of an aldehyde with alkynes and deoxygenation of the carbonyl group, to the best of our knowledge, such reactions are unprecedented.

Although aldehydes are among the most common unsaturated substrates, transition metal-mediated cycloaddition reactions of aldehydes with alkynes are rare.^[6] Tsuda, Saegusa et al. reported the first cycloadditions of diynes with aldehydes to give six-membered oxacycles such as pyrans with catalysis by Ni^0 .^[6a] The reaction proceeded with a formal [2+2+2] pattern. Here we report the first cyclization of two alkyne molecules with an aldehyde and deoxygenation of the C=O bond to give multiply substituted cyclopentadiene derivatives; the reactions are mediated by AlCl_3 and zirconocene compounds [Eq. (1); $\text{Cp} = \eta^5\text{-C}_5\text{H}_5$].



Two molecules of the same or different alkynes readily underwent cycloaddition on a low-valent zirconocene complex to afford zirconacyclopentadienes **1**.^[7, 8] Addition of two equivalents of benzaldehyde and two equivalents of freshly sublimed AlCl_3 to a solution of **1a**, prepared in situ in toluene, led to a rapid reaction [Eq. (2)]. Gas chromatographic (GC) analysis showed that the reaction was complete within 30 min

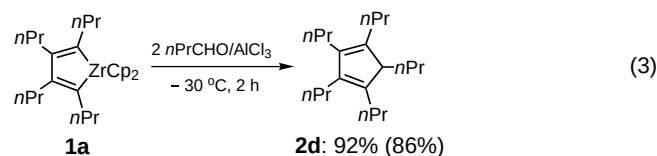


and **2a** was formed in 89% yield (yield of isolated product: 65%). Similarly, **2b** was isolated in 45% yield from the reaction of **1b** with benzaldehyde in the presence of AlCl_3 . Different regioisomers of cyclopentadiene derivatives can be obtained, depending on the reaction conditions and work-up procedures, but under our reaction conditions, only the isomer shown in Equation (2) was obtained. The reaction of **1c** with benzaldehyde proceeded comparatively slowly at room temperature to give **2c** as colorless crystals in 47% yield. The NMR spectroscopic data and m.p. of **2c** are consistent with those reported earlier.^[9] Reactions of metallacyclopentadienes with C_1 units or C_1 unit equivalents to form cyclopentadiene derivatives are rare.^[10] Reaction (2) is the first of this kind in which an aldehyde behaves formally as a C_1 unit. It is noteworthy that, when zirconacyclopentadienes were prepared from $[\text{Cp}_2\text{ZrCl}_2]/\text{EtMgBr}/\text{alkynes}$,^[11] the above reaction was not observed, even at an elevated temperature.

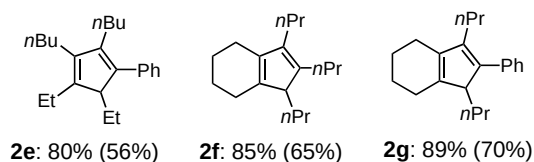
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To investigate the scope of the reaction, aliphatic aldehydes were also used. Reaction of **1a** with butyraldehyde at room temperature in the presence of AlCl_3 resulted in a messy mixture. When the reaction was carried out at about -30°C , **2d** was formed cleanly as a single product within 2 h in 92 % yield (GC) [Eq. (3)].

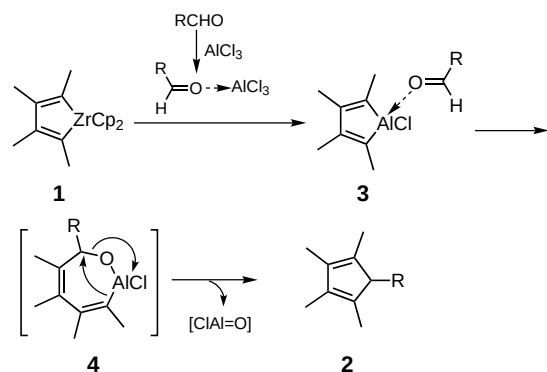


Highly selective cross-coupling between two different alkynes on $\text{Cp}_2\text{Zr}^{\text{II}}$ species can be easily performed to afford unsymmetrically substituted zirconacyclopentadienes.^[7b] The deoxygenative cycloaddition of hex-3-yne, dec-5-yne, and benzaldehyde gave the multiply substituted cyclopentadiene derivative **2e** in 80 % yield as a 1:1 mixture of two positional double-bond isomers. Tetrahydroindene derivatives **2f** and **2g** could be also prepared in good yields by the reaction of the



corresponding bicyclic zirconacyclopentadienes and aliphatic (**2f**) or aromatic aldehydes (**2g**) in the presence of AlCl_3 . Compound **2f** was obtained as a 3:1 mixture of two double-bond positional isomers. The major isomer is given.

A possible reaction mechanism is shown in Scheme 1. Insertion of aldehydes into zirconacyclopentadienes^[12] and titanacyclopentadienes^[13] has been reported. However, aldehydes did not react with zirconacyclopentadienes under these

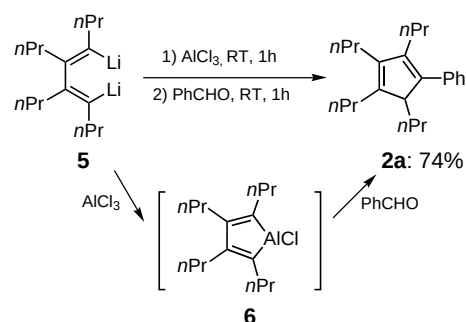


Scheme 1. Proposed mechanism for the AlCl_3 -mediated reaction of zirconacyclopentadienes with aldehydes.

reaction conditions. The ^1H NMR chemical shift of the Cp ligand in **1c** was unchanged after treatment with benzaldehyde for 1 h at room temperature. Because transmetalation of **1** with AlCl_3 was expected, we treated **1c** with AlCl_3 at room temperature for 1 h. However, no change in the chemical shift of the Cp ligand of **1c** was observed. These results indicate

that transmetalation of zirconacyclopentadienes **1** with AlCl_3 in the absence of aldehydes is unlikely. Since interaction of aldehydes with AlCl_3 is well known, formation of an adduct between AlCl_3 and aldehyde is presumably favored.^[14] Indeed, addition of $\text{PhCHO} \cdot \text{AlCl}_3$ to **1a** in toluene afforded **2a** in 85 % yield. Obviously, the adduct reacts with **1** to form aluminacyclopentadienes **3**.^[15] The activated carbonyl group then inserts into an Al–C bond of **3** to afford the oxaluminacycles **4**. We assume that formation of a cyclopentadiene ring and an oxo aluminum species is the driving force for the formation of **2** from **4**.

To further investigate the reaction mechanism, we carried out the reactions shown in Scheme 2. The 1,4-dilithio-1,3-diene compound **5** was prepared in situ by the reaction of the



Scheme 2. Investigations into the reaction mechanism.

corresponding 1,4-diiodo compound with $t\text{BuLi}$.^[16] An equimolar amount of AlCl_3 was then added to the reaction mixture.^[15a,e,f, 17] After the mixture was stirred at room temperature for 1 h, benzaldehyde was added. The reaction was complete after 1 h at room temperature and gave **2a** in 74 % yield (GC). This indicates the formation of aluminacyclopentadiene **6** from the reaction of **5** with AlCl_3 .^[17]

Experimental Section

Typical procedure: oct-4-yne (2.0 mmol, 0.29 mL) was added to a solution of $[\text{Cp}_2\text{ZrBu}_2]$ (Negishi reagent),^[7a] prepared in situ from $[\text{Cp}_2\text{ZrCl}_2]$ (1.0 mmol, 0.29 g) and $n\text{BuLi}$ (2.0 mmol, 1.3 mL, 1.60 M in hexane) in toluene (10 mL) at -78°C . The reaction mixture was stirred at room temperature for 1 h. Then, PhCHO (2.0 mmol, 0.21 mL) and AlCl_3 (2.0 mmol, 0.27 g) were added. The reaction mixture was stirred at room temperature for 1 h. Hydrolysis with 3 N HCl followed by normal work up and evaporation in vacuo gave a bright yellow oil, which was purified by column chromatography (silica gel, hexane) to afford **2a** as a colorless liquid in 65 % yield (202 mg). A yield of 89 % was determined by GC with dodecane as internal standard.

^1H NMR: 300 MHz, ^{13}C NMR: 75 MHz; CDCl_3 , TMS, unless stated otherwise.

2a: ^1H NMR: $\delta = 0.77\text{--}1.02$ (m, 14H), $1.30\text{--}1.62$ (m, 8H), $2.02\text{--}2.44$ (m, 6H), 3.61 (t, $J = 6.4$ Hz, 1H), $7.10\text{--}7.38$ (m, 5H); ^{13}C NMR: $\delta = 14.50$ (3 CH_3), 14.58 (CH_3), 16.18 (CH_2), 23.70 , 23.96 , 24.05 , 27.86 , 28.69 , 29.12 , 30.57 , 52.69 (CH), 125.38 , 128.02 , 128.46 , 137.96 , 140.61 , 141.76 , 142.45 , 144.33 ; HRMS calcd for $\text{C}_{23}\text{H}_{34}$: 310.2661; found: 310.2656.

2b: Colorless liquid, 45 % yield (89 mg); ^1H NMR: $\delta = 1.02$ (d, $J = 7.4$ Hz, 3H), 1.82 (s, 3H), 1.94 (s, 3H), 2.04 (s, 3H), 3.20 (m, 1H), $7.15\text{--}7.50$ (m, 5H); ^{13}C NMR: $\delta = 11.10$, 11.89 , 12.68 , 14.75 , 50.05 (CH), 125.37 , 128.07 , 128.35 , 134.99 , 137.09 , 140.67 , 142.68 ; HRMS calcd for $\text{C}_{15}\text{H}_{18}$: 198.1409; found: 198.1410.

2c:^[9] Colorless crystals, 47 % yield (210 mg); m.p. $255\text{--}257^\circ\text{C}$; ^1H NMR: $\delta = 5.0$ (s, 1H), $6.82\text{--}7.20$ (m, 25H); ^{13}C NMR (C_6D_6): $\delta = 63.15$ (CH),

126.56, 126.73, 126.86, 127.64, 127.85, 128.57, 128.65, 129.26, 130.30, 136.08, 136.43, 144.16, 147.03.

2d: Colorless liquid, 86% yield (237 mg); ^1H NMR: δ = 0.79–0.94 (m, 17H), 1.29–1.59 (m, 10H), 2.03–2.30 (m, 8H), 2.86 (t, J = 6.6 Hz, 1H); ^{13}C NMR: δ = 14.32, 14.41, 14.68, 16.41, 23.87, 24.10, 27.93, 29.01, 30.46, 50.95 (CH), 139.73, 141.43; HRMS calcd for $\text{C}_{20}\text{H}_{36}$: 276.2817; found: 276.2820.

2e: 1:1 mixture of positional double-bond isomers, combined yield 56% (347 mg, 2.0-mmol scale); ^1H NMR of the mixture: δ = 0.62–1.80 (m, 20H), 1.96–2.62 (m, 8H), 3.34–3.52 (m, 1H), 7.02–7.40 (m, 5H); ^{13}C NMR of the mixture: δ = 13.92, 14.05, 15.35, 19.82, 20.70, 22.81, 22.92, 23.07, 25.43, 26.33, 32.76, 33.15, 52.34 (CH), 52.49 (CH), 125.41, 127.76, 128.04, 128.07, 128.25, 128.32, 128.37, 137.76, 140.59, 140.85, 143.04, 145.12; HRMS calcd for $\text{C}_{25}\text{H}_{34}$: 310.2661; found: 310.2663.

2f: 3:1 mixture of positional double-bond isomers, combined yield 65% (320 mg, 2.0-mmol scale); ^{13}C NMR of the major isomer: δ = 14.28, 14.37, 14.42, 15.01, 24.08, 24.14, 24.20, 27.53, 27.90, 28.47, 28.72, 30.43, 52.48, 138.16, 139.03, 139.55, 141.27; HRMS calcd for $\text{C}_{18}\text{H}_{30}$: 246.2348; found: 246.2354.

2g: Colorless liquid, 70% yield (392 mg, 2.0-mmol scale); ^1H NMR: δ = 0.64–0.94 (m, 8H), 1.44–1.62 (m, 8H), 2.27–2.35 (m, 6H), 3.31 (t, J = 5.2 Hz, 1H), 7.14–7.34 (m, 5H); ^{13}C NMR: δ = 14.38, 14.45, 16.98, 23.21 (2 CH_2), 23.25, 23.36, 24.34, 28.48, 30.62, 54.08 (CH), 125.41, 128.04, 128.44, 137.71, 138.91, 141.47, 141.85, 142.32; HRMS calcd for $\text{C}_{21}\text{H}_{28}$: 280.2191; found: 280.2187.

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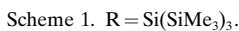
Synthesis and Structure of an Anionic Ga_8R_8 Cluster with a Metalloid Core**

Alexander Rodig and Gerald Linti*

In anionic partial structures of compounds of gallium with more electropositive metals, gallium clusters are found, for example the Ga_8 dodecahedron in RbGa_3 ,^[1] the Ga_{12} icosahedron in RbGa_7 ,^[2] and the Ga_{11} octadecahedron in K_3Ga_{13} .^[3] Up to now, the nonagallane **1**^[4] and the two Ga_{22} clusters **2a**, **b**^[5, 6] have been the only examples of metalloid^[7] gallium cluster compounds (Scheme 1). All the other gallium cluster compounds such as the tetragallane R_4Ga_4 ^[8, 9] and **4**^[4] have a

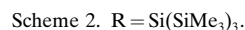
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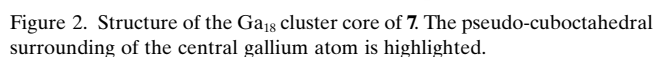
For the synthesis of **1** and **2b** in particular, but also for a number of other gallium cluster compounds such as **3**^[9] and **4**^[4] (Scheme 1), a mixture of gallium subhalides “GaI”,^[10] prepared sonochemically from gallium and iodine in a 1:1 ratio, proved to be a suitable starting material. Herein, we describe the reactions of the latter and similar mixtures of gallium subhalides, respectively, with tris(trimethylsilyl)silyllithium (=hypersilyllithium).^[11]

Furthermore, black cube-shaped crystals with a silvery reflection can be obtained from the mother liquor. The X-ray structure analysis of these crystals reveals^[14] their composi-



typical range. (Ga–Si distances in gallium–hypersilyl cluster compounds: 237.6 (**6**), 240.6 (**3**), 242.6 (**2a**), 244.3 (**1**), 245.3 pm (**4**)). One central gallium atom (Ga(1)) is surrounded by eight neighbors (Ga(2)–Ga(5) and Ga(2a)–Ga(5a)) which form a distorted square prism ($d_{\text{Ga(1)}-\text{Ga}} = 278.5\text{--}286.6$ pm). Four faces of this prism are capped, three of them by one more gallium atom each ($d_{\text{Ga(1)}-\text{Ga(6,6a,7)}} = 307.5\text{--}315.2$ pm), the fourth by a Ga₂ unit ($d_{\text{Ga(8)}-\text{Ga(9)}} = 260.9$ pm, $d_{\text{Ga(1)}-\text{Ga(8, 9)}}(\text{av})$ 345.8 pm). The center of this unit is 320 pm from Ga(1). Thus, a “cuboctahedral” environment results for the central gallium atom, consisting of 11 Ga atoms and the center of a Ga₂ unit (Figure 2). The arrangement of Ga atoms in the cluster anion can be described basically as a section of the body-centered tetragonal packing found in a certain GaIII high-pressure modification.^[15] In contrast to the (8+3+2) coordination of the central Ga atom in **7**, the coordination in the elemental modification is (4+8) ($d_{\text{Ga-Ga}} = 281$ and 299 pm, respectively).

In this unusual 13-membered polyhedron, two edge-sharing pentagonal faces and two vertices sharing rhomboid faces are each capped by a hypersilyl-bearing gallium unit at an average



distance of 272.4 and 259.8 pm respectively. Two opposite faces of the central prism are capped by folded $\text{Ga}_4\{\text{Si}(\text{SiMe}_3)_3\}_2$ units. From an alternative viewpoint, one can recognize another structural element common in the cluster chemistry of metallic elements:^[16] The central gallium atom, four Ga atoms of the first sphere and these outer four, rhomboidally arranged Ga atoms form a tricapped prism.

In the crystal, the Ga_{26} units form a distorted cubic close packing (packing sequence ABC). The charge of the dianion is balanced by two $[\text{Li}(\text{thf})_4]^+$ cations, which occupy all tetrahedral sites in this packing. Therefore the packing type corresponds to the antiferrotype type.

Density functional theory (RI-DFT) calculations^[17] on $[\text{Ga}_{26}(\text{SiH}_3)_8]^{2-}$ show, in spite of the drastic reduction of the steric demand of the substituents, a good agreement of the structure of the Ga_{26} framework with that of **7**. Thus, the central Ga(1) atom is surrounded by eight closest neighbors at distances between 279.5 and 285.2 pm and three more at distances between 308.5 and 315.5 pm. The twelfth coordination site is occupied by a Ga_2 unit ($d_{\text{Ga-Ga}(4)} = 269.8$ pm). The Ga–Si distances average 243.5 pm. The two negative charges in this model are, according to Roby–Davidson population analyses,^[18] mainly distributed to the central gallium atom and the outer, silyl-bearing Ga atoms.

The combination of hypersilyl groups as a protective ligand-sphere and “GaI” as an easy source for subvalent gallium has afforded a number of novel gallium clusters, which can in part be described according to the Wade rules. Compound **7** in particular, but also **2**, represent new types of clusters in main group chemistry, to which these proven rules cannot be applied. Consequently, the aim of future research has to be not only the synthesis of further cluster species of this type, using ligands with different steric and electronic properties, but the investigation of whether special electric or magnetic properties result from these unusual structures. After all, the dimensions of these clusters, being almost 2 nm in diameter along with an elliptical metallic core which is 1 nm long, imply the formation of nanostructures on a molecular level.

Experimental Section

Analogously to the synthesis of “GaI”,^[10] gallium (1.2 g, 17.2 mmol) and iodine (3.28 g, 25.8 mmol) in toluene (40 mL) were treated with ultrasound until the color of the mixture turned yellowish-green. A solution of $[\text{Li}(\text{thf})_3\{\text{Si}(\text{SiMe}_3)_3\}]$ (11.75 g, 9.98 mmol) in toluene (100 mL) was added to this slurry at -78°C . The mixture was allowed to warm to ambient temperature over a period of 6 h and was stirred for an additional 12 h. Then all volatiles were removed in vacuo and the residue was extracted with pentane (50 mL). The pentane extract contains mainly colorless to yellow compounds ($[\text{Li}(\text{thf})]\text{I}$, $[\text{Li}(\text{thf})_3\{\text{I}_3\text{GaSi}(\text{SiMe}_3)_3\}]$ ($x = 2, 3$)), which crystallize first, and the well-known gallatetrahedrane **3**. Then the dark residue insoluble in pentane was extracted with toluene (100 mL). At -10°C , black crystals of **6** (1.28 g, 20% with respect to Ga) and dark orange needles of **5** (0.76 g, 9% with respect to Ga) precipitated. From the concentrated mother liquor, **2a** (0.09 g, 3% with respect to Ga) and **7** (0.21 g, 7% with respect to Ga) were isolated after some weeks. Slight modifications of the reaction conditions did not result in an increased yield of **2a** and **7**. Once crystallized, **2a** and **7** are insoluble in common solvents, thus preventing their NMR spectroscopic characterization. An EPR spectrum of crystals of **7** did not display any signals,^[19] indicating a diamagnetic compound. Compounds **3**, **5**, and **6** were identified by NMR spectroscopy, **2a** by X-ray structure determination.

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Cover Picture

P. Kraft et al.

The cover picture shows, on the lip of *Oncidium tigrinum* La Llave et Lex., the superposition of β -ionone and a tailor-made bicyclo[6.4.0]dodecene. The structural similarity of the two molecules accounts for their close odors. β -Ionone is present in the scent of the depicted tropical orchid at the unusually high level of 14.5 %. An overview of the olfactory spectrum is shown in the background. More on scents and their molecules can be found in the review of P. Kraft et al. on p. 2980 ff.





Odds and Trends: Recent Developments in the Chemistry of Odorants**

Philip Kraft,* Jerzy A. Bajgrowicz, Caroline Denis, and Georg Fráter

Fragrance chemistry is, together with the closely related area of flavor chemistry, one of the few domains, if not the only one, in which chemists can immediately experience structure–activity relationships. This review presents structure–odor correlations and olfactophore models for the main odor notes of perfumery: “fruity”, “marine”, “green”, “floral”, “spicy”, “woody”, “amber”, and “musky”. New trendsetters and so-called captive odorants of these notes are introduced, and recent activities and highlights in fragrance chemistry are summarized. The design of odorants, their chemical synthesis, and their use in modern

perfumery is discussed. Our selection is guided and illustrated by creative fragrances, and features new odorants which encompassed current trends in perfumery. New odorants for grapefruit and blackcurrant, for galbanum, and leafy top notes are presented. Compounds with fashionable marine, ozonic, and aquatic facets are treated, as well as new odorants for classical lily-of-the-valley, rose, and jasmine accords. Compounds with sweet and spicy tonalities are also discussed, as are the most recent developments for woody notes such as sandalwood and vetiver. We conclude with musky and ambery odorants possessing uncom-

mon or unusual structural features. Some odor trends and effects are illustrated by microencapsulated fragrance samples, and areas where there is need for the development of new synthetic materials and methodologies are pointed out. Thus, chemists are invited to explore fragrance chemistry and participate in the design and synthesis of new odorants. This review gives the latest state of the art of the subject.

Keywords: fragrances • macrocycles • molecular modeling • structure–activity relationships • terpenoids

1. Introduction

Albert Eschenmoser once said,^[1] “We all agree [...] that the emphasis in synthetic research is shifting toward the *synthesis of properties*, and not just compounds”. In this sense, fragrance chemistry is certainly a very interesting area for research; indeed, one of the few where synthetic chemists can immediately explore and experience structure–activity relationships: simply by smelling.

In fact, the *synthesis of properties* is not just en vogue, it was already the goal at the very beginning of modern organic

chemistry—and the isolation and identification of olfactorily active principles of valuable odoriferous natural products was an important chapter. For example, the olfactory properties of coumarin (**1**), previously accessible only by isolation from tonka beans, were key for Perkin to work on its structure elucidation and an industrially feasible synthesis.^[2] In 1876, coumarin (**1**) became available on the market, and inspired Jean-François Houbigant to break with the traditional perfumery of Eaux de Cologne. By combining coumarine with oakmoss, geranium, and bergamot he created an accord that was not an attempt to imitate a natural scent, but a new fantasy smell. The result was “Fougère Royale” (1882), a fragrance that set a trend and finally resulted in a whole family of related perfumes, the so-called Fougère family. Thus, the birth of modern perfumery began with coumarin (**1**). Scheme 1 presents a brief overview of other odorants that played a decisive role in the evolution of perfumery, each one together with an illustrative example of an important use in a fine fragrance.

New perfumery trends were almost always directed by new fragrance synthetics, or as Ernest Beaux, the creator of “Chanel N° 5”, said in 1952: “We’ll have to count on chemists to find new substances, if we are to make new and original notes. Yes, the future of perfumery is in the hand of

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[**] *Note on trademarks:* Words which we know or have reason to believe constitute registered trademarks ® are designated as such. However, neither the presence nor absence of such designation should be regarded as affecting the legal status of any trademark. *Note on perfume analysis:* The quoted percentages of perfume raw materials in market products are rounded figures. They are often derived from area percentages from the GC (FID) analysis, and are thus subject to analytical error.

chemistry".^[3] In this review we intend to show the chemistry behind the recent trends in perfumery, to illustrate the design of new molecules, and to summarize some recent developments in fragrance chemistry. Processes and perfumery compounds with a more general technical and industrial relevance were treated in complementary reviews.^[4]

2. Fruity Odorants

2.1. An Olfactophore Model for Fruity Odorants

Even without any knowledge of perfumery every chemist will remember the fruity odor of aliphatic esters from his early studies in chemistry. In our discussion of odor notes we will therefore also start with fruity odorants, and by following the odor transitions of prominent raw materials of perfumery we will continue with green, leafy notes, marine-smelling molecules, floral, and then spicy odorants. We will conclude with woody, ambery, and finally musky perfumery synthetics that form the base note, the dry-down of a perfume.^[4] Some of the

macrocyclic musks also possess fruity aspects so that the olfactory spectrum presented becomes a full circle at the end of our overview (Figure 1).

Fruity notes are very popular in perfumery. They are now present in almost every feminine fragrance, and are used more and more in masculine perfumes as well. However, not many new fruity odorants were introduced in recent years; instead, already known materials such as lactone **6** have become increasingly used.

Esters are certainly prototypes for fruity odorants. But even if they are very simple in structure, their odor is difficult to predict. Boelens et al.^[5] stated the following structure–odor correlations for aliphatic esters:

- 1) Aliphatic esters up to C₈ are strongly fruity.
- 2) For the acyl moiety there is a decrease in fruitiness along the series C₃–C₅ > C₁, C₂ > C₆–C₈.
- 3) For the alkoxy group there is a decrease in fruitiness with C₁–C₅ > C₆–C₁₀.

But apples are not pears, and their odors are quite distinct and cannot be grouped together for structure–odor correlations.^[6]



P. Kraft



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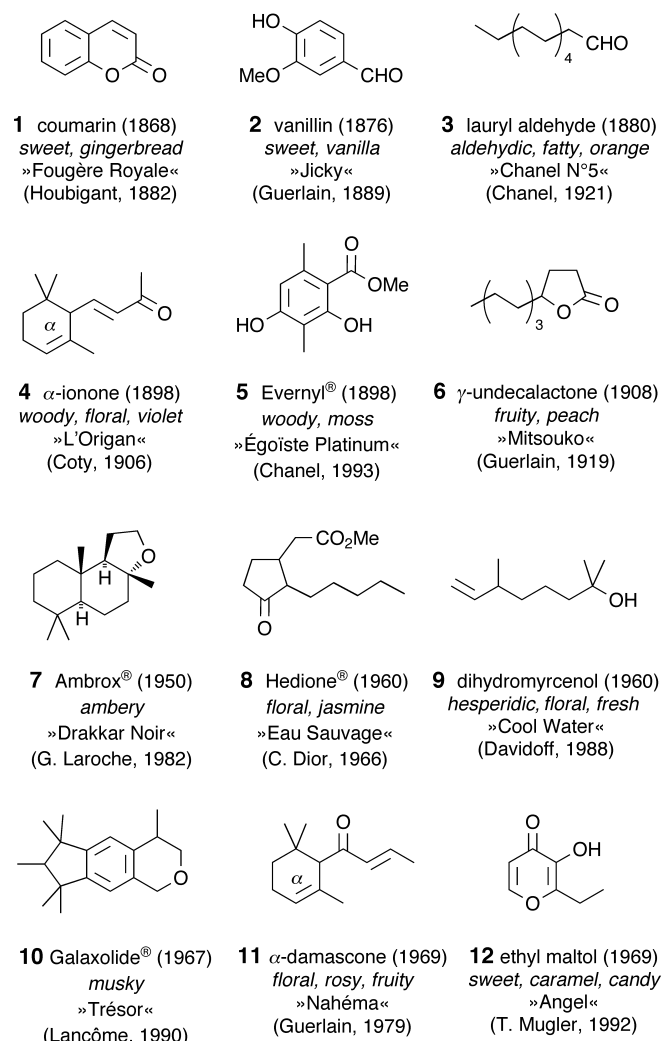
G. Fráter

Philip Kraft, born in 1969, received his diploma and doctorate degree from the University of Kiel, by carrying out syntheses of medium and large ring compounds under the supervision of Professor W. Tochtermann. From 1990–1994, he was a scholarship holder of the Studienstiftung des Deutschen Volkes, and from 1994–1996 recipient of a Chemiefond Stipendium des Verbandes der Chemischen Industrie. In 1996, he joined Givaudan Roure Research Ltd. and is currently laboratory head in Organic Synthesis.

Jerzy A. Bajgrowicz, born in 1950, graduated from the Technical University of Cracow, and received his doctorate from the Technical University of Wrocław in 1977. Following several years of research on azaaromatics and amino acids at his alma mater, the Ecole Normale Supérieure of Rabat, and the University of Montpellier he joined Laboratoires Fournier in 1985. After four years in the pharmaceutical industry he moved to Givaudan Roure, where he is currently leading the Fragrance Ingredients group.

Caroline Denis, born in 1973, studied chemistry at the University of Nice. In 1996, she joined the Givaudan Roure Perfumery School in Grasse, continued her perfumery training in Applied Research in Argenteuil, and became junior perfumer at the start of 1999. Currently, she is working as perfumer in Dübendorf on perfumery briefs as well as in the evaluation of new research compounds and on the composition of demo formulas with new odorants.

Georg Fráter, born in 1941, obtained his doctorate working with Professor H. Schmid at the University of Zürich, and then spent postdoctoral years in Leiden and Edmonton. After more than ten years of agrochemical research with Hoffmann-La Roche, he joined Givaudan Roure, and is currently head of its Research Center in Dübendorf. He is also a senior lecturer at the University of Zürich.



Scheme 1. Trendsetting odorants of the past.

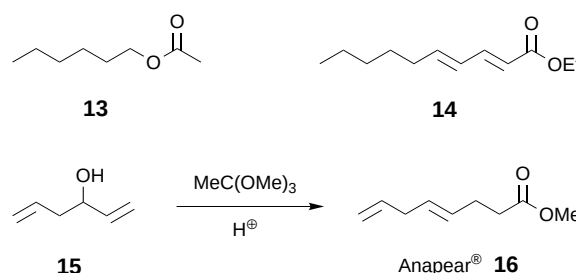


Figure 1. The olfactory spectrum.

After peach, coconut, and apple, it is now pear, grapefruit, and blackcurrant that are in fashion. The pear trend started in personal care products such as “Elsève alpha jojoba” shampoo (L'Oréal, 1995), which contained 3.5 % of hexyl acetate (**13**), and “P'tit DOP Douche + Bain Super Relaxing” (Lascad/L'Oréal, 1997), which contained about 25 % of **13**.

This trend then spread to fine fragrances such as “Tempore Donna” (Laura Biagiotti, 1999), where **13** was used in a pear-chocolate accord that was blended with vanilla. We also find **13** in “D&G feminine” (Dolce & Gabanna, 1999) with its sensual pear-musk interplay. A blackcurrant note joins the pear-musk theme in “2020 Courrèges” (Courrèges, 1997). Here the pear note is mainly a consequence of about 5 % of ethyl (2*E*,4*E*)-deca-2,4-dienoate (**14**), which like **13** is a natural constituent of pears.^[7]

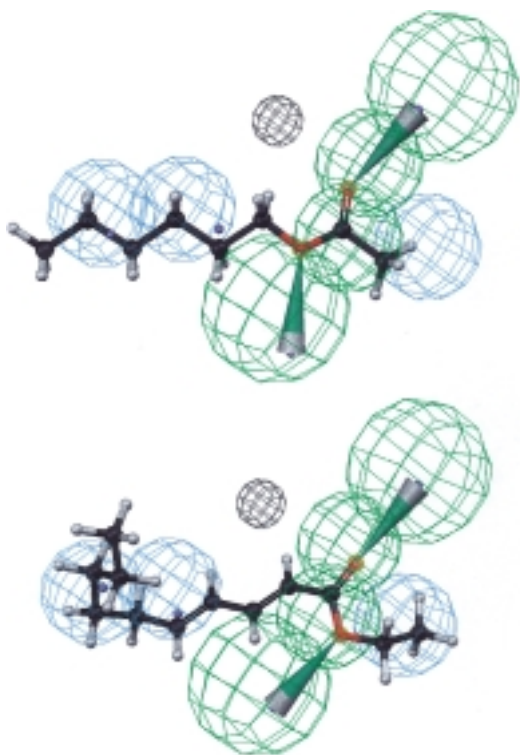
Anapear (**16**) is a new powerful odorant with a fruity-green pear note. It is synthesized by an orthoester–Claisen rearrangement of hexa-1,5-dien-3-ol (**15**), as outlined in Scheme 2.^[8] It has just started to be used, for example, in “Emporio Armani Elle” (G. Armani, 1998).



Scheme 2. Pear odorants.

A data set of quantitatively assessed odorants for pear esters was published by Sell,^[6] and this has been used in the generation of several models that relate the molecular structure to the pear odor of the compounds.^[6, 9, 10] A distance of approximately five carbon atoms, corresponding to approximately 6.3 Å, between a hydrophobic part and a hydrogen-bond-accepting oxygen atom as well as the absence of steric bulk in the vicinity of the alkoxy oxygen atom were found to be important requirements for a pear-type odor. By using the data set of Sell^[6] and including the commercially important pear odorants **13**, **14**, and **16**, we generated the CATALYST^[11] olfactophore model shown in Scheme 3.

An olfactophore model (a special case of a pharmacophore model) is a representation of generalized molecular features (hydrophobic groups, hydrogen-bond donors and acceptors) that are key for a given odor. It corresponds to the binding properties and geometries of the receptor. In the pear model of Scheme 3 both oxygen atoms of each ester moiety fit with the hydrogen-bond acceptors (depicted in green) that orient the molecule within the binding site of the receptor. Two hydrophobic pockets (depicted in cyan) are located 7.2/7.9 Å and 5.3/5.9 Å away from the hydrogen-bond acceptors on one side, and a third one is situated on the opposite side at a distance of 2.5/3.1 Å. An excluded volume (shown in black), inaccessible to the odorant molecule, was placed 2.9/4.0 Å away to account for steric demands of the receptor. The model covers a conformational energy barrier of 3 kcal mol^{−1}. Within this energy barrier the conformer that best fits the model is selected, and the estimated activity is calculated on the basis of its mapping with the features. Scheme 3 illustrates the mapping of **13** and **14**, respectively, to this pear olfactophore.



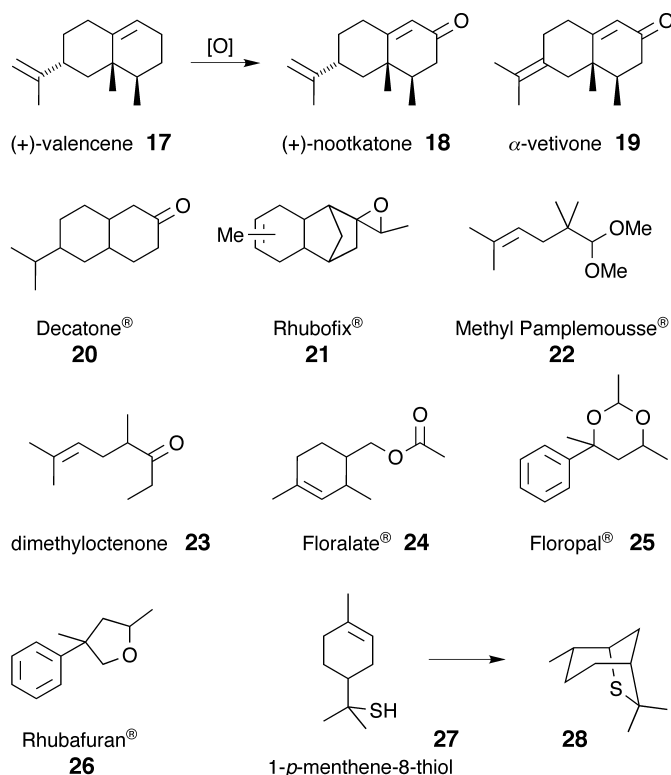
Scheme 3. Olfactophore model for pear odorants. Top: with **13**; bottom: with **14**.

These olfactophore models (hypotheses based on the *molecular similarity* of known odorants) can provide some insight into the molecular parameters responsible for a given odor, and they are helpful for the computer-aided design of new odorants. Despite all the uncertainties of these models, such a pragmatic approach is inevitable, because our limited knowledge on the mechanism of olfaction^[12–14] and the odorant receptors does not yet allow a *de novo* design of odorants based on the *molecular complementarity* to a receptor surface.^[4]

2.2. Grapefruit and Blackcurrant

“Pleasures for Men” (E. Lauder, 1997), “Aqua Allegoria Pamplelune” (Guerlain, 1999), and “L’eau de Kenzo masc” (Kenzo, 1999) with around 3.5 % of Methyl Pamplemousse (**22**; see Scheme 4), are recent examples of the grapefruit trend. Bergamot, lemon, and orange oil (so-called hesperidic notes) have always been used to give freshness and lift to a fragrance. Grapefruit accords offer a new way of doing so without being reminiscent of the traditional Eaux de Cologne.

The prototype odorant of this note, the naturally occurring nootkatone (**18**), can be obtained by oxidation of valencene (**17**; Scheme 4). Despite its good substantivity, **18** remains too expensive for many applications. It differs from α -vetivone ((+)-isonootkatone, **19**), one of the main components of vetiver oil (see Section 7.3), only by the position of the nonconjugated double bond. The olfactory properties of these two octalinones are similar in many respects, and thus grapefruit aspects are also present in vetiver oil. The odor of



Scheme 4. Grapefruit odorants.

nootkatone (**18**) is mimicked by the perfumery synthetics Decatone (**20**) and Rhubofix (**21**), the latter being a complex mixture of regio- and stereoisomers.

Grapefruit odorants have many facets, hesperidic–citrusy, fruity rhubarb, and cassis-type, as well as green and woody tonalities. Many of these facets are present in “Good life” (Davidoff, 1998), and they all revolve around an overdose of 8.5 % of Methyl Pamplemousse (**22**): fruity aspects are reinforced by blackcurrant notes, green by a fig-leaf tonality, and woody by a classical moss accord. Other grapefruit odorants with mainly hesperidic, citrusy, fresh facets are dimethyloctenone (**23**) and Floralate (**24**; Scheme 4). Floropal (**25**) and Rhubafuran (**26**) are two examples of grapefruit odorants with fruity rhubarb undertones.

Grapefruit accords are also often based on sulfur-containing compounds in chemically and olfactorily complex mixtures such as thiolimonene, thiocineole, or thiogeraniol. These are obtained by treating the corresponding terpenes with sulfur, hydrogen sulfide, or thiourea. Their main odor vectors are the enantiomers of 1-*p*-menthene-8-thiol (**27**),^[13] which are the potent character-donating constituents of grapefruit juice^[14] and easily cyclize to 2,8-*epi*-thio-*p*-menthane (**28**). 8-Mercapto-*p*-menthone (**29**; Scheme 5), identified in buchu leaf oil,^[15, 16] is also an olfactorily important component of the thioterpenes mixtures. It possesses a blackcurrantlike odor.

Other blackcurrant odorants include 4-methoxy-2-methylbutan-2-thiol (**30**), a major constituent of the cassis bud aroma,^[17, 18] Corps Cassis (**31**), and Oxane (**32**, Scheme 5), the 4*S*-*cis* and 4*S*-*trans* stereoisomers of which were found in yellow passion fruits.^[19–21] Labienoxime (**33**)^[22] and the bicyclic Buccoxime (**34**) belong to another important family

“Khas Khas”

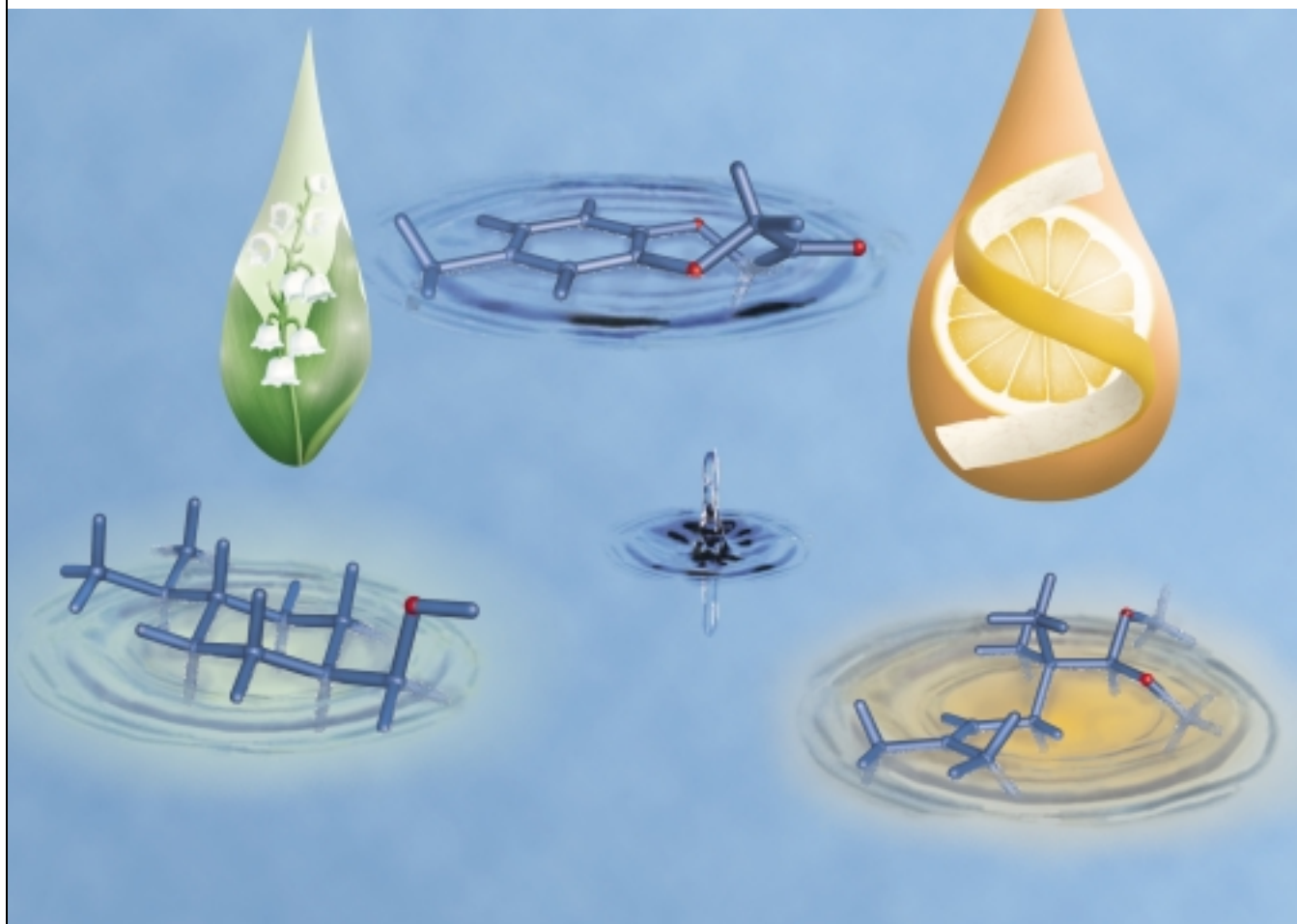
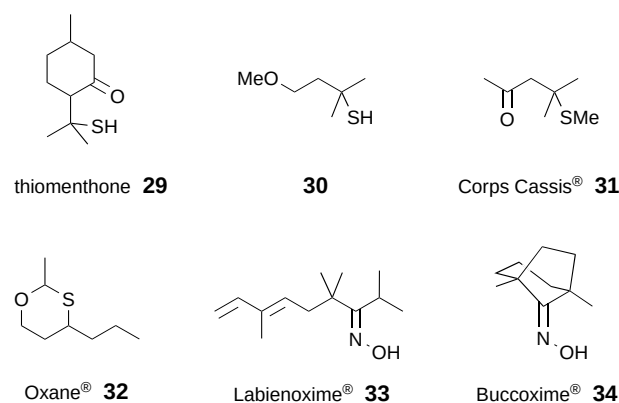


Figure 2. “**K**has Khas”—a modern feminine fragrance featuring Methyl Pamplemousse (**22**), Calone 1951 (**84**), Melonal (**90**), Tropional (**85**), Mayol (**96**), Florol (**97**), and vetiver oil.

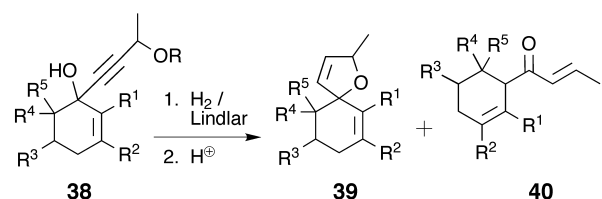
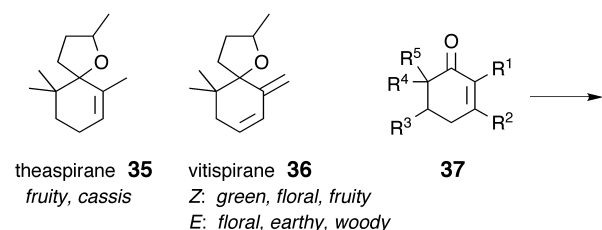
••• *Microencapsulated fragrance: Rub and smell!*



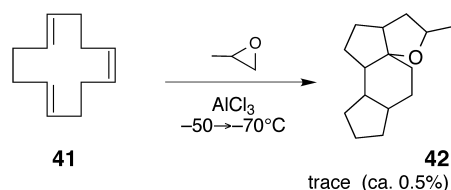
Scheme 5. Sulfur- and nitrogen-containing blackcurrant-type odorants.

of chemicals with blackcurrant smell, the ketoximes, which usually have a more green and metallic odor.

The family of the blackcurrant-smelling spirocyclic ethers **39** (Scheme 6) is synthetically demanding. They resemble natural vitispiranes (**36**, found in grape juice, wine, spirits,



		R ¹	R ²	R ³	R ⁴	R ⁵
39a	Etaspirorene®	Et	H	H	Me	Me
39b	Isospirene®	Me	H	Me	Me	H
39c	Neocaspirene®	H	Me	H	H	<i>i</i> Pr



Scheme 6. Spiroethers with a fruity, blackcurrant scent.

vanilla, blackberry, etc.), and theaspiranes (**35**, found in raspberries, tea, rose, quince, etc.), but seem to originate from the damascone (**11**)/damascenone chemistry, as presented in Scheme 6.^[23, 24] An interesting structure–odor relationship study with nineteen spirocyclic ethers was published by van der Weerd.^[25] Recently, the polycyclic ether **42**, which also possesses the 1-oxaspiro[4.5]decane substructure, was

patented^[26] as having a very intense and substantive blackcurrant odor.

In general, blackcurrant odorants are extremely powerful, and are therefore usually used in the form of perfumery bases: Oxane (**32**) in the green-fruity-floral “Cassis base 345 B”, and Neocaspirene (**39c**) in the fruity-exotic “Dewfruit”. These bases are easy to work with, even in higher concentration. For instance, about 18% of cassis bases were employed in the floral-fruity feminine fragrance “Le monde est beau” (Kenzo, 1997).

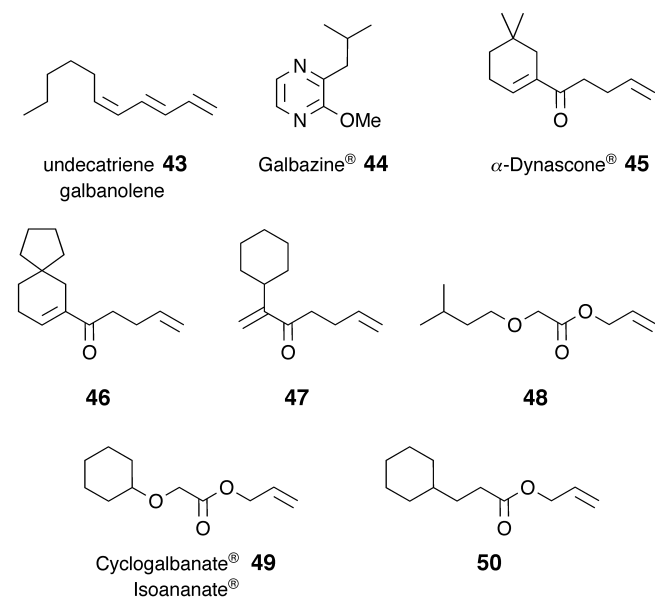
Last but not least, there are also examples for the direct use of blackcurrant odorants. Etaspirorene (**39a**) gives a lot of volume and diffusivity to “Pleasures” (E. Lauder, 1995), and Buccoxime (**34**) links the blackcurrant and grapefruit accords in “Good life”. Furthermore, these odorants opened the way to fantasy notes, such as the tomato-leaf accord of “Les Belles” (N. Ricci, 1996).

In Figure 2 the microencapsulated feminine fragrance “Khas Khas” illustrates how a grapefruit accord, composed of Methyl Pamplemousse (**22**) can exalt the hesperidic aspects of vetiver. This vetiver note is modernized by a marine accord (see Section 4.2), and blended with trendy floral odorants such as Mayol (**96**) and Florol (**97**; see Scheme 17). This perfume demonstrates how modern perfumery synthetics enable the use of a rather traditional ingredient of masculine perfumery, such as vetiver oil, in a floral-woody-aquatic feminine fragrance.

3. Green Notes

3.1. An Olfactophore Model for Galbanum Notes

The characteristic, harsh green odor of galbanum oil (*Ferula galbaniflua* Boiss. et Buhse and *Ferula rubicaulis* Boiss) arises to a great extent from minor constituents, of which (3*E*,5*Z*)-undeca-1,3,5-triene (**43**)^[27] and 2-methoxy-3-isobutylpyrazine (Galbazine, **44**) are the most important (Scheme 7). Both



Scheme 7. Galbanum-pineapple odorants.

“ChocoMinty”

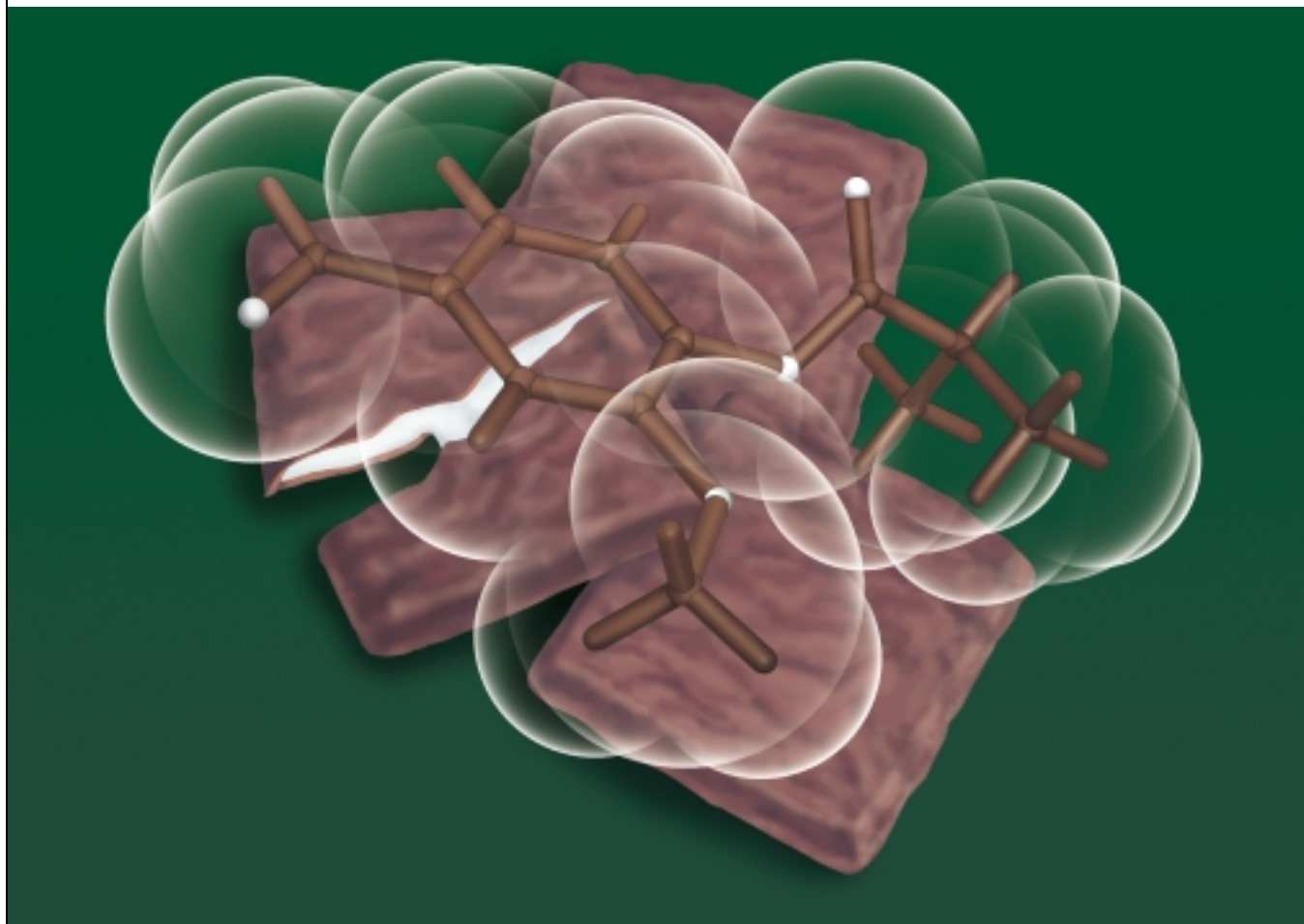
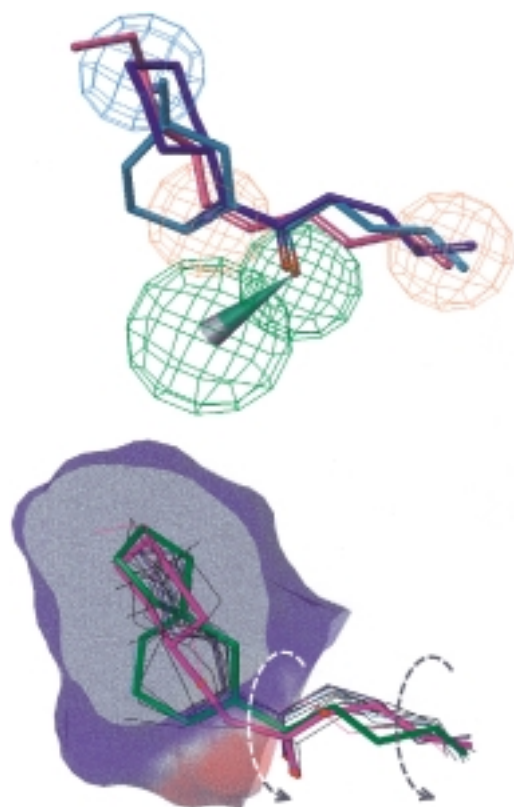


Figure 3. “ChocoMinty”—a fresh gourmande note featuring Isobutavan (**134**).

••• *Microencapsulated fragrance: Rub and smell!*

compounds are available to the perfumer as synthetic products. The galbanumlike odor of another important perfumery raw material, Dynascone (**45**, main odor vector), is accompanied by a fruity, pineapple-type note. Compound **45** was found by serendipity among the impurities of synthetic α -damascone (**11**).^[28, 29]

An analysis of the structural requirements for the green, galbanum odor^[30] led to a family of hypothetical olfactophores composed of one nonaromatic hydrophobic function (blue sphere), one oriented hydrogen-bond acceptor (green spheres), and two customized functions representing an electron-rich fragment that is either an ether or an aliphatic double bond (orange spheres).^[11] The generated olfactophore hypotheses consist of a bulky hydrophobic part with an electron-rich link to the pent-4-en-1-one or allyloxycarbonyl side chain. Different positions of the side chain are possible because of the flexibility of the latter. A proposal for the 3D shape of the hydrophobic pocket, corresponding to the Connolly surface of the aligned active structures that are colored according to their electrostatic potential, is also depicted in Scheme 8.^[31] This feature is of important discriminating power; slight changes, for example, ring enlargement by one carbon atom or shift of a methyl substituent by one position in the ring, can lead to a total loss of the green-galbanum component in the olfactory profile. Even more restrictive is the presence of both hydrogen-bond acceptors. Two recently revealed galbanum odorants of very low odor



Scheme 8. Galbanum olfactophore model. Top: One of the best hypotheses mapped by undecatriene **43** (magenta), α -Dynascone (**45**, cyan), and **47** (blue). Bottom: All the active structures of the training set aligned on this hypothesis. The galbanum-like **46** (green) and much more pineapple-smelling **49** (pink) are highlighted. Hydrogen atoms are omitted for clarity.

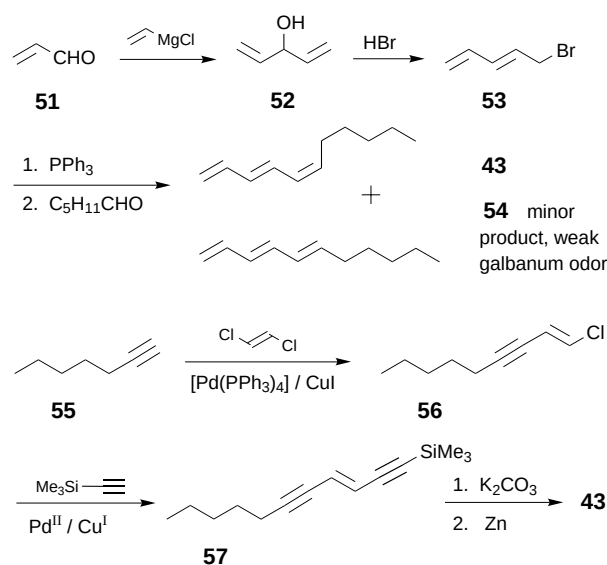
threshold and outstanding substantivity, **46** and **47** (Scheme 7),^[30, 32, 33] map very well this *combinatorial*^[34, 35] olfactophore. Not surprisingly, the even more flexible undecatriene **43** with a green-galbanum odor without fruity aspects also fits to it, except for the hydrogen-bonding function. It can be postulated that the C-3 double bond is playing the role of a weak hydrogen-bond acceptor. Another possibility consists of the generation of an oxygen function at this position (through a metabolic process), prior to the receptor–ligand interactions.

3.2. Galbanum Notes

The complex galbanum odor blends with many different accords. As early as 1945, galbanum was overdosed in “Vent Vert” (Balmain, 1945) in a green-floral accord. In “Chanel 19” (Chanel, 1970) galbanum shows its efficiency with floral and woody notes. More recently, with the development of synthetic galbanum odorants, galbanum is used in fragrances to impart fruity-green, especially pineapple notes.

The main representatives of this odorant family are the 1-substituted pent-4-en-1-ones **45–47** and the allyl glycolates **48** and **49** (Scheme 7), which can be considered as their 2-oxa analogues. The odors of the esters are much more fruity, reminiscent of the pineapple scent. Their weaker galbanum note disappears completely in the absence of the ether function close to the carbonyl group, which mimics the α,β -conjugated double bond of the corresponding ketones. This is the case for allyl cyclohexylpropionate (**50**), which smells almost exclusively pineapple-like.

Since the first isolation of undecatriene **43** from galbanum oil in 1964,^[27] several synthetic methods for the preparation of this impact chemical have been developed. One of the most industrially viable processes, using vinyl chloride, acrolein, and hexanal as building blocks, is depicted in Scheme 9, together with the most recently published one.^[36] The main step of the latter is the Pd/Cu-catalyzed coupling of tri-



Scheme 9. Syntheses of (3*E*,5*Z*)-undeca-1,3,5-triene (**43**).

“W-A-S”

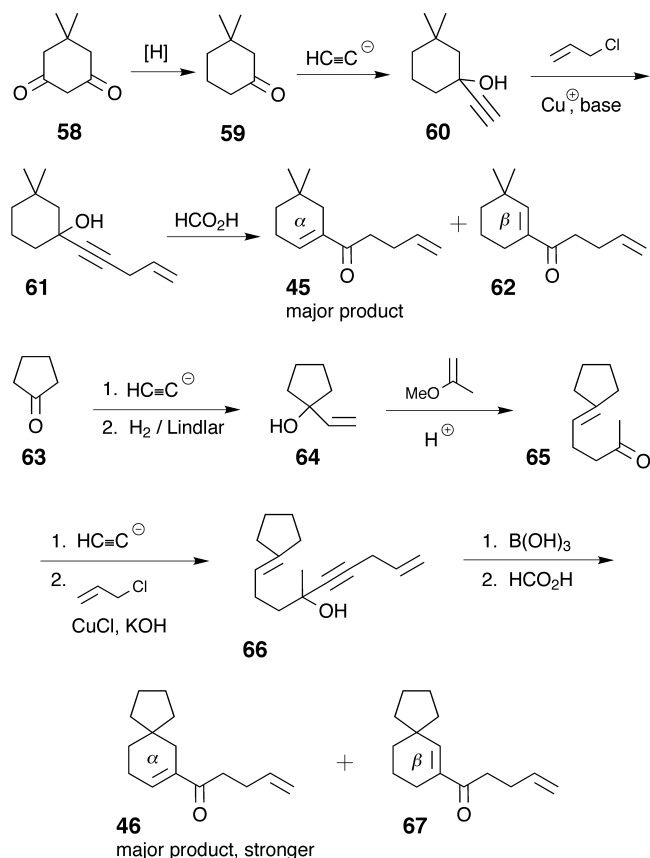


Figure 4. “**W**-**A**-**S**.”—a woody-ambery-spicy masculine perfume featuring Georgywood (**170**), Spirambrene (**200**), Okoumal (**196**), and black and pink pepper oil.

••• *Microencapsulated fragrance: Rub and smell!*

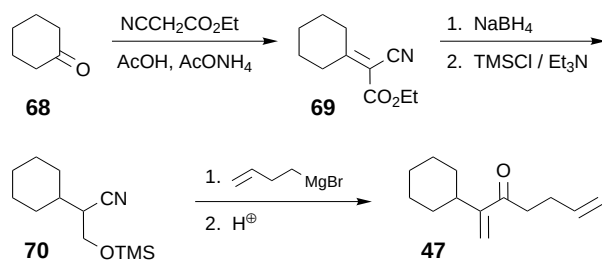
methylsilylacetylene with chloroenynes. Interestingly, **43** (=galbanolene) was also found in algae, where it plays the same pheromone role (sperm attractant) as another structurally similar arachidonic acid metabolite,^[37] dictyopterene A (**77**, see Scheme 13; Section 4.1).

α -Dynascone (**45**), in a mixture with its weaker β isomer (**62**), had long been used as a captive material (that is, a material used only in-house and not sold to others) before it was introduced recently to the market. One of the possible synthetic routes to this valuable material starts with a partial reduction of dimedone (**58**), with the crucial step being the last Rupe rearrangement of the propargylic alcohol **61** (Scheme 10).



Scheme 10. Syntheses of **45** and **46**.

The more substantive galbanum odorant **46** can be obtained starting from cyclopentanone. The synthesis ends by dehydration followed by a tandem ring closure and Rupe rearrangement (Scheme 10).^[32] Significantly simpler and less expensive is the synthesis of **47** (Scheme 11), which comprises



Scheme 11. Preparation of **47**. TMS = Me₃Si.

an elegant one-pot reaction: Grignard addition, imine hydrolysis, hydroxyl deprotection, and dehydration.^[30]

Dynascone (**45/62**), allyl amyl glycolate (**48**), and Cyclogalbanate (**49**) are often used in perfumery, with or without natural galbanum oil. We find galbanum oil, allyl amyl glycolate (**48**), and Cyclogalbanate (**49**), for example, in “Elements Aqua” (H. Boss, 1997). Dynascone (**45/62**) and allyl amyl glycolate (**48**) have also contributed in the development of the modern Fougère family with the important accord of **48**, **45/62**, dihydromyrcenol (**9**), and Ambrox (**7**). “Cool Water” (Davidoff, 1988) and “Egoïste Platinum” (Chanel, 1993) are prominent representatives of modern Fougère perfumes.

3.3. The Green of Leaves

Green notes in perfumery are very diverse. Very often green odor means freshly cut grass, but it can also mean the smell of leaves. In “Premier Figuier” (L’Artisan Parfumeur, 1995), galbanum oil is combined with many other green notes around Glycolierral (**72**) to give a fig-leaf accord. Glycolierral (**72**) has an ivy-leaf odor, and for this note it is used in “J’adore” (C. Dior, 1999). Another fresh green, leafy note with floral undertones is Undecavertol (**74**), which possesses more violet-leaflike facets. This compound requires a careful dosage because of its strength. In “Extravagance d’Amarige” (Givenchy, 1998) it was used in around 0.1% and blended with allyl amyl glycolate **48** and some other violet-leaf odorants, such as methyl non-2-enoate (Neofolione) and methyl non-2-ynoate (methyl octine carbonate). Undecavertol (**74**) was even used in around 0.4% and blended with 0.3% of Cyclogalbanate (**49**) in “Sunwater” (Lancaster, 1997). Birch leaves are trendy too, and “Birch Leaf Givco” was for example used in “Escape for Men” (C. Klein, 1993). It is a base with a very natural smell that contains Undecavertol (**74**) together with a natural, fresh, green, leafy, aldehydic odorant called Vernaldehyde (**76**).

Glycolierral (**72**) and Vernaldehyde (**76**) are prepared starting from the common terpenes phellandrene (from eucalyptus oil) and myrcene, respectively (Scheme 12). The Diels–Alder condensations with acrolein and methacrolein (formed in situ by Mannich condensation of propional and formaldehyde) lead to the corresponding aldehydes that are transformed to the final regioisomer mixtures **72** and **76** by simple ketalization or partial hydrogenation. Undecavertol (**74**)^[38] is also prepared in a simple two-step process, which consists of an aldol condensation of propanal, and the Grignard addition of pentylmagnesium bromide.

4. Marine-Type Molecules

4.1. The Olfactory Impression of a Seashore

Which molecules hit our olfactory receptors when we walk along a wet seashore? At least some of them are linear or alicyclic C₁₁H₁₄ to C₁₁H₁₈ hydrocarbons, which serve as mating pheromones of various species of brown algae.^[39]

“Muskberry”

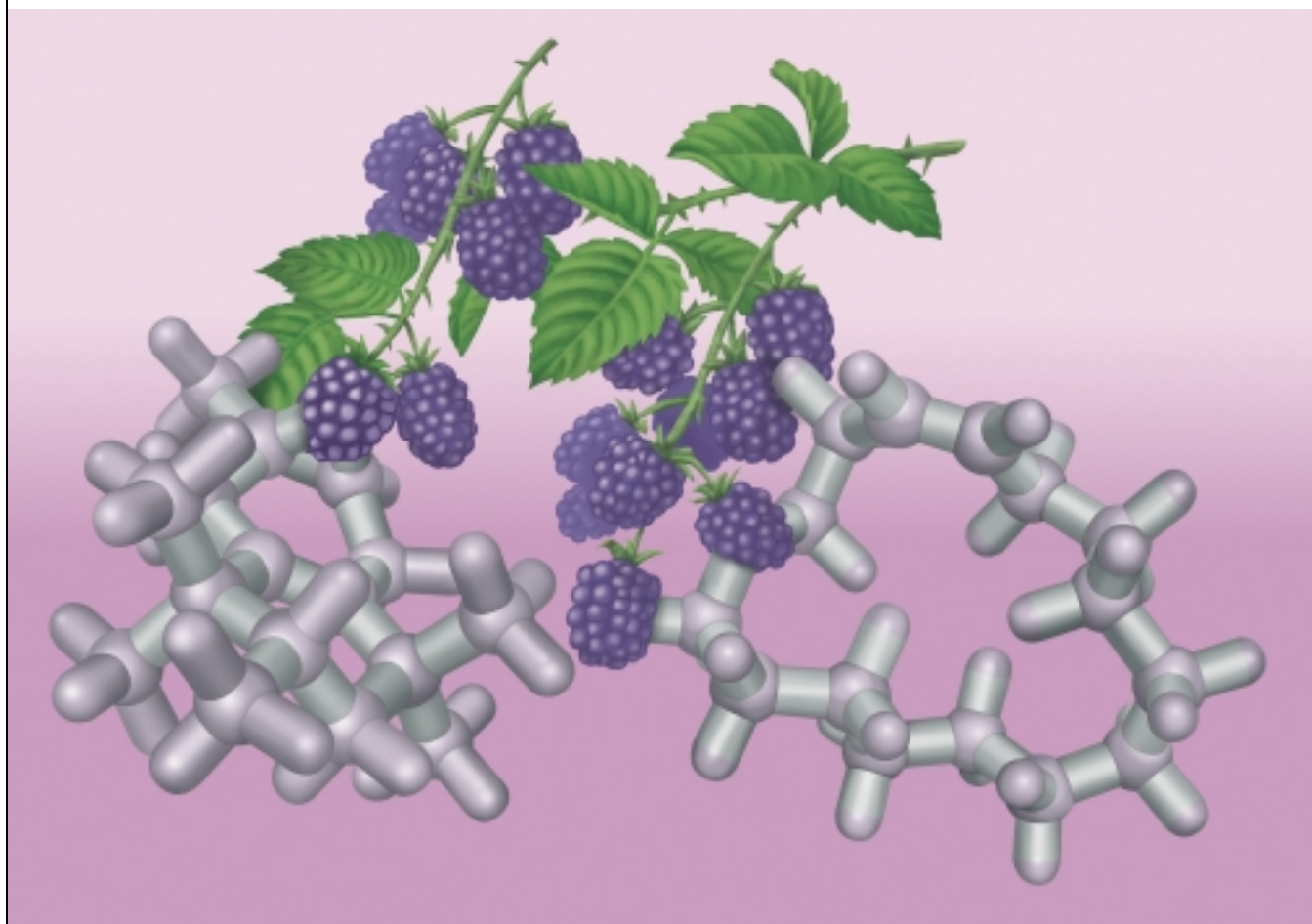
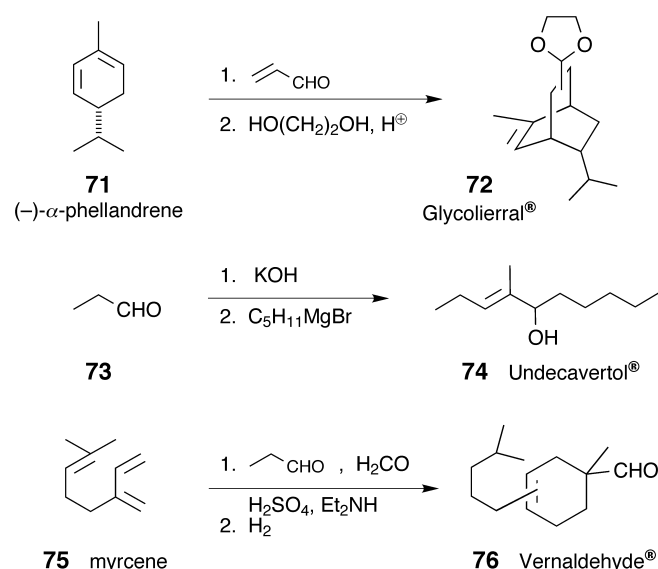


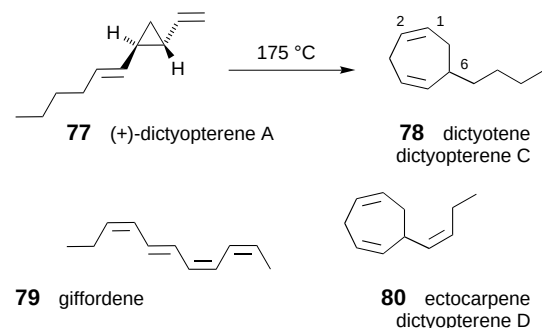
Figure 5. “**M**uskberry”—a fruity-musky accord featuring Habanolide (**254**) and Moxalone (**212**).

••• *Microencapsulated fragrance: Rub and smell!*



Scheme 12. Synthesis of some green-leaf odorants.

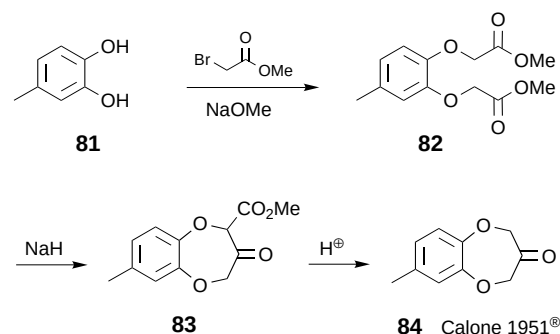
(+)-Dictyopterene A (**77**), which possesses a characteristic odor of seawater, was the first of these odoriferous hydrocarbons to be isolated from algae.^[40] The Cope rearrangement of **77** gives dictyotene (**78**; Scheme 13),^[41] the (–) isomer of which also was found to be present in marine brown algae. The odor of **78** is fishy, somewhat reminiscent of salmon roe. Giffordene (**79**) with its typical sea-algae odor is the odoriferous principle of *Giffordia mitchellae* (Harvey) Hamel,^[42] and the green, tomato-leaflike smelling ectocarpene (**80**) is secreted by the brown algae *Ectocarpus siliculosus*.^[43]



Scheme 13. Some odorants of algae.

When it comes to creating the olfactory impression of a seashore in perfumery, Calone 1951 (**84**) is the material of choice. The benzodioxepanone **84**,^[44] a very unusual structure for a fragrant substance, possesses a typical marine sea-breeze

note with floral nuances, and is manufactured from homopyrocatechol (**81**) as outlined in Scheme 14. A Williamson reaction with methyl bromoacetate is followed by a classical Dieckmann condensation, and hydrolysis and decarboxylation of the resulting intermediate **83** in the last step provides the commercial product **84**.^[44]

Scheme 14. Industrial synthesis of Calone 1951 (**84**).

4.2. Marine, Watermelon, and Ozonic Notes

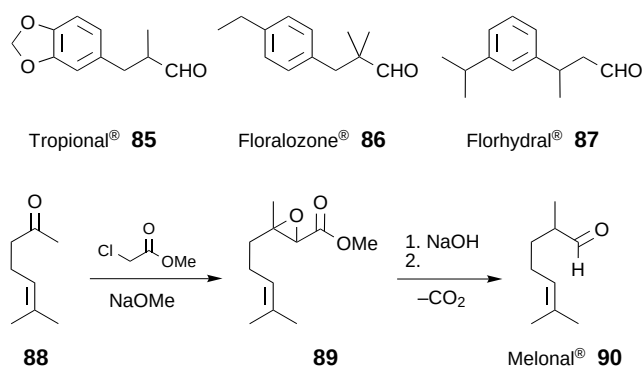
With the launches of “New west for her” (Aramis, 1990) and “Escape for her” (C. Klein, 1991) a marine trend was initiated in feminine perfumery, based on a very simple combination of two raw materials: Calone 1951 (**84**) and Tropional (**85**). In “L’Eau d’Issey” (I. Miyake, 1992), a watermelon note was added to this accord with Melonal (**90**). Finally, ozonic aspects were added to these marine and watery facets by Floralozone (**86**), to form a typical aquatic accord that can be found in fragrances such as “Cool Water fem” (Davidoff, 1996), “L’Eau d’Eden” (Cacharel, 1996), and “Polo Sport Woman” (R. Lauren, 1996). The evolution of the aquatic accord is shown in Table 1.

These accords were also employed in masculine perfumes. “L’Eau d’Issey masc” (I. Miyake, 1994) with about 13 % of Tropional (**85**), 0.4 % of Calone 1951 (**84**), and 0.4 % of Melonal (**90**) is a prominent example. However, the compounds can also be used to give just an ozonic or marine inflection. In “Allure” (Chanel, 1996) we find some 2 % of Tropional (**85**) and around 0.05 % of Floralozone (**86**), and in “Good life woman” (Davidoff, 1999) a trace of Calone 1951 (**84**) and some Florhydral (**87**), which conveys a fresh, marine, and ozonic touch.

The chemical structures of these aldehydes are depicted in Scheme 15. The industrial synthesis of Melonal (**90**) by Darzen’s glycidic ester condensation of 6-methylhept-5-en-2-one (**88**), subsequent hydrolysis, and decarboxylation is also presented.

Table 1. Evolution of the aquatic accord.

	“New West for her” (1990)	“Escape for her” (1991)	“L’Eau d’Issey” (1993)	“L’Eau d’Eden” (1996)	“Cool water fem” (1996)	“Polo Sport W” (1996)
Calone 1951 (84) [%]	1.2	0.8	0.6	0.17	0.4	0.45
Tropional (85) [%]	7.5	3.7	2	1.5	4.3	4.8
Melonal (90) [%]	–	–	0.02	0.03	0.02	0.12
Floralozone (86) [%]	–	–	–	0.1	0.07	0.04
ratio 84 : 85	0.21	0.16	0.3	0.11	0.09	0.09



Scheme 15. Aldehydes with marine odor notes.

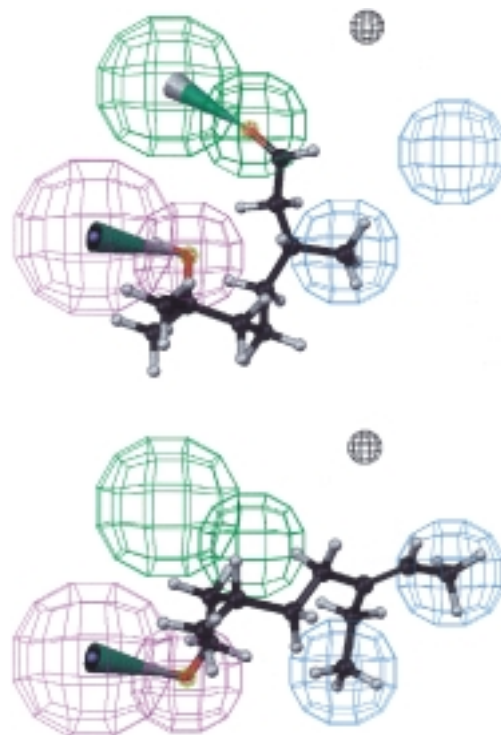
5. Floral Odorants

5.1. A Muguet Olfactophore

The complex scent of lily of the valley flowers (*Convallaria majalis* L.), in perfumery often called muguet, is delicate floral, with a distinct touch of greenness, surrounded by fresh, rosy-lemony nuances. It has something in common with the scent of rose, jasmine, and lilac blossoms; and therefore, cannot be imitated by a single compound. Odorants with muguet character also often possess other different aspects, which makes structure–odor correlations difficult.^[10] However, even though no single odorant duplicates the natural scent, hydroxycitronellal (**91**; scheme 17)^[45] reproduces the odor of lily of the valley quite closely. Therefore, it is generally considered as the prototype of muguet odorants, and can be found in the typical muguet perfume “Diorissimo” (C. Dior, 1956).

Boelens et al.^[46, 47] assumed an aldehyde group, a γ,δ -double bond or oxygen atom, and a maximum length of the molecules of 12–14 Å with a bulky group at the end to be necessary structural features of muguet odorants. However, 2,3-dihydrofarnesol (**92**; Scheme 17), which lacks an aldehyde group as well as a γ,δ -double bond or an ether function is an outstanding muguet odorant, and by headspace-GC analysis was even shown to be a true fragrance component of the living lily of the valley flowers.^[48] On the basis of the observation that bifunctional compounds possess a muguet odor only if they can adopt a conformation in which the distance between the functional groups is less than 3 Å, Ohloff and Giersch^[49] postulated a proton-donor-proton-acceptor unit at the binding site of the muguet receptor. But Pelzer et al.^[48] pointed out that these conformations are relatively strained, and their work with energetically minimized muguet odorants resulted in two different structural fragments for each functional group. The hydroxy fragment is 2,5-disubstituted and consists of two hydrophobic residues 4.7 and 6.0 Å from the carbinol carbon atom, while the carbonyl fragment is 2,4-disubstituted and bears a C-4 double bond and two hydrophobic substituents 3.9 and 5.0 Å from the carbonyl carbon atom.^[10, 48]

By allowing a relatively high conformational freedom of 15 kcal mol^{−1} for the compounds, we could however generate a CATALYST^[11] olfactophore model (Scheme 16), which features a bifunctional unit of a hydrogen-bond donor

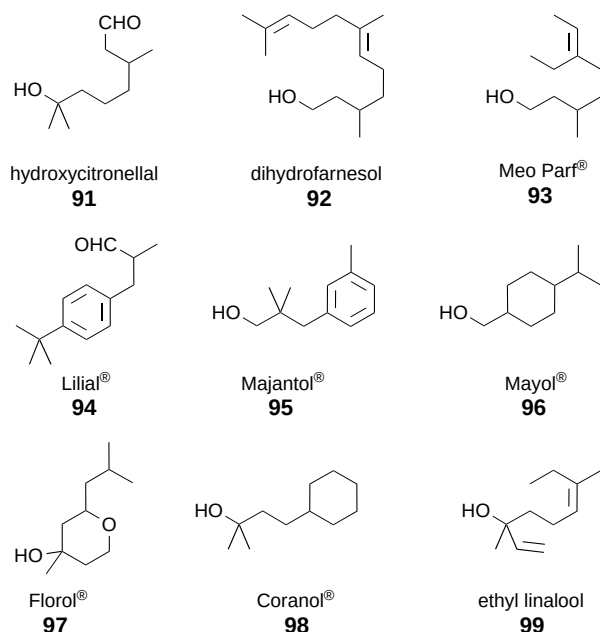
Scheme 16. Muguet olfactophore model. Top: with **91**; bottom: with **93**.

(magenta) and acceptor (green), and maps well with both fragments of Pelzer’s model. The donor, acceptor, and one of the two hydrophobic functions form a quasi equilateral triangle with a side length of 4.5 Å. The second hydrophobic binding pocket is situated 4.2 Å away from the first, 5.7 Å from the hydrogen-bond acceptor, and 8.1 Å from the hydrogen-bond donor. A small excluded volume (in black) accounts for steric constraints. The model incorporates structure–threshold data of a series of muguet alcohols. Scheme 16 presents the fit of (+)-(3*R*)-hydroxycitronellal^[45] (3*R*)-**91** (upper part) and Meo Parf (**93**, lower part), a powerful muguet alcohol with rosy undertones, to this muguet olfactophore model.

5.2. Muguet-Type Alcohols

The traditional muguet odorants, such as the well-known example Lilial (**94**; Scheme 17), were aldehydes, and in general, these muguet aldehydes are more powerful than muguet alcohols. However, aldehydes are more unstable, especially in oxidizing or alkaline media. The growing usage and scope of application of muguet odorants triggered a big demand to find alcohols of this odor note. Besides Meo Parf (**93**), Majantol (**95**), Mayol (**96**), and Florol (**97**), a derivative of rose oxide with a muguet odor and rose-petallike aspects, are representative muguet alcohols. In terms of odor, Mayol (**96**) is the closest to hydroxycitronellal (**91**), followed by Florol (**97**), Majantol (**95**), and Meo Parf (**93**), which already has a strong rosy inflection.

A muguet accord that contains Lilial (**94**) and Majantol (**95**) in the ratio of around 3:2 has been used in “Good Life”



Scheme 17. Floral, muguet-type odorants.

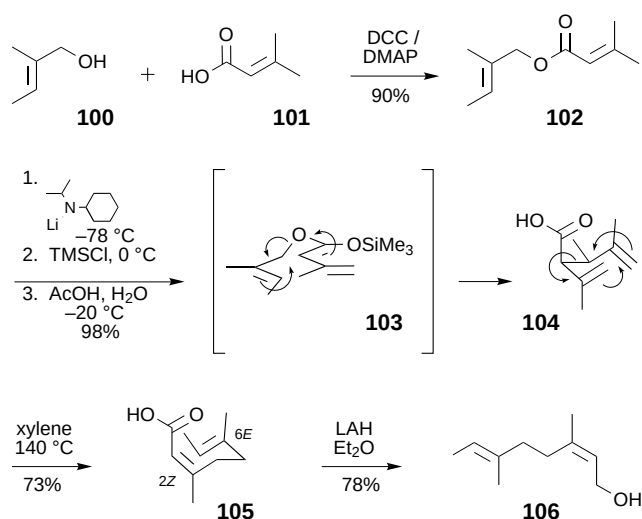
(Davidoff, 1998) and “Good Life woman” (Davidoff, 1999). In “Dolce vita” (C. Dior, 1995) overdoses of around 15 % of Majantol (**95**) and 6 % of Mayol (**96**) have been combined to harmonize the different floral parts, and to convey smoothness to the fragrance. Florol (**97**) was often used in combination with hydroxycitronellal (**91**) and Lilial (**94**) in muguet accords. Some examples are summarized in Table 2.

Table 2. Recent fragrances with a classical muguet accord.

	“XS for her” (1994)	“O oui” (1998)	“Dazzling Gold” (1998)	“J’adore” (1999)	“Fragile” (1999)
Florol (97) [%]	0.5	5.5	7	5.6	2.7
Lilial (94) [%]	3.5	5.5	8.5	7.5	3.2
hydroxycitronellal (91) [%]	2	2.5	5	1.7	3.3

Coranol (**98**), a captive odorant of Firmenich^[50] with an intense floral odor similar to ethyl linalool (**99**) is only slightly reminiscent of lily of the valley. One of the first uses was in “Jean-Paul Gaultier” (J.-P. Gaultier, 1993) at a concentration of 0.3 %. Coranol (**98**) also possesses a very fresh, clean dihydromyrcenol facet, and therefore has been used in high dosage in masculine fragrances, both in modern Fougère perfumes as well as in the oriental family, for example, in “L’Eau d’Issey masc” (I. Miyake, 1994) at 8 %, “Bvlgari” (Bulgari, 1995) at 4.5 %, “Dune masc” (C. Dior, 1997) at 8 %, and “Jungle homme” (Kenzo, 1998) at 3.7 % of **98**. Florol (**97**) and Coranol (**98**) were combined in the feminine “L’Eau d’Eden” (Cacharel, 1996), and more recently in “Romance” (R. Lauren, 1998) as well as in the masculine “L’Eau de Kenzo masc” (Kenzo, 1999) at 0.16, 3, and 1 %, respectively, for Florol (**97**), and 3, 3.5, and 7 % for Coranol (**98**).

An interesting analogue of Meo Parf (**93**) has recently been found in the constitutional nerol isomer **106**.^[51] This was synthesized as outlined in Scheme 18 by Steglich esterification



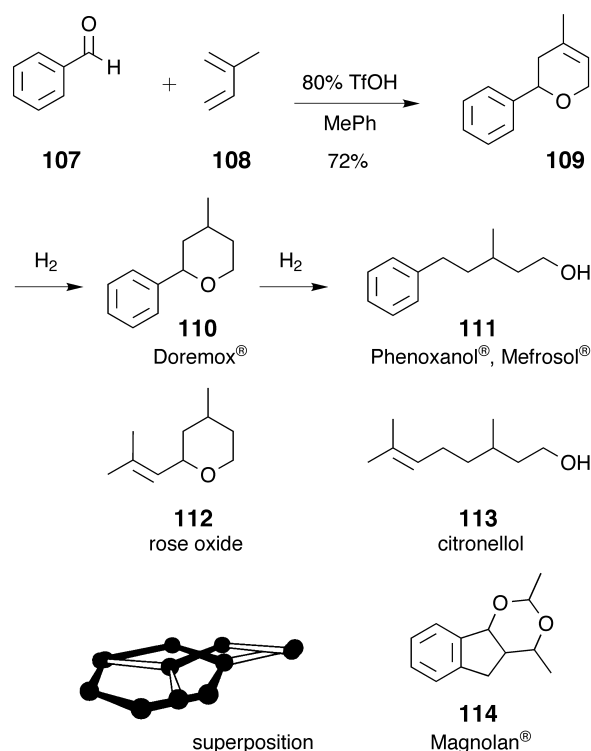
Scheme 18. Synthesis of a constitutional isomer (**106**) of nerol. DCC = dicyclohexylcarbodiimide; DMAP = 4-dimethylaminopyridine; LAH = lithium aluminum hydride.

of tiglic alcohol (**100**) with senecioic acid (**101**), followed by a selective Ireland–Claisen rearrangement of the resulting ester **102**, subsequent Cope rearrangement, and LAH reduction of the formed product **105**. The shift from the typical rosy odor of nerol to the more muguet tonality of **106** by transposition of only one methyl group in the hydrophobic prenyl tail is impressive.^[51]

5.3. Substantive Rose Scents

Substantivity^[52] refers to the persistence of perfume materials or fragrances on blotters, on skin, or in the intended application. One important parameter that determines the substantivity of an odorant is its vapor pressure, which depends on the molecular weight. One way to increase the substantivity is consequently to increase the mass of a given molecule without affecting its shape a great deal. In terms of shape, the isobutenyl group, a very common structural element of terpenes, is similar to a phenyl ring (see the superposition in Scheme 19). Thus, both structural elements can be interchanged without alteration of the main odor characteristics. This isosterism has been demonstrated for many examples,^[53, 54] whereby substituting a phenyl for an isobutenyl moiety has allowed more substantive, that is, long-lasting, odorants to be designed.

The most prominent case for this odorant design is Phenoxanol (**111**), which is constructed by replacing the isobutenyl group of citronellol (**113**) by a phenyl ring (Scheme 19). This results in a decrease of the vapor pressure by factor of 10, from 130000 ngL⁻¹ for **113** to about 12000 ngL⁻¹ for **111**, while the odor threshold of **111** at 0.8 ngL⁻¹ is also ten times lower than that of **113**. Doremox (**110**),^[55] the corresponding analogue of rose oxide (**112**), is another precedent for increasing the substantivity of an odorant by replacing an isobutenyl moiety by a phenyl group. From the olfactory point of view, Phenoxanol (**111**) is richer and smoother than citronellol (**113**), and slightly fruity.

Scheme 19. Substantive rose odorants. Tf = F₃CSO₂.

Doremix (**110**) and rose oxide (**112**) both have floral, rosy, green odors, but Doremix (**110**) is less metallic, and has a pear-type undertone.

As illustrated in Scheme 19, the two perfumery synthetics Phenoxanol (**111**) and Doremix (**110**) are linked by common chemistry, which starts from the hetero-Diels–Alder reaction of benzaldehyde (**107**) with isoprene (**108**).^[56] Recently, a lot of different catalyst systems have been elaborated for this reaction, including cationic palladium(II) complexes^[57] and triflic acid,^[58, 59] as shown for the synthesis of **109** in Scheme 19. The hetero-Diels–Alder adduct **109** is either hydrogenated to provide Doremix (**110**), or hydrogenolytically cleaved to afford Phenoxanol (**111**).

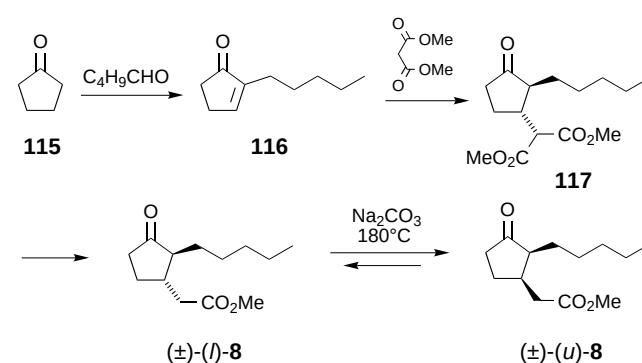
In “Birmane” (Van Cleef&Arpels, 1999), 0.6 % of Phenoxanol (**111**) was mixed with other floral odorants such as Lilial (**94**) and additional rosy materials such as citronellol (**113**) to bring richness and volume, and to give a nice natural effect of rose absolute. In the floral-fruity “Bulgari E.D.P.” (Bulgari, 1994), around 2.5 % of Phenoxanol (**111**) was used. Its effect was reinforced by natural rose absolute.

Roses are very diverse and all of them smell differently. This is also true for rosy odorants. Another substantive floral, rosy odorant is Magnolan (**114**),^[60] the Prins reaction product of indene with acetaldehyde in the presence of formic acid. Compound **114** can convey the freshness of rose flower dew in compositions. Magnolan (**114**) is often used with muguet odorants. In “Comme des garçons 2” (Comme des garçons, 1999) about 9 % was combined with around 2.8 % of Mayol (**96**), and in “Sunwater” (Lancaster, 1997) 1.4 % of **114** was used with 0.5 % of Florol (**97**). In “In Love Again” (Yves Saint Laurent, 1998) 2.7 % of Magnolan (**114**) was employed in combination with jasmine notes.

5.4. Jasmine and Transparent

Methyl dihydrojasmonate (Hedione, **8**) had its debut with around 2 % incorporated in “Eau Sauvage” (Dior, 1966).^[61] It confers a floral-jasmine, transparent, warm note on fragrances with the fresh-floral softness of lemons, and was already used in the high dosage of 8 % in “Diorella” in 1972 (C. Dior). Just four years later, we find it in over 20 % in “First” (Van Cleef&Arpels, 1976). Methyl dihydrojasmonate (**8**) indeed became a real phenomenon in fine perfumery, and today it is hard to find a formula without it. Up to now, the highest level of Hedione (**8**) is present in “Odeur 53” (Comme des garçons, 1998), at about 63 %.

Scheme 20 details the industrial synthesis of **8** by aldol condensation of cyclopentanone with pentanal, subsequent Michael addition of methyl malonate, and decarboxylation.

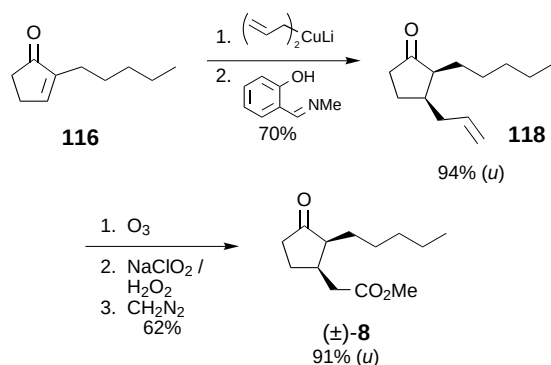
Scheme 20. Industrial Synthesis of Hedione (**8**).

thermodynamic equilibrium of about 90 % (*l*)-**8** and 10 % (*u*)-**8**. However, the *u* isomers of **8** are much more powerful (about 70 times) than the *l* isomers, and the odor of (*u*)-**8** is almost exclusively a result of the presence of the 1*R*,2*S*-(+) enantiomer, while (1*S*,2*R*)-**8** is very weak, and more earthy than floral in smell. Therefore, different processes have been worked out to increase the content of the *u* isomers by fractional distillation from the thermodynamic equilibrium, which is adjusted by heating **8** with bases such as sodium carbonate. Cepionate with around 30 % of (*u*)-**8** was the first quality methyl dihydrojasmonate enriched in the *u* isomers.^[62] Kharismal, Super Cepionate, and Hedione HC with up to 75 % of (*u*)-**8** (Table 3) followed.^[63] These new higher quality methyl dihydrojasmonates (**8**) avoid a dilution effect by the weak *l* isomers; however, (*u*)-**8** isomerizes back into the thermodynamic equilibrium outside the narrow range of pH 5–7. Hedione HC was used for instance in the floral, green, musky bestseller “Pleasures” (E. Lauder, 1995).

Table 3. Qualities of methyl dihydrojasmonate (**8**).

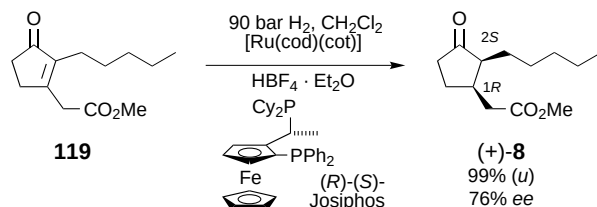
Product	<i>l</i> : <i>u</i>	Odor threshold [ng L ⁻¹]
Hedione	ca. 90:10	0.3
Cepionate	ca. 70:30	0.1
Kharismal	ca. 40:60	0.05
Super Cepionate	ca. 30:70	0.04
Hedione HC	ca. 25:75	0.03

An interesting approach to the synthesis of (*u*)-**8** was developed by S. Ebert and N. Krause.^[64, 65] As outlined in Scheme 21, 2-pentylcyclopent-2-en-1-one (**116**) was alkylated by lithium diallylcuprate. The protonation of the chiral enolate intermediate with *N*-methylsalicylaldehyde proceeded with high diastereoselectivity, and **118** was isolated in 70 % yield with a *u*:*l* ratio of 94:6. Ozonolysis, oxidation, and diazomethane esterification led to a methyl dihydrojasmonate (**8**) containing 91 % of the *u* isomers.



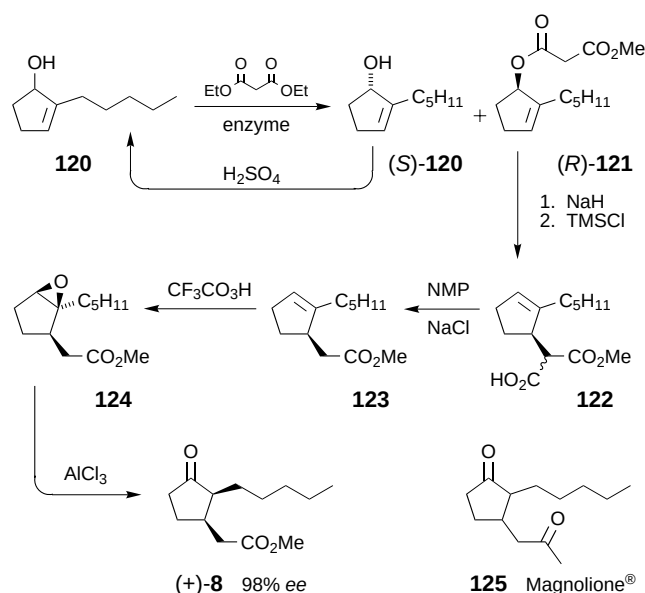
Scheme 21. Synthesis of (*u*)-methyl dihydrojasmonate (($\pm$)-**8**) by diastereoselective protonation.

The next step in improving the odor intensity of methyl dihydrojasmonate (**8**) was the catalytic asymmetric hydrogenation of the cyclopentenone **119** with chiral ruthenium catalysts.^[66, 67] Scheme 22 details the application of a recently claimed catalyst system comprising a Ru(II)-Josiphos complex in the presence of tetrafluoroborate to this reaction. (+)-(1*R*,2*S*)-Methyl dihydrojasmonate ((1*R*,2*S*)-**8**) was obtained in a *u*:*l* ratio of 99:1 with 76 % *ee*.^[67]



Scheme 22. (1*R*,2*S*)-(+)-Methyl dihydrojasmonate ((+)-**8**) by enantioselective hydrogenation. cod = 1,5-cyclooctadiene; cot = cyclooctatetraene; Cy = cyclohexyl.

An alternative industrially feasible route to (1*R*,2*S*)-**8** was elaborated by Fehr.^[68, 69] As outlined in Scheme 23 the key step of this approach is the *syn*-selective epoxidation of the methyl cyclopent-2-enyl acetate **123** by highly electrophilic peracids, such as trifluoroperacetic acid.^[70] The chiral starting material (*R*)-**121** was prepared either by kinetic enzymatic resolution of 2-pentylcyclopent-2-en-1-ol (**120**) or by asymmetric hydrogenation of the corresponding ketone. Chirality transfer by Claisen rearrangement of the malonate-derived allylic silyl ketene acetal led, after decarboxylation, to the central intermediate **123**. The already mentioned diastereoselective epoxidation, and a subsequent aluminum trichloride mediated rearrangement complete this synthesis of (1*R*,2*S*)-**8**.



Scheme 23. An alternative route to (+)-**8**. NMP = *N*-methylpyrrolidone.

Its advantages are the easy availability of **120** and the high enantiomeric purity of the final product.

Related to methyl dihydrojasmonate (**8**) both in structure and in odor is Magnolione (**125**, Scheme 23), which bears an acetyl group instead of the methyloxycarbonyl moiety. This substitution results in an increased odor strength, a better stability, and a more floral, intense jasminic note. In analogy to the synthesis of methyl dihydrojasmonate (**8**) in Scheme 20, it is accessible by reaction of 2-pentylcyclopent-2-en-1-one (**116**) with ethyl acetoacetate and decarboxylation of the resulting adduct.^[71] Magnolione (**125**) was used in 13 % in the chypre “Coriandre” (Couturier, 1973), but even the use of 0.7 % of it in “Eden” (Cacharel, 1994) emphasizes the floral note of that fragrance.

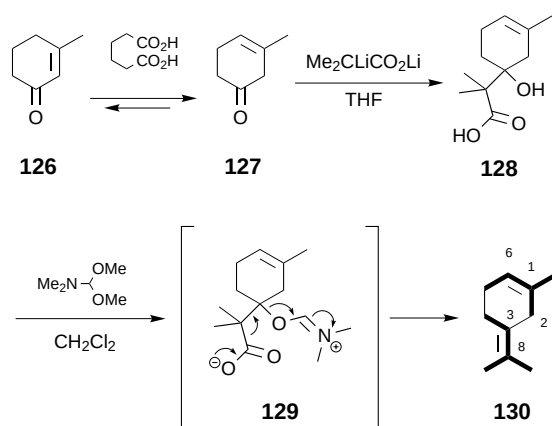
6. Spicy Tonalities

6.1. Pepper

Spices have always been associated with warmth and sensuality, and thus, just as in the kitchen, diverse spices are also used in perfumery. One prominent example is “Parfum Sacré” (Caron, 1990) with a combination of coriander (6 %), nutmeg (2 %), cardamom (0.5 %), clove (0.7 %), cinnamon bark (0.5 %), and black pepper oil (1 %). Recently, in “Les Belles: Délices d’Epices” (N. Ricci, 1999), cardamom, cinnamon, clove, and pepper were also combined.

Pepper is not only the most important spice of international trade, its essential oil has also become increasingly fashionable in perfumery. In the spicy-aromatic-musky male fragrance “Extreme Polo Sport” (R. Lauren, 1998), we even find an overdose of around 6 % that determines the overall pepper note of that fragrance. The use of black pepper oil in “Contradiction” (C. Klein, 1997) is much more subtle; 0.2 % of it provides more of a sparkling and piquant effect than conferring a peppery note.

Black pepper oil has an intense, spicy, and peppery odor, reminiscent of cubebs (the dried unripe berry of an Indonesian pepper shrub) but with a milder taste that lacks the pungency of the spice. Around 90 % of the oil is made up of hydrocarbons, and about 77 % of this hydrocarbon fraction consists of monoterpenes,^[72] of which α - and β -pinene (ca. 10 % and 16 %, respectively), 3-carene (ca. 36 %), and limonene (ca. 24 %) are the main constituents.^[73] It is assumed that trace constituents of polar sesquiterpenoids of yet unknown structure play a decisive role in the characteristic pepper note of this essential oil;^[74] however, as we just saw, the sparkling and piquant effects of black pepper oil, which are mainly due to the monoterpene fraction, are also very important in perfumery. In 1992, Kollmannsberger et al.^[74] reported on two unidentified menthadienes in the monoterpene fraction that could contribute to this interesting effect. The mass spectrum of one of these unknown trace constituents of black pepper oil was rather similar to that of terpinolene, and by a short synthesis it was shown to be the corresponding *meta*-monoterpene *meta*-mentha-3(8),6-diene (isosylveterpinolene, **130**), the first established example of a naturally occurring *meta*-menthadiene.^[75] The synthetic reference was prepared as outlined in Scheme 24 by deconjugation



Scheme 24. Synthesis of isosylveterpinolene (**130**).

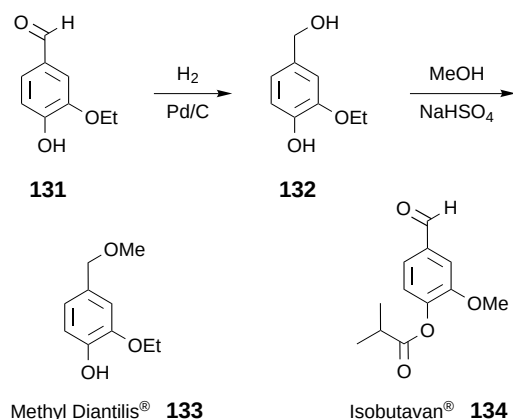
of 3-methylcyclohex-2-en-1-one (**126**), subsequent alkylation with lithium 2-lithio-2-methylpropanoate, and decarboxylative dehydration^[76] of the resulting hydroxycarboxylic acid **128** by employing dimethylformamide dimethyl acetal. *meta*-Mentha-3(8),6-diene (**130**) possesses a pleasant green-herbal, terpenic odor reminiscent of parsley and carrot leaves, and a slightly metallic nuance; it is however, much weaker than limonene, and thus does not contribute considerably to the odor of black pepper oil.^[75]

A different quality of pepper oil, that of pink pepper berries (*Shinus molle*), has been used in “Pleasures” (E. Lauder, 1995). It brings diffusivity, adds a creamy aspect, and blends well with musks.

6.2. Sweet Spice

Some spices and some spicy odorants also possess sweet aspects, close to vanilla. Methyl Diantilis (**133**) with its clove-

carnation-powdery note is one example. It is synthesized as presented in Scheme 25 by hydrogenation of ethyl vanillin (**131**), and methylation of the benzylic hydroxy function formed in intermediate **132**.^[77]



Scheme 25. Two derivatives of vanillin (**2**).

Methyl Diantilis (**133**) resulted from the search for derivatives of vanillin (**2**) that possess the sweet odor characteristics of **2** but do not lead to discoloration problems. Isobutavan (**134**), the isobutyrate of vanillin (**2**), is another product of this research, and is indeed less discoloring than vanillin (**2**). It has a sweet and creamy vanilla odor reminiscent of white chocolate, and at around 2 % plays an important part in the base note of the oriental-ambery-gourmand fragrance “A*Men” (T. Mugler, 1996).

The interplay of spicy and sweet perfumery materials is very important in the oriental-spicy-gourmand perfume “Angel” (T. Mugler, 1992), where nutmeg, cardamom, and pepper oil were blended with a candy accord made mainly of ethyl maltol (**12**), vanillin (**2**), and coumarin (**1**). Many other feminine fragrances followed this gourmand trendsetter, for example “Sonia Rykiel” (S. Rykiel, 1997) and “Just me” (Montana, 1997).

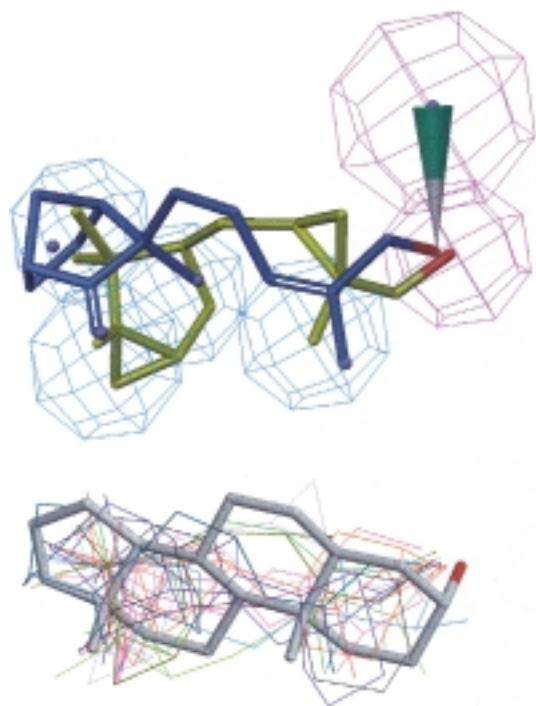
The microencapsulated perfume base “ChocoMinty” in Figure 3 (see p. 2987) is a gustative note, in which a fresh minty accord is married with a sweet chocolate accord, formulated with 4 % of Isobutavan (**134**). You can easily recognize the odor of a delicious candy!

7. Woody Odorants

7.1. Sandalwood Olfactophores

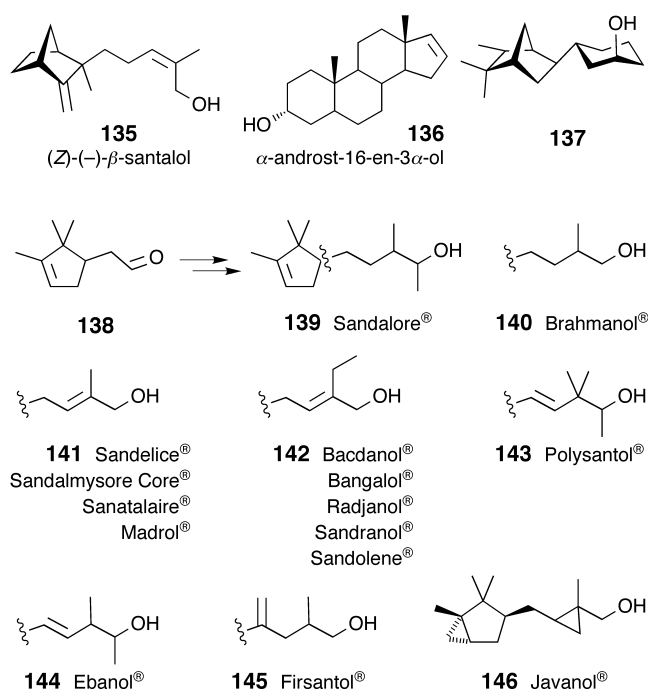
The East Indian sandalwood oil (*Santalum album* L.) represents one of the most precious perfumery raw materials. It plays an important religious and cultural role in India, and has always been an indispensable constituent of fine fragrances worldwide. The rarity, and therefore high price of sandalwood oils made the search for synthetic substitutes inevitable, which has provided numerous structure–odor relationship data. Unfortunately, because of the complex stereoisomeric composition of sandalwood-smelling products—almost al-

ways of terpenic origin—most of these data were only of limited use for the generation of precise olfactophore models. Nevertheless, several such models have been proposed and reviewed.^[4, 10] They all include a distance constraint between the osmophoric hydroxyl group (which orients the molecule on the receptor) and a bulky, lipophilic moiety. These models are, however, a source of some controversy. The situation improved during the last decade when more information on optically pure sandalwood odorants and their structurally related inactive counterparts became available. Recent models, based on this new data, and generated with the help of advanced molecular modeling tools, take into consideration the flexibility of structures within defined strain energy limits: 10 and 15 kcal mol⁻¹ in the studies of Buchbauer et al.^[78] and Bajgrowicz et al.,^[35] respectively. The latter proposed the concept of a combinatorial olfactophore model based on the recent discovery of combinatorial receptor coding schemes that the mammalian olfactory system uses to encode odor entities.^[79] The best hypothesis of this model is depicted in Scheme 26 together with the alignment of all the active stereoisomers used in the generation of the model.^[11]



Scheme 26. Sandalwood olfactophore model. Top: Best hypothesis mapped by (*Z*)-(-)- β -santalol (**135**, cyan) and by Javanol (**146**, yellow). Bottom: Alignment of all the active structures used in the generation of the model on the best hypothesis; α -androst-16-en-3 α -ol (**136**) is highlighted. Hydrogen atoms are omitted for clarity.

The lipophilic, bulky moiety is decomposed into three hydrophobic regions in this olfactophore model. The fourth one, which usually belongs to the flexible spacer, is placed in the vicinity of the osmophoric hydrogen-bond donor. It corresponds to the 18-Me group of α -androst-16-en-3 α -ol (**136**, Scheme 27), which astonishingly also possesses a fine sandalwood scent, in contrast to the unpleasant, steroidal, animalic, and urinous odor of the ketone derivative, which is the sex pheromone of pigs.



Scheme 27. Strong sandalwood-smelling compounds.

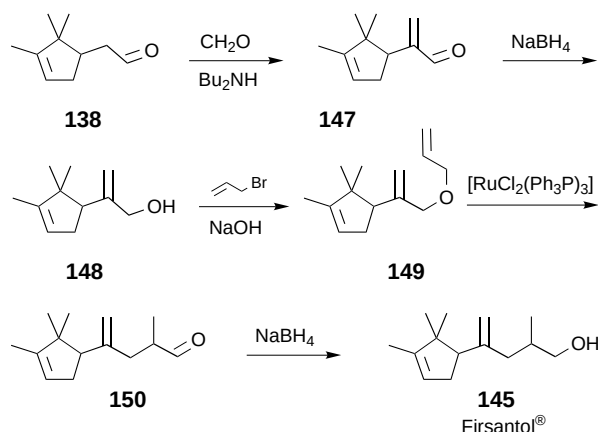
7.2. Derivatives of α -campholenic aldehyde

Despite many attempts no industrially viable process leading to β -santalol, the main olfactory constituent of sandalwood oils (ca. 25% of the weight of the oil), has yet been elaborated. The synthetic difficulties can be illustrated by the fact that the natural (*Z*)-(-)- β -santalol (**135**, Scheme 27) was first prepared only in 1990 in an 11-step synthesis starting with the Diels–Alder addition of cyclopentadiene to the *trans*-crotonate of (*R*)-pantolactone.^[80] In relation to the price of natural sandalwood oils, which, however impressive for the fragrance market, does not allow a total synthesis of **135** to be competitive.

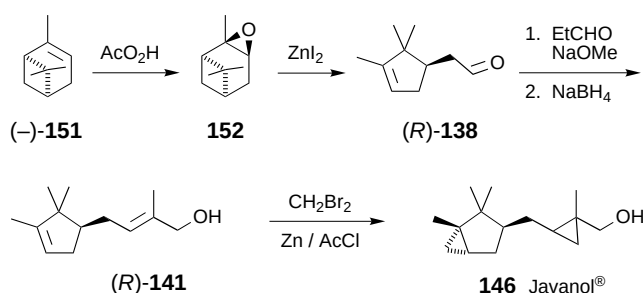
The recourse to synthetic analogues was a natural step. Complex mixtures of terpenylcyclohexanols, prepared by hydrogenation of acid-catalyzed condensation products of phenols (for example, guaiacol) with inexpensive terpenes (the most used was camphene) constituted the first generation of such products (for example, Sandela). Their main odor vectors of defined absolute configuration were claimed only recently. Compound **137** possesses the lowest odor threshold.^[81] Today, the best and most used sandalwood oil substitutes (**139**–**146**, Scheme 27) are derivatives of α -campholenic aldehyde (**138**), which is prepared from inexpensive α -pinene (**151**), most of which comes as a by-product from the paper industry.

Initially, the products were prepared from mixtures of enantiomers of **138**, often of variable ratio. More recent studies pointed at dramatic odor differences of the diastereoisomers and enantiomers in this series, with the derivatives of (*R*)-(+)- α -campholenal ((*R*)-**138**) being most often, but not always, the better sandalwood odorants.^[35, 82, 83] These studies gave rise to a series of “chiral switch” patents, an already well-established procedure in the pharmaceutical domain.

Among the usually simple and straightforward syntheses of campholenic aldehyde derivatives those of Firsantol (**145**, Scheme 28) and Javanol (**146**, Scheme 29) merit citation. The first one resulted from an elegant and cost-effective sequence consisting of an α -methylenation, reduction, etherification, and isomerization/Claisen rearrangement;^[84] the latter from the first example of a successful industrial application of a double Simmons–Smith cyclopropanation process.^[83]



Scheme 28. Synthesis of Firsantol (**145**).



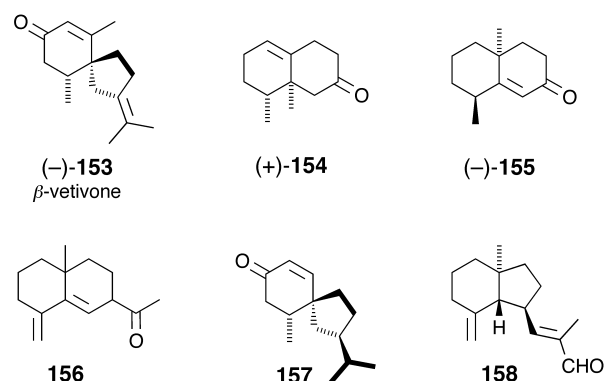
Scheme 29. Preparation of Javanol (**146**).

Sandalwood notes are often used in mixtures, together with sandalwood oil or without it. In the floral-oriental-woody “Samsara” (Guerlain, 1989) sandalwood oil was blended with Polysantal (**143**), while the sandalwood note of the oriental-spicy “Jako” (K. Lagerfeld, 1997) was created with Sandalore (**139**), Radjanol (**142**), and Ebanol (**144**). The method of using sandalwood odorants as well as the dosage varies a lot. As an example, two extremes for the use of Sandalore (**139**) illustrate this: “Boudoir” (V. Westwood, 1998) with 0.7% and “Chaos” (D. Karan, 1996) with 5% of Sandalore (**139**). Curiously, around 1% of the intermediate Firsantol (**150**) gives a woody-sandal-floral touch to the middle note of the recently launched “Ultra Violet” (P. Rabanne, 1999), while around 3% of the end product Firsantol (**145**) was employed in the woody-aromatic-musky fragrance “212 for men” (C. Herrera, 1999).

7.3. Vetiver Oil

The odor of vetiver oil (*Vetiveria zizanoides* Stapf) still remains one of the unrevealed secrets of nature. This oil is

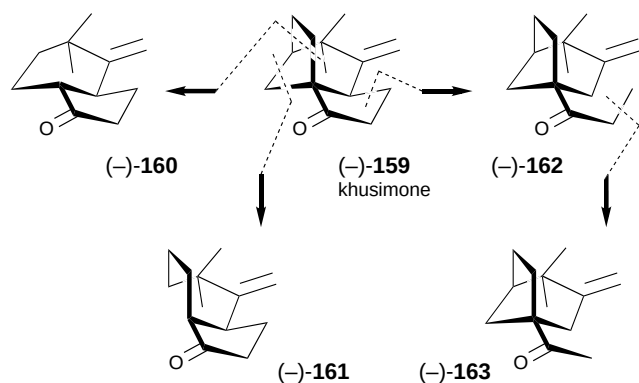
available on the market in a lot of different qualities, and consequently a lot of different odor tonalities, but they all share a suave and sweet woody odor with earthy, green, fungi-type aspects and grapefruit, rhubarb undertones. However, there is a big controversy about which compounds contribute to this highly appreciated scent, which arises mainly from the carbonyl fraction that makes up some 12% of the vetiver oils.^[85] Around 60% of this fraction consists of α - (**19**) and β -vetivone (**153**; Scheme 30), but their odor was said to be weak and uninteresting for perfumery.^[85] According to Maurer^[85] khusimone (**159**) and the two dimethyloctalones **154** and **155**,^[86] which are present in the essential oil to about 0.15%, are responsible for the characteristic odor of vetiver oil. A technically feasible partial synthesis of khusimone (**159**) starting from vetivenic acid was worked out,^[85] but **159** has not (yet) been introduced into perfumery.



Scheme 30. Compounds supposed to contribute to the typical odor of vetiver oil.

In contrast to this work at Firmenich, Mookherjee et al. of International Flavours & Fragrances (IFF) considered β -vetivone (**153**) the key odorant of vetiver oil,^[87] together with the 2-acetyl-4a-methyl-8-methyleneoctaline **156**, which has a powerful woody-amber odor, and nordihydro- β -vetivone **157**, which has a strong rich, woody-peppery odor.^[87] Mookherjee et al. described compounds **154** and **155** of Maurer as weak, green, rooty, and woody. Recent work by Spreitzer et al. on $(-)$ - and $(+)$ - β -vetivone (**153**)^[88] as well as on their chiral partial structures^[89, 90] showed, however, that neither β -vetivone (**153**) nor the partial structures possess the pleasant vetiver scent. The odor of the naturally occurring $(-)$ -**153** was evaluated by Dragoco perfumers and found to be quinoline-like, reminiscent of cassis and grapefruit, while $(+)$ -**153** exhibited an unpleasant cresolic, medicinal note.^[88]

Therefore, khusimone $(-)$ -**159** seems to be the only compound of vetiver oil which perfumers agree possesses a typical vetiver odor. This was the reason why Spreitzer et al.^[91–93] synthesized *seco* structures of $(-)$ -**159** to study the structure–odor relationship of the vetiver odor. Scheme 31 details three different *seco*-khusimones. $(-)$ -**160** smelled weakly camphoraceous with a woody by-note,^[91] while $(-)$ -**162** was camphoraceous with a sweet-herbal by-note, and $(-)$ -**163** cedarwoodlike.^[92] *seco*-Khusimone $(-)$ -**161** could not be evaluated since epimerization favored the corresponding *trans*-fused 3a*S*,8a*R* isomer. The odor of this isomer was



Scheme 31. *seco*-Khusimones (**160**–**163**) used to study the structure–odor relationship of the vetiver scent.

intense woody-camphoraceous, but lacked the typical vetiver odor descriptors.^[93] Consequently, the typical odor descriptors of vetiver seem not to stem from these partial structures, and we are still far away from a synthetic vetiver odorant.

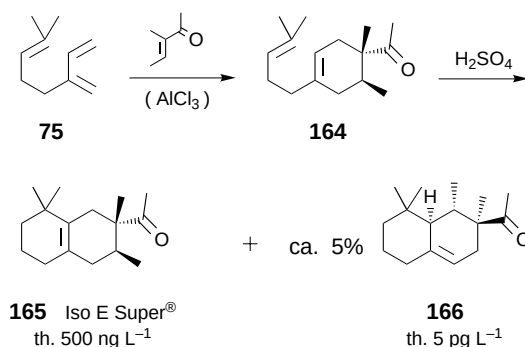
Only very recently, Weyerstahl et al.^[94] discovered a new olfactorily important constituent of Haïtian vetiver oil, (*E*)-opposita-4(15),7(11)-dien-12-al (**158**; Scheme 30), which possesses a strong, green odor with undertones of mimosa and orris, and a woody tonality. The secrets of the vetiver odor remain, however, far from being unveiled, and much more work will be necessary to find synthetic substitutes.

Vetiver oil was used a great amount in the past, with “Vetiver” (Carven, 1957) and “Vetiver” (J. P Guerlain, 1959) being the best examples. But vetiver oil was also indispensable in the chypre family, for example, in “Mitsouko” (J. P Guerlain, 1919). Nowadays, vetiver oil is still used, but in much lower dosage. “Hugo Dark Blue” (H. Boss, 1999), “Christian Lacroix” (C. Lacroix, 1999), and “Azzura” (Azzaro, 1999) are some recent examples.

7.4. Woody!

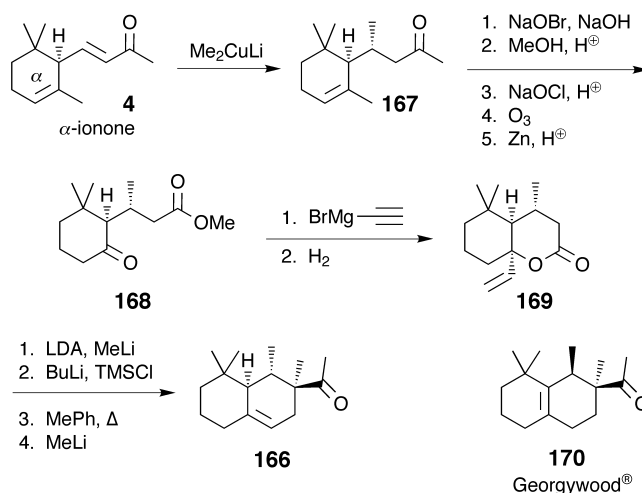
What Hedione (**8**) is to florals, IsoE Super is to woody notes. IsoE Super (**165**; see Scheme 32) is a very rich product with many facets, and some people smell a cedarwood note while others perceive it as transparent or musky, ionone-like. The musky-violet facets were exploited in “Fahrenheit” (C. Dior, 1988) with 25 % of IsoE Super (**165**) and in “Trésor” (Lancôme, 1991) with 18 % of it. The cedarwood side was important for “Féminité du Bois” (Shiseido, 1992) with 43 %, and for “Déclaration” (Cartier, 1998) with 35 % of it.

The industrial synthesis of IsoE Super (**165**) is shown in Scheme 32. The aluminum trichloride catalyzed Diels–Alder reaction of myrcene (**75**) with (3*E*)-3-methylpent-3-en-2-one gives predominantly regioisomer **164**, which is cyclized in the presence of sulphuric acid to provide **165** as the major product. However, it is not this compound that causes the woody odor of the commercial product, but a 5 % impurity that is formed during the cyclization step.^[95] The odor threshold of this very powerful impurity **166** is about 100 000 times lower than that of the main product **165**.



Scheme 32. Industrial synthesis of IsoE Super (**165**). th. = threshold value.

The powerful impurity **166** was isolated by epoxidation of a commercial IsoE Super mixture, and a structure was proposed by 2D NMR experiments;^[95] however, this required conformation by a directed synthesis. The stereoselective synthesis of **166** is summarized in Scheme 33.^[95] Diastereoselective conjugate addition of lithium dimethylcuprate to α -ionone (**4**) gave **167**, which was further transformed by a haloform



Scheme 33. Synthesis of the powerful impurity (**166**) of IsoE Super and the structure of a related woody odorant **170**. LDA = lithium diisopropylamide.

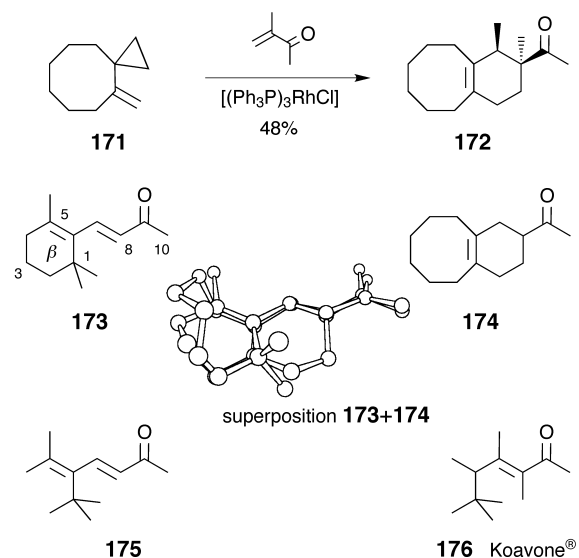
reaction, esterification, and ozonolysis of the double bond after isomerization with sodium hypochlorite. The resulting oxo methyl ester **168** reacted diastereoselectively with ethynylmagnesium bromide, and the Grignard intermediate was hydrogenated in the presence of Lindlar's catalyst to furnish vinyl lactone **169**. Ireland–Claisen rearrangement of **169** followed by methylation with methyllithium afforded the target molecule **166**, which was identical by all analytical methods to the material isolated from commercial IsoE Super.^[95]

Clearly, this synthesis of **166** is not suited for an industrial scale, and the structure turned out to be too complex for an alternative synthetic procedure. However, out of several synthesized analogues, **170** was found to have an almost equal odor threshold, and it was even preferred in terms of its odor characteristics. Its industrial synthesis by a Diels–Alder reaction of homomyrcene with methyl isopropenyl ketone is

straightforward,^[4] and **170** has been introduced into perfumery by Givaudan as Georgywood. Among many examples, “Golden Moments” (P. Presley, 1999) with 5% of Georgywood (**170**) is the most prominent one.

What is “W-A-S”? Smell the microencapsulated fragrance in Figure 4 (see p. 2989): An original woody-ambery-spicy masculine fragrance with anisic and floral facets. The woody part is mainly composed of Georgywood (**170**), combined with musky notes. The ambery part consists of Okoumal (**196**) and Spirambrene (**200**, see Scheme 37), which bring warmth and sensuality to the fragrance and harmonize with the spicy accord mainly based on black and pink pepper oil.

Similar to Georgywood (**170**) in odor, structure, and excellent threshold value is **172**, which is accessible by an uncommon Diels–Alder-type reaction of 4-methylene-spiro[2.7]decane (**171**) with methyl isopropyl ketone using Williamson’s catalyst (Scheme 34).^[96] A dimethylcyclohexene ring is almost equivalent in terms of shape to a cyclooctene moiety, and this is also reflected in the remarkable olfactory resemblance of 1-bicyclo[6.4.0]dodec-1(8)-en-10-yl-ethan-1-one (**174**) to β -ionone (**173**). The superposition in Scheme 34 shows the excellent overlap of both molecules.

Scheme 34. Odorants **172** and **174** with the bicyclo[6.4.0]dodecene

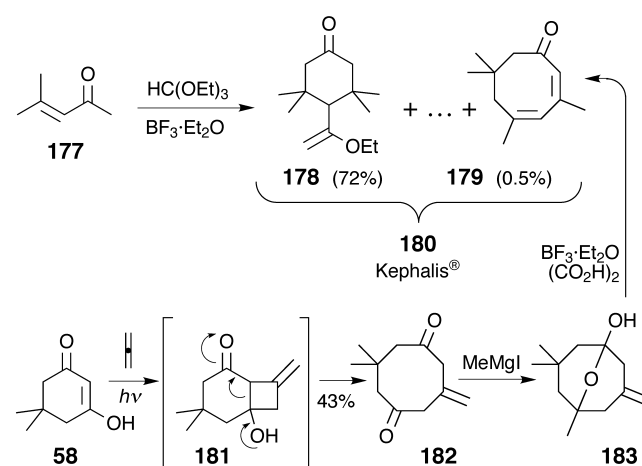
In 1962, Sestanji^[97] had already found that cutting out carbon atom C-3 does not alter the odor characteristics of β -ionone (**173**), and 5-*tert*-butyl-6-methylhepta-3,5-dien-2-one (**175**) indeed possesses a woody, floral odor close to that of **173**. This result incited researchers at IFF to synthesize related *seco*-ionones, and one of these compounds, Koavone (**176**), indeed became a commercial product. An analysis of the superposition of **176** and 10-methylionone,^[98] one of the first applications of molecular modeling in fragrance chemistry, rationalized the similar odor profiles. Koavone (**176**) smells woody, floral, and ambery, and has mainly been used in consumer goods, in many different medias: In shampoos such as “Elsève multivitaminé 2 in 1” (L’Oréal, 1995), fabric softeners such as “Vernel cool fresh” (Henkel, 1996), soaps such as “Fa aqua” (Henkel, 1997), as well as deodorants such

as “Dove sans alcool” (Lever, 1998). Nevertheless, the interest of Koavone (**176**) for fine fragrance perfumery was demonstrated by “Jazz” (Yves Saint Laurent, 1988) with a dosage of around 2%.

8. Ambery Notes

8.1. Ambery Ketones and Ketals

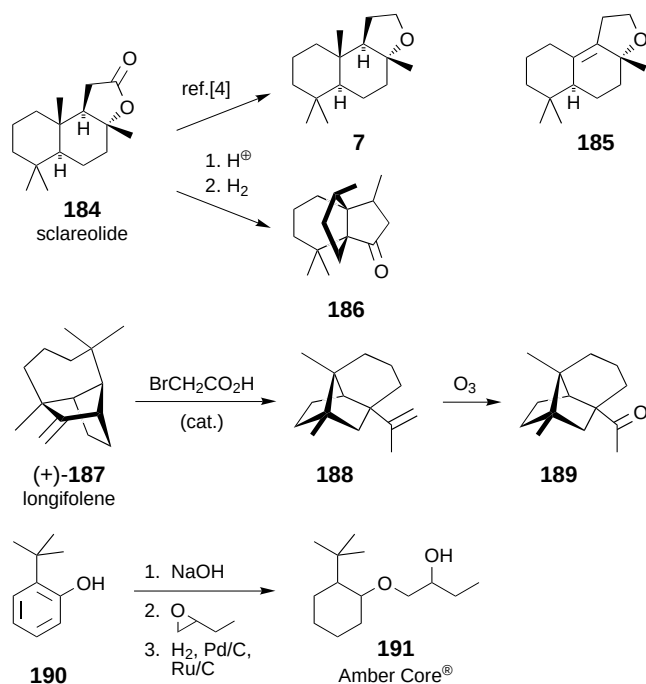
Many odorants share woody and ambery odor characteristics. Kephalis (**180**) is a typical example, since it possesses woody-piney as well as ambery-tobacco facets. These various facets have been exploited in “Yohji Homme” (Y. Yamamoto, 1999) with around 4% of Kephalis (**180**). This valuable perfumery raw material **180**^[99] consists of a complex mixture of ketones, alcohols, and ethers obtained in an acid-catalyzed reaction of mesityl oxide with ethyl orthoformate.^[100] In an attempt to improve the efficiency of the manufacturing process, a detailed analytical and synthetic study was undertaken and the synthesis of one of the trace constituents (**179**) contributing to the overall rich ambery scent was published (Scheme 35).^[101]



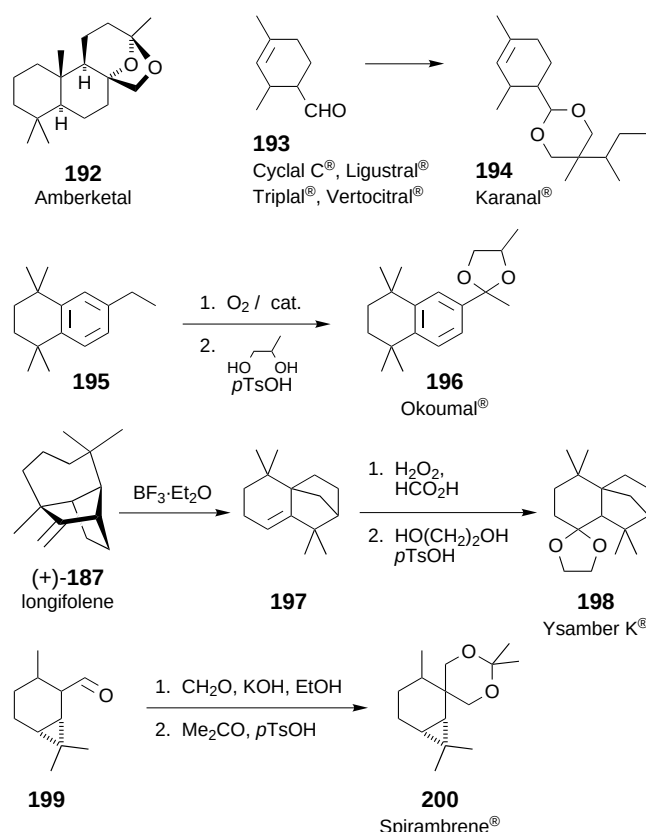
Scheme 35. The perfumery raw material Kephalis (**180**).

All three steps in the preparation of **179** were tandem transformations: de Mayo photocycloaddition/retroaldol ring enlargement, followed by Grignard addition/transannular acetalization, and ketal ring opening/dehydration/double-bond migration, which led to the conjugated system of **179**. Ambery and tobacco facets are indeed present in the odor profile of **179**, but these are astonishingly accompanied by a fruity, α -damascone note.^[100]

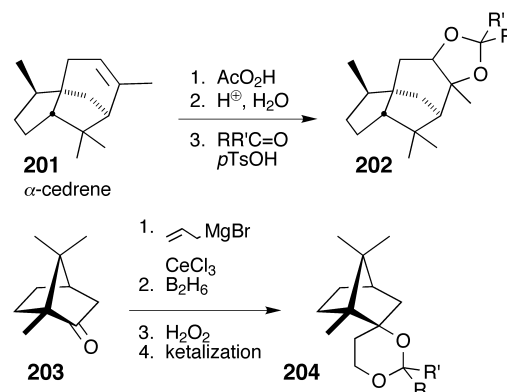
Chemicals that smell like amber are mainly ethers, ketones (Scheme 36), and cyclic ketals (Scheme 37, Scheme 38) that are almost always derived from commonly occurring natural terpenes. The cyclic ether Ambrox (**7**) remains the prototype of what perfumers understand as ambery, which is actually a very unclear term derived from the multifacet odor of natural ambergris.^[102] Interestingly, its nordidehydro analogue **185**^[103] still exhibits an intense amberlike odor, which corroborates the amber olfactophore model discussed below. From a molecular



Scheme 36. Some of the best ambery odorants; cyclic ketals are excluded.

Scheme 37. Cyclic ambery ketals already introduced into perfumery. $p\text{TsOH} = p\text{-H}_3\text{CC}_6\text{H}_4\text{SO}_3\text{H}$.

modeling point of view, it is also interesting to compare the shapes of propellane **186**, obtained by an interesting acid-catalyzed transposition of sclareolide (**184**),^[104, 105] and the recently published^[106] 12-noralloisolongifolan-11-one (**189**,



Scheme 38. New cyclic ambery ketals. R, R' = H, C1–C6 alkyl.

obtained from naturally abundant longifolene (**187**)) with those of the woody odorants Iso E Super (**166**), Georgywood (**170**), and their analogues (see Section 7.4). An ambery-woody borderline case is Amber Core (**191**),^[107, 108] which should be envisaged as a noroxa analogue of the active stereoisomers of Timberol and Norlimbanol,^[4] which are usually classified as woody.

Cyclic ketals are steadily gaining ground among ambery odorants (Scheme 37 and 38). Amberketal (**192**)^[109], Karanal (**194**),^[110, 111] derived from the well-known green-smelling Ligustral (**193**), Okoumal (**196**)^[112], Ysamber K (**198**),^[113] and Spirambrene (**200**),^[114] produced in two steps from 2-formylcarane (**199**), are the most prominent amongst the already commercialized perfumery products. Potential future introductions include the dioxolanes **202**^[115] and the 1,3-dioxanes **204**,^[116] obtained in straightforward transformations from α -cedrene (**201**) and camphor (**203**), respectively.

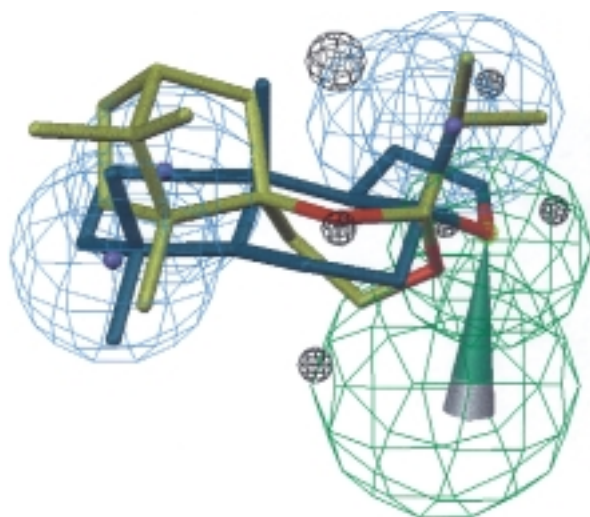
The use of Spirambrene (**200**) is very diverse and versatile. It is found in the floral-oriental feminine fragrance “Eden” (Cacharel, 1994) as well as in the marine masculine perfume “Kenzo pour Homme” (Kenzo, 1991). Okoumal (**196**) is a very powerful ambery odorant, which blends well with woody, especially sandalwood notes. The combination Okoumal/Ebanol (**196/144**) was found in “Escape for men” (C. Klein, 1993) and in “Hugo” (H. Boss, 1995). Karanal (**194**) and Amberketal (**192**) are also very powerful and long-lasting odorants. They have been used in “Diesel++masc” (Diesel, 1997) and in “Boss” (H. Boss, 1998), respectively.

8.2. Amber Olfactophore

Attempts to rationalize the structure–odor relationship of amber odorants have a long history.^[4] From Ohloff’s “triaxial rule”, through Vlad’s “ambergris triangle”,^[119] and oxygen accessibility criteria to the more recent CATALYST study^[11, 117] based on the alignment of “chemical functions” in flexible structures, and repeated electron-topological investigations,^[118] different concepts have been investigated to explain the presence or the absence of this noble scent, which depends on very slight structural modifications. An important argument for carrying out structure–odor correlations in this odor note is the relative rigidity of most of the

compounds that smell like amber. In the most recent study in this domain, which uses the electronic-topological matrices of contiguity, Gorbachov and Rossiter^[118] propose a new “active ambergris fragment” consisting of one oxygen atom and three carbon atoms, possessing certain atomic charges, and being separated by certain key distances.

Our 3D model of the amber olfactophore^[117] (Scheme 39) contains four hydrophobic functions (blue spheres), and one oriented hydrogen-bond acceptor (green). The six excluded



Scheme 39. Ambrey olfactophore model mapped by Ambrox (**7**, blue) and one of the epimers of the best derivative **204** ($R, R' = H, iPr$, yellow). Hydrogen atoms are omitted for clarity.

volumes (black), which tentatively correspond to the geometrical limits of the binding pocket of the putative amber olfactory receptor and significantly improve the predictability of the presence or absence of the ambrey odor in examined structures, were added by a trial and error process. However imperfect, this model has been successfully applied to the design and lead development of the camphor derivatives **204** (Scheme 39). The best of these compounds **204** ($R, R' = H, iPr$) is depicted.

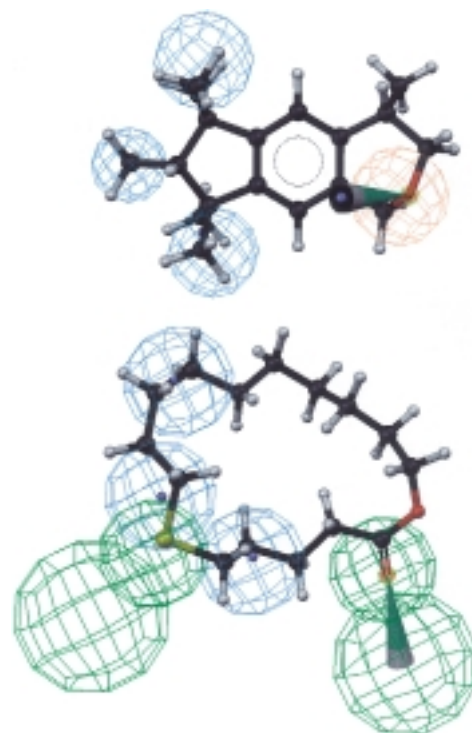
9. Musk Odorants

9.1. Musk Olfactophore Models

Although quite a lot of data on substances with musk odor are accessible in the open literature,^[119] our insight into the molecular parameters of musk odorants remains rather limited. This is because musk odorants belong to structurally very different chemical classes (for example, macrocyclic aliphatic compounds, polycyclic benzene derivatives, and nitro arenes) with different possible binding features.

Bersuker et al.^[119] stated two independent features to be responsible for a musk odor. The first is a hydrogen-bond acceptor symmetrically flanked at a distance of 6.7 ± 0.5 Å by two hydrophobic binding pockets that are 2.5 ± 0.5 Å apart from each other. The second feature consists of two hydrophobic groups within a distance of 5.5 ± 0.5 Å to each other.

However, Kansy et al.^[120] could not confirm the prediction rate of Bersuker's hypothesis. Using the same data set^[119] they proposed an olfactophore model consisting of three hydrophobic features 6.7, 8.0, and 5.6 Å away from the hydrogen-bond acceptor.^[120] As is shown in the upper part of Scheme 40, the methyl groups of the pentamethylindane moiety of (4*S*,7*R*)-Galaxolide ((4*S*,7*R*)-**10**) fit well to these hydrophobic functions; however, this olfactophore model could not explain



Scheme 40. Olfactophore models for musk odorants. Top: with **10**; bottom: with 6-thiahexadecanolid.

the importance of the *S* configuration of the 4-methyl group, which is crucial for the musk odor of **10**.^[121] Thus, the conformationally constrained (4*S*,7*R*)-**10** seems to be better suited as a template for the design of musk odorants than this olfactophore model.

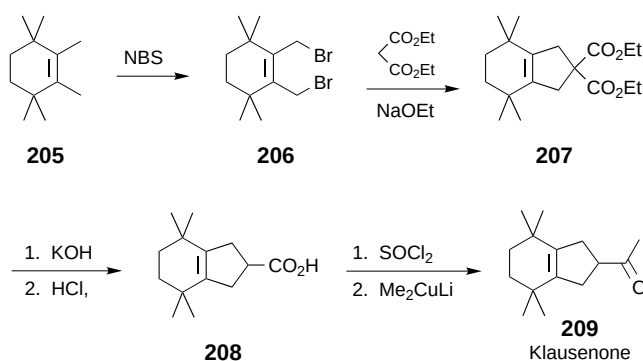
Other questions also remain open: Why is the threshold of the nitro musks about 20–30 times lower than that of the natural alicyclic lead substance (*R*)-(-)-muscone (**223**; see Scheme 44)?^[122] Does the musk receptor possess more than one binding site for polar groups? Can more powerful macrocycles be designed by introducing additional polar groups? Is the function of double bonds restricted to conformational effects, or is it also of electronic importance?

To find some answers to these questions a series of thiamacrolides was synthesized.^[123] A sulfur atom can mimic a double bond without restricting the conformational freedom of the macrocycle. A 1,7-distance of the sulfur atom and the carbonyl group in even-membered rings, and a 1,6-position of the sulfur atom and carbonyl group in odd-membered rings significantly lowered the thresholds of the compounds. A CATALYST^[11] hypothesis was generated using the threshold data of 13 thiamacrolides smelling of musk, which allowed threshold values to be calculated with good accuracy. In the

lower part of Scheme 40 the fit of 6-thiahexadecan-16-olide to this olfactophore hypothesis is shown. The model consists of two hydrogen-bond acceptors 6.9 Å away from each other, and three hydrophobic functions 4.1/2.8, 7.1/1.7, and 8.3/5.0 Å, respectively, away from the hydrogen-bond acceptors. The second hydrogen-bond acceptor could explain why some dicarbonyl compounds, such as for example 5-oxomusccone,^[124] possess intense musk notes, while others are practically odorless.

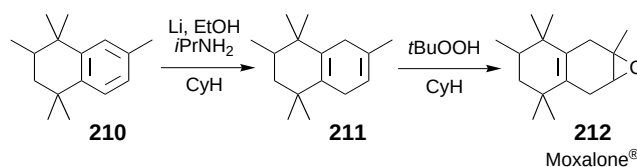
9.2. The Klausenone Story

In 1979, while working on the synthesis of aromatic retinoids, M. Klaus of F. Hoffmann-La Roche discovered by serendipity a compound with an intense musky odor and interesting damascone-like, woody nuances. Allylic bromination of 1,2,3,3,6,6-hexamethylcyclohex-1-ene (**205**) with NBS, followed by a malonic ester synthesis led to the diethyl ester **207**, which was hydrolyzed and decarboxylated to **208**, and finally transformed into the unexpected musk odorant **209** (Scheme 41). In comparison with other polycyclic musk odorants such as Galaxolide (**10**), the intense musk odor of the non-benzenoid 2-acetyl-2,3,4,5,6,7-hexahydro-1*H*-indene **209** was also truly amazing from a structural point of view, and the substance therefore soon became known as Klausenone—also at Givaudan Roure, at that time the flavor and fragrance division of Roche.



Scheme 41. The original synthesis of Klausenone (**209**). NBS = *N*-bromosuccinamide.

Efforts were first directed towards an industrially feasible synthetic route to Klausenone (**209**).^[125] However, this turned out to be rather difficult, and the synthesis of derivatives was soon also investigated. Surprisingly, it turned out that the corresponding alcohol of Klausenone (**209**), obtained by LAH reduction of **209**, also possesses an intense musk odor, which showed that a carbonyl function was not a necessary requirement. Finally, the epoxide intermediate **212**, en route to a methylated Klausenone by an acid-catalyzed rearrangement, turned out to be the best musk odorant of all the derivatives synthesized (Scheme 42). It was introduced into perfumery by Givaudan as Moxalone (**212**). The industrial synthesis^[126] starts from 1,1,2,4,4,7-hexamethyltetralin (**210**). As depicted in Scheme 42, an industrial Birch reduction and epoxidation



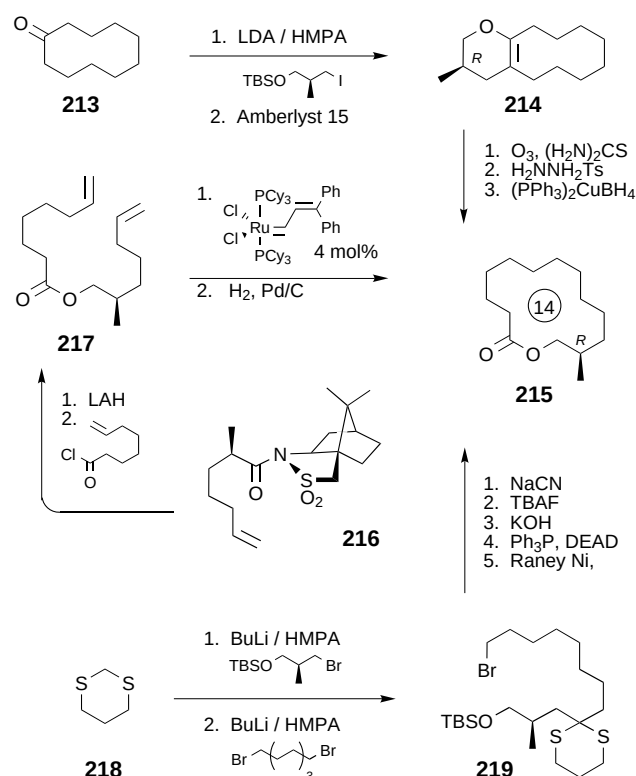
Scheme 42. Industrial synthesis of Moxalone (**212**).

employing *tert*-butylhydroperoxide complete the Moxalone synthesis.

Moxalone (**212**) was the first musk with an oxirane moiety. It possesses a rich musk odor with woody and fruity undertones, which distinguish it from the other musk odorants. Recently, it has been used in “Paris Baby Doll” (Yves Saint Laurent, 1999) at around 1.2 %, and also shows its efficiency in the unisex fragrance “CK be” (C. Klein, 1996).

9.3. Macrocyclic Musks

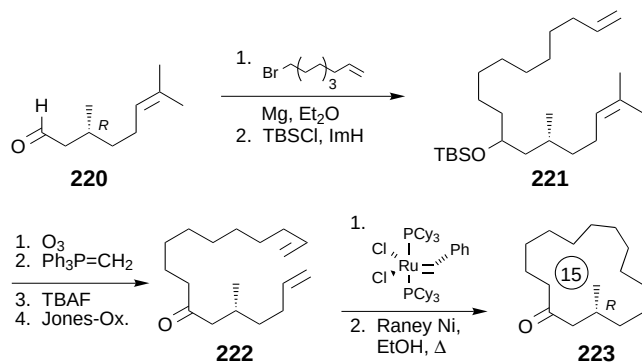
12-Methyl-13-tridecanolide (**215**; Scheme 43) was the first example showing the significance of methyl substituents in macrocyclic musk odorants. While the 13-tridecanolide possesses no musk odor, the enantiomers of 12-methyl-13-tridecanolide (**215**) differ distinctly in their musk odors: The 12*R*-(+) stereoisomer exhibits a sandalwood-musk note, while its 12*S*-(-) enantiomer has an animal-like musk odor with camphoraceous aspects.^[127, 128] Both enantiomers occur in Angelica root oil (*Archangelica officinalis* Hoffm.) in the ratio of 72:28 ((*R*)-**215**:(*S*)-**215**).^[129]



Scheme 43. Synthetic approaches towards (12*R*)-(+)-methyl-13-tridecanolide (**215**). HMPA = hexamethyl phosphoramide, TBS = *t*BuMe₂Si, Ts = H₃CC₆H₄SO₂, TBAF = tetrabutylammonium fluoride, DEAD = diethyl azodicarboxylate.

So far three different enantioselective approaches to this interesting natural product have been published (Scheme 43). The first one makes use of a ring-enlargement sequence with a chiral building block.^[127] Cyclodecanone (**213**) was alkylated with a chiral building block derived from the methyl ester of (2*S*)-(+)-3-hydroxy-2-methylpropanoic acid. Amberlyst 15 mediated the cyclization to the bicyclic enol ether **214**, which was then subjected to ozonolysis and selective reduction of the resulting ketone group to provide the target molecule (12*R*)-(+)-**215** in 98.8% *ee*. Fürstner et al.^[130, 131] applied a ring-closing metathesis to the synthesis of (12*R*)-(+)-**215**. The enantiomerically pure starting material **216** was prepared by alkylation of *N*-propionylbornane-10,2-sultam with 5-iodopent-1-ene. Reduction of amide **216** followed by esterification of the resulting alcohol provided the dienoate **217**, which was cyclized by the action of a Grubbs catalyst. As outlined in Scheme 43, reduction of the metathesis product then furnished the target molecule (12*R*)-(+)-**215**. The most recent approach of Noda et al.^[132] utilizes 1,3-dithiane (**218**) as an auxiliary.^[133] The carbon chain of **215** was constructed by alkylation of **218** with first a chiral building block and then 1,8-dibromooctane. Interconversion of the functional groups of **219**, subsequent macrolactonization, and reductive desulfurization completed this synthesis of enantiopure (12*R*)-(+)-**215** (Scheme 43).

As a consequence of the recently developed ruthenium and molybdenum alkylidene catalysts,^[131, 134] ring-closing olefin metathesis has advanced to a widely applicable synthetic method for the preparation of macrocycles. An interesting application is the synthesis of enantiopure (*R*)-(-)-muscone (**223**; Scheme 44), the principal odorous constituent of the

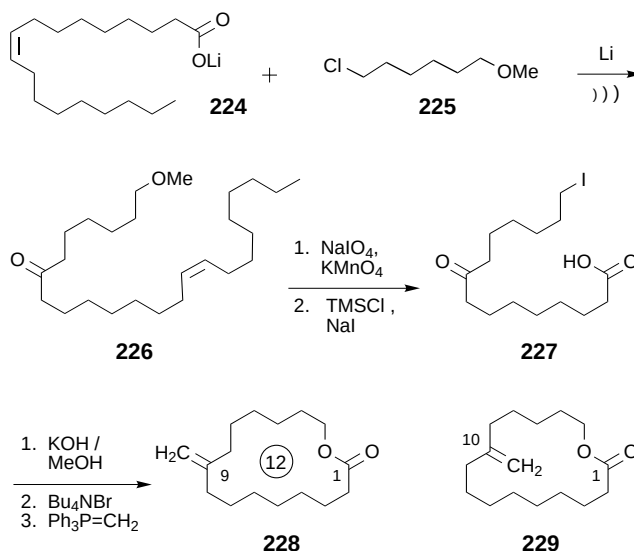


Scheme 44. Synthesis of (*R*)-(-)-muscone (**223**) by ring-closing metathesis. ImH = imidazole.

male musk deer.^[135] The Grignard reaction of 1-bromodec-9-ene with commercially available (*R*)-(+)-citronellal (**220**) provided, after oxidation or protection of the alcohol function, a substrate that was already suitable for a ring-closing metathesis to form (*R*)-(-)-muscone (**223**). However, the *gem*-dimethyl substitution of one double bond of **221** rendered ring closure by metathesis impossible. Therefore, **221** had to be transformed into diene **222** with two terminal double bonds. As outlined in Scheme 44, this substrate smoothly underwent ring closure in the presence of 5 mol % of bis(tricyclohexylphosphine)benzylidene ruthenium(IV) di-

chloride to furnish the desired target molecule **223** after hydrogenation.

The synthesis of the first methylene macrolides is shown in Scheme 45.^[136, 137] A Barbier reaction of chloroalkyl methyl ether **225** with lithium oleate led, after Lemieux oxidation and deprotection with Olah's reagent, to the ω -iodooxocarboxylic

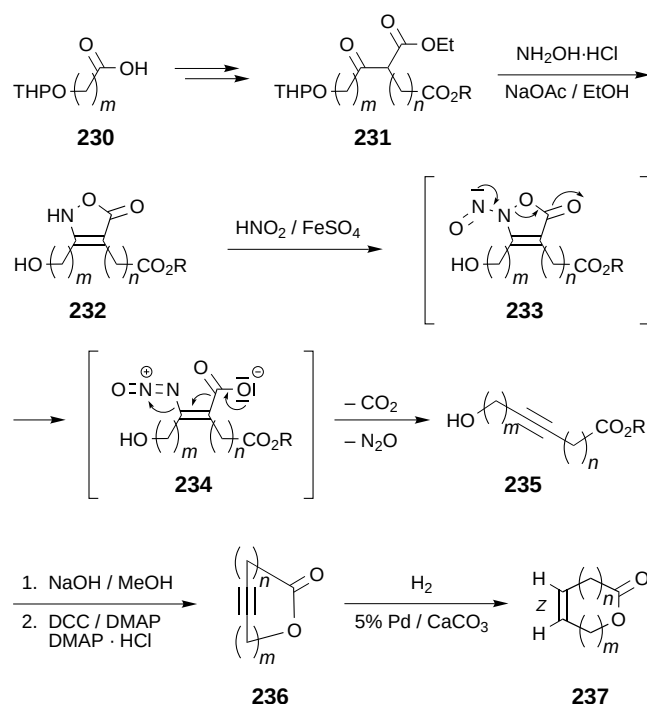


Scheme 45. The first methylene macrolides.

acid **227**. This compound was cyclized and methylenated by a standard Wittig reaction to provide 9-methylene-15-pentadecanolate (**228**), which emanated a strong sweet musk note with an animal-like tonality. The 10-methylene analogue **229**, which is available from undecylenic acid, is even sweeter and more animal-like, but has a waxy undertone.^[136, 137]

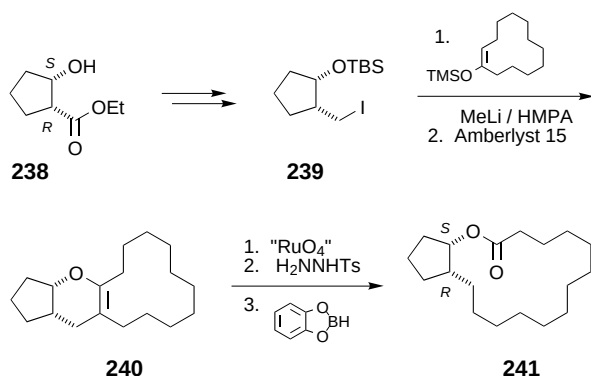
A general sequence for the synthesis of *Z*-configured alkenolides is presented in Scheme 46. Starting from the THP-protected ω -hydroxycarboxylic acids **230**, Lehmann and Tochtermann^[138] synthesized α -branched β -oxo esters **231** that were converted into isoxazolones **232** by treatment with hydroxylamine hydrochloride. By employing the method of Zard et al.,^[139, 140] these compounds fragmented upon nitrosation to afford ω -hydroxyalkynoic acids **235**. Cyclization and hydrogenation with Lindlar's catalyst gave the 16-membered *Z*-alkenolides **237**, the olfactorily most interesting of which turned out to be (7*Z*)-pentadec-7-en-15-olide (**237**, $n = 5$, $m = 7$).^[138]

As (9*E*)-hexadec-9-en-16-olide (*iso*-ambrettolide), synthesized from a main constituent of shellac (aleuritic acid), became an important perfumery material^[4] it also seemed interesting to utilize fatty acids from other natural products in the synthesis of macrocyclic musks. Baba et al.^[141] converted chaulmoogric acid (cyclopent-2-ene-1-tridecanoic acid), a main component of Chaulmoogra oil, into a mixture of macrobicyclic lactones that was reported to possess a musk odor. This process led to the synthesis of related macrobicyclic lactones with defined regio- and stereochemistry.^[142] The preparation of (1*S*,16*R*)-(+)-2-oxabicyclo[14.3.0]nonadecan-3-one (**241**) by ring enlargement with a chiral building block, derived by yeast reduction from 3-oxocyclopentanecarboxy-



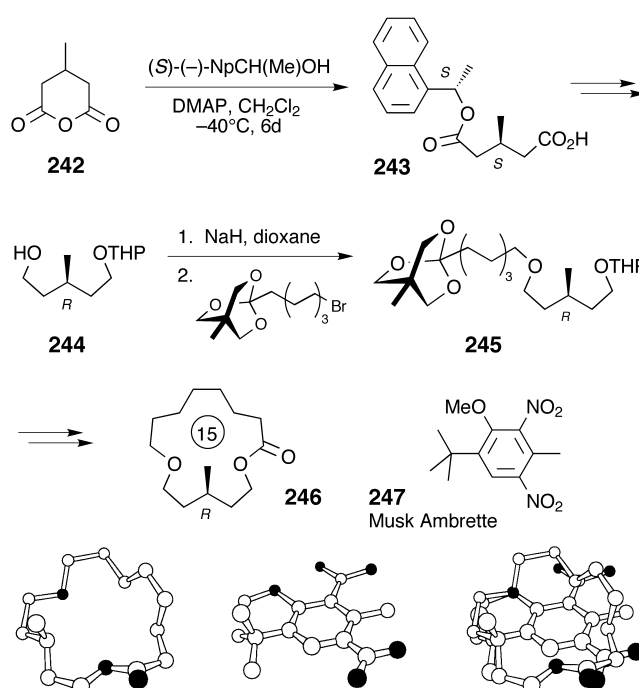
Scheme 46. A synthetic sequence towards *Z* alkenolides. THP = tetrahydropyran.

late, is outlined in Scheme 47. The smell of this macrobicyclic lactone was reminiscent of the olfactory impression of clean skin, with aldehydic, fatty, and woody aspects but only a slight musky touch.^[142]



Scheme 47. Stereoselective synthesis of a macrobicyclic lactone (**241**) by ring enlargement.

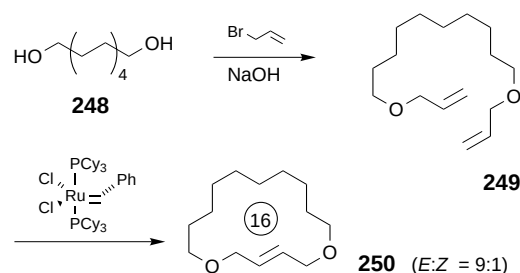
The synthesis of a macrocyclic musk that closely resembles the nitro musk Musk Ambrette (**247**) is presented in Scheme 48.^[143, 144] The observation that only the *R* isomer **246** was responsible for these interesting odor characteristics, while its enantiomer was odorless in the GC olfactometry was unexpected.^[143] The key step in the stereoselective synthesis of **246** was the desymmetrization of 3-methylglutaric anhydride (**242**) with (1*S*)-(-)-1-(1-naphthyl)ethanol.^[145] Borane reduction, THP protection, LAH reduction, and a Williamson reaction with ω -bromoheptyl-OBO (OBO = 4-methyl-2,6,7-trioxabicyclo[2.2.2]octane) led to the extremely powerful musk odorant **246** via intermediate **245**. The superposition



Scheme 48. A macrocyclic musk that closely resembles the nitro musk Musk Ambrette (**247**). (S)-(-)-Np = (S)-(-)-naphthyl.

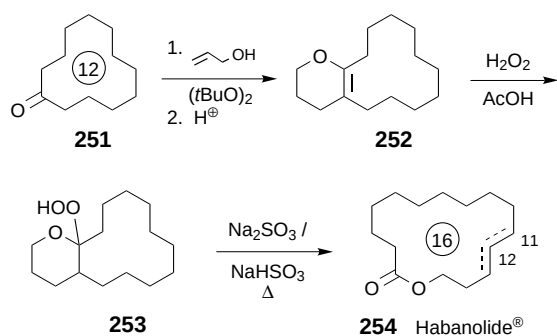
of the minimum-energy conformer of **246** with Musk Ambrette (**247**), also shown in Scheme 48, may account for the odor similarity of both compounds. An (ω -2)-methyl substituent as well as a 1,7-dioxa distance, both of which are present in **246**, were assumed to intensify the musk odor of macrocycles.^[143]

A recent Takasago patent^[146] claims 1,6-dioxamacrocycles to be outstanding musk odorants. 1,6-Dioxacyclohexadec-3-ene (**250**) possesses a powdery musk note, and was synthesized as outlined in Scheme 49 by a Williamson reaction of decan-1,10-diol (**248**) with allyl bromide and subsequent ring-closing metathesis. This simple two-step process illustrates the search of industrial chemists for inexpensive routes to macrocyclic structures.



Scheme 49. 1,6-Dioxacyclohexadec-3-ene (**250**).

Habanolide (**254**)^[147] is one of the least expensive macrocyclic musks currently available to the perfumer, and thus has recently become important in perfumery. Its industrial synthesis (Scheme 50) starts from cyclododecanone (**251**), and is the result of over 20 years of process research on the synthesis of pentadecan-15-olide.^[148–151] Habanolide (**254**) blends very well with other macrocyclic musks and traditional ambery



Scheme 50. Industrial synthesis of Habanolide (254).

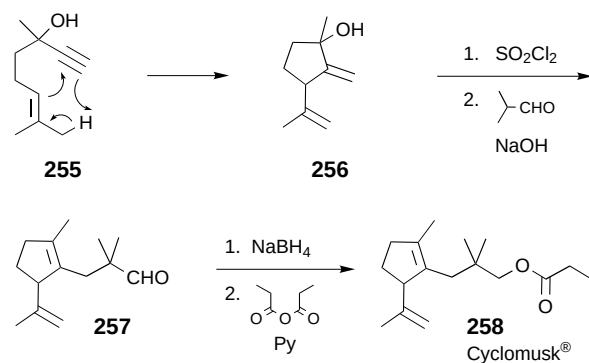
notes. “Bulgari for men” (Bulgari, 1995) was one of the first examples where it was used in large amounts of around 10%. In the oriental-amber-musk family, we find an overdose of Habanolide (254) in “Salvatore Ferragamo pour Homme” (S. Ferragamo, 1999), and “Hypnotic Poison” (C. Dior, 1998) at around 16 and 17%, respectively.

Habanolide (254) also goes well with Helvetolide (262), and this combination of perfumery raw materials was used for example in “2020” (Courrèges, 1997) in the ratio of 2.3:1 and in “Herve Leger” (H. Leger, 1998) in the ratio of 0.6:1.

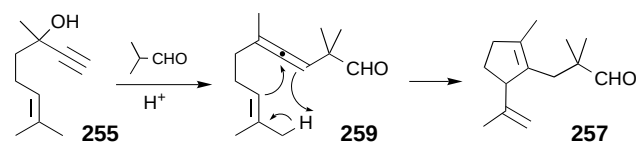
The microencapsulated base “Muskberry” in Figure 5 (see p. 2991) was built around a blackberry accord. It illustrates how the musk odorants Habanolide (254) and Moxalone (212) bring sensuality to a composition, and accentuate the perfumery side of this gustative odor.

9.4. Neither Macrocyclic Nor Benzenoid

When musks are mentioned, one usually thinks of macrocycles, polycyclic benzene derivatives, or nitro arenes.^[4, 124] However, in 1975 BASF had already discovered a cyclopentenyl ester with a musky, warm, powdery odor and fruity, strawberry-type nuances.^[152, 153] The compound, which was introduced as Cyclomusk (258), was manufactured as summarized in Scheme 51 by thermal cyclization of dehydrolinalool (255), subsequent halogenation under migration of the double bond, reaction of the resulting halide with isobutyraldehyde, reduction, and esterification of the resulting product with propionic anhydride. As shown in Scheme 52, the central intermediate 257 of this process is also accessible



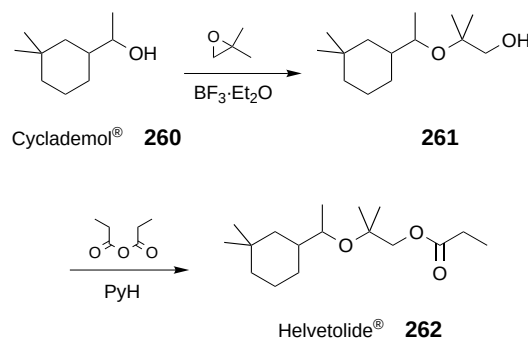
Scheme 51. Synthesis of Cyclomusk (258). Py = pyridine.



Scheme 52. Alternative route to the intermediate aldehyde of the Cyclomusk (258) synthesis.

by direct thermal cyclization of the allene 259, which is obtained by heating dehydrolinalool (255) with isobutyraldehyde in the presence of acid.^[154]

Fifteen years later, in 1990, another representative of this uncommon class of musk odorants was discovered:^[155] Helvetolide (262) with its musky-floral, fruity, pear-type odor. Its synthesis, as described in the corresponding patent,^[155] is outlined in Scheme 53. An acid-catalyzed ring opening of isobutylene oxide with Cyclademol (260) afforded regioselectively the 2,2-dimethyl-3-oxaolcohol 261, which was esterified to the corresponding propionate Helvetolide (262). In the floral-woody-musky feminine perfume “Ultra Violet” (P. Rabanne, 1999) around 3% of Helvetolide (3) was used in combination with other musks.



Scheme 53. Synthesis of Helvetolide (262).

10. Fragrance Forecast

With the pear-type nuances of the new musk Helvetolide (262) we return to the starting point of our journey into modern perfumery. We have seen how new odorants lead to new accords and then to new trends.

The traditional pyramidal building of perfumes seems to have changed, and perfumes become more and more rectangular. This allows the possibility of getting directly into the middle and base notes which give the character to a fragrance. Nevertheless, the top note is still important, and some perfumes can be distinguished most easily by this. That is also why impact odorants become more and more crucial.

We do not want to conclude this overview on recent developments in the chemistry of odorants without recalling the main trends, and without daring to take a look into the future. Again, we follow the olfactory circle of Figure 1. Fruity odorants are used to a great extent nowadays, and therefore, there seems to be a real need for innovative new fruity odorants such as Anapear (16) in the future. “Salvatore Ferragamo pour homme” (S. Ferragamo, 1999) is a recent

example of a high dosage of fruity-lactonic notes, which previously were only used in such high concentrations in feminine fragrances. Grapefruit is still trendy in feminine as well as in masculine perfumes, and here the end is not yet in sight. Green perfumery materials have very diverse nuances, but the galbanum direction will continue to be important. New materials such as **46** and **47** with increased performance will open new possibilities for creation. In nature, the scent of leaves is ubiquitous and now begins to enter more and more into perfumery, with new accords such as that of tropical leaves in "Cerruti Image" (Cerruti, 1998). In the future, the family of green odors could be even further extended with vegetable odors. Marine and ozonic notes are still used, but not anymore in overdoses. However, odorants such as Calone 1951 (**84**), Tropional (**85**), Floralozone (**86**), and Florhydral (**87**) have made their way into the perfumer's palette, and are now commonly used. With the comeback of sensuality in perfumery, floral notes will probably boom in the next few years; even masculine perfumes will probably become more and more floral. Everybody is waiting for a new Hedione (**8**), not one just enriched in the powerful isomers, but a really new floral building block with different tonalities, maybe in the rosy, magnolia direction. Also Coranol (**98**) could get used in a similar way to Hedione (**8**), even though it is completely different in smell. But sensuality is not only evoked by floral but also by spicy materials, and this has been demonstrated in perfumery for decades. "L'Air du Temps" (N. Ricci) of 1948 was already composed of spicy odorants, and "Opium pour Homme" (Yves Saint Laurent, 1995) as well as "Jungle for men" (Kenzo, 1998) are some recent examples in place of many other masculine oriental-spicy perfumes. "Oscar for men" (O. de la Renta, 1999), a spicy woody perfume, shows that this spicy trend is not over yet; and new spicy odorants would give a boost to it. Likewise, new sweet and suave compounds such as Methyl Diantilis (**133**) would reinforce the sweet-spicy notes. Woody notes are clearly indispensable in perfumery, and they are well represented by synthetic odorants. Nevertheless, not all aspects of the woody family are equally well covered. Vetiver and patchouli are less common notes by far for perfumery synthetics than cedar and sandalwood notes. Therefore, perfumers are waiting for a transparent vetiver odorant, and an inexpensive patchouli-type material. Ambery compounds are well established in the perfumer's palette; thus, a real revolution is not expected in this family, unless we could really get close to the natural odor of ambergris tincture. After nitro musks, polycyclic musks, and macrocyclic musks, linear musks are now being developed. Completely new structures for musk odorants could also bear new side notes such as, for example, the anisic tonality in **246**, and this can inspire new signature accords.

However that may be, the ambition of the perfumer to create something outstanding will continue to depend on the fascination of the chemist to invent something never smelled before.

The 3D illustrations were designed by Maikel Das, Hamburg, whom we profoundly thank for his outstanding empathy and his excellent computer skills. For insights into the secrets of

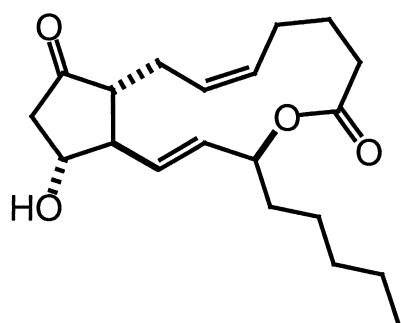
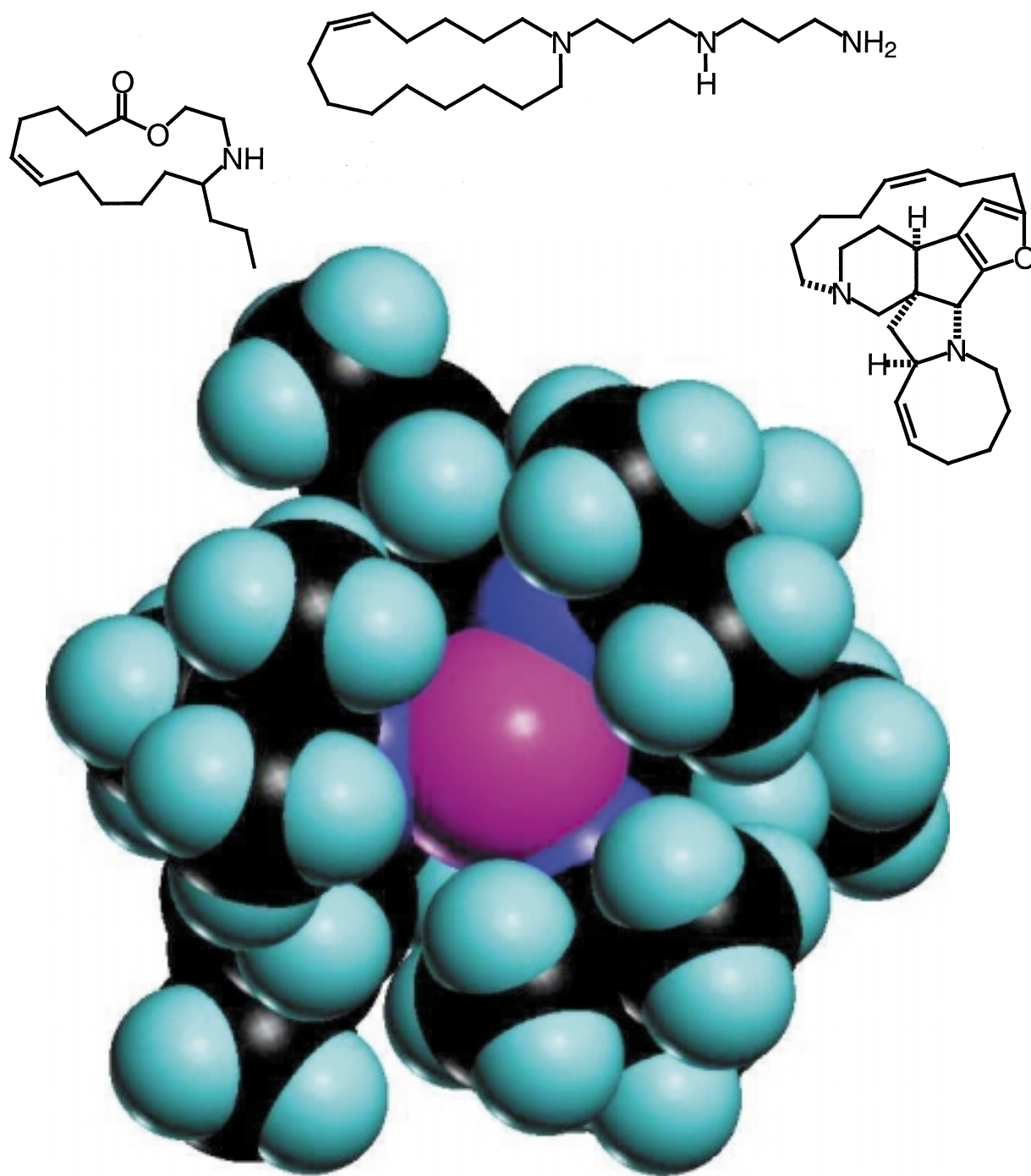
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Space-filling model of $[(\text{Ar})(t\text{Bu})\text{N}]_3\text{MoCl}$, a novel catalyst for ring-closing alkyne metathesis, and structures of some natural products formed with this emerging new methodology.

Olefin Metathesis and Beyond**

Alois Fürstner*

The advent of well-defined catalysts for olefin metathesis which combine high activity, durability, and excellent tolerance towards polar functional groups has revolutionized the field. The past decade has seen the rapid embrace of these reagents as tools for advanced organic and polymer chemistry and the success of this development is witnessed by a plethora of

elegant applications to the synthesis of natural and nonnatural products. This review article provides an overview of these developments and intends to familiarize the reader with some very recent advances which hold the promise to expand the scope of the reaction even further. Moreover, the positive impact of metathesis on the fundamental logic of retrosynthetic planning is

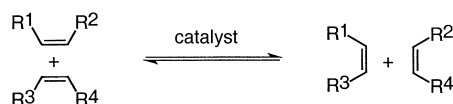
demonstrated by means of typical examples. Finally, it will be shown that metathesis is by no means restricted to alkenes as substrates, and some comments on metathesis reactions following unconventional mechanistic pathways will also be presented.

Keywords: alkenes • alkynes • carbenes • carbynes • metathesis

1. Introduction

The fundamental discovery by Karl Ziegler that catalysts formed in situ from certain transition metal salts and main group alkylating agents promote the polymerization of olefins under unprecedentedly mild conditions has had a tremendous impact on chemical research as well as on industrial production.^[1] Early on it was discovered that some “Ziegler-type” catalysts do not only lead to the addition polymerization of olefins, but can also effect a mechanistically entirely different process, that is, the mutual alkylidene exchange reaction of alkenes.^[2] This transformation, comprising the cleavage and the formation of double bonds, is nowadays generally referred to as “alkene metathesis” (Scheme 1);^[3] it was soon emancipated from its polyolefin roots and it has evolved into an independent and highly prosperous field of research.

The first generation of metathesis catalysts exhibits the characteristics of “mixed” Ziegler catalysts:^[2] Specifically,



Scheme 1. The principle of olefin metathesis.

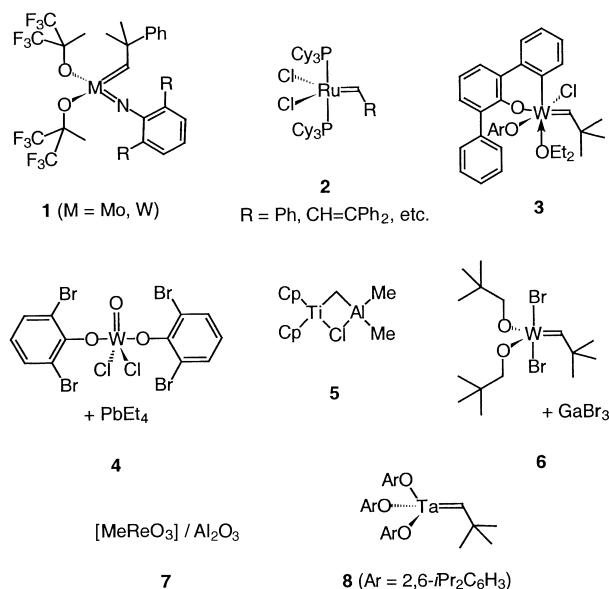
they show high activity at the expense of a poor compatibility with polar functional groups, due to a strongly Lewis-acidic and alkylating character. It is obvious that this property makes them hardly attractive for applications in advanced organic synthesis and it essentially restricted the scope of olefin metathesis to the production of unfunctionalized polymers.

Basic research in organometallic chemistry, however, has completely changed this situation. The discovery of metal alkylidene complexes,^[4] followed by the insight that some species of this type constitute well-behaved single-component catalysts for olefin metathesis, gave the essential clue for settling the long lasting debate on the actual mechanism of this transformation (“Chauvin mechanism”)^[5] and has triggered the development of a new generation of high-performance, reasonably stable, and—most importantly—exceedingly tolerant catalysts or catalyst precursors. Scheme 2 depicts some of these catalysts (or precatalysts that convert into metal alkylidenes in situ),^[6] among which the tungsten or molybdenum alkylidene complexes **1**^[7] developed by Schrock and co-workers and the ruthenium carbene complexes **2**^[8] introduced by Grubbs and co-workers are undoubtedly the most popular and versatile ones. Both reagents are now commercially available. The last decade has seen the rapid embrace of these tools by advanced organic synthesis as well as polymer chemistry, and the tremendous success of this development is documented in several excellent monographs and review articles.^[9–11]

Although one might guess that the explosive growth of this field will now slowly cease after reaching a certain level of maturity, the very recent advent of even more advanced catalysts holds the promise of unabated pace for the next few

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[**] A list of abbreviations can be found at the end of this article.



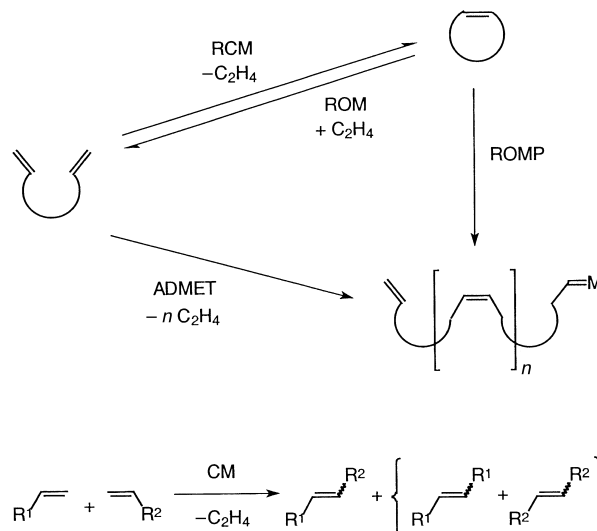
Scheme 2. Some typical metathesis catalysts and precatalysts.

years. The following review article is meant to delineate and accentuate this striking development and does not intend to provide a fully comprehensive treatise. The focus is on the advancements in catalyst design and on prototype applications illustrating the profound synthetic implications of metathesis on the logic of retrosynthetic planning. Finally, it will be shown that metathesis reactions of π systems other than olefins also bear great potential, and a short excursion into the, as yet hardly explored, “non-Chauvin world” will be undertaken. Despite the enormous relevance of metathesis for polymer chemistry, this field of applications will not be covered; the reader is referred to some authoritative review articles and monographs for further information in this respect.^[9–12]

2. Mechanism and Fundamental Types of Applications

The generally accepted mechanism of metathesis reactions (“Chauvin mechanism”)^[5] consists of a sequence of formal [2+2] cycloadditions/cycloreversions involving alkenes, metal

carbenes, and metallacyclobutane intermediates (for possible exceptions, see Section 8). Since all individual steps of the catalytic cycle are reversible, an equilibrium mixture of olefins is obtained. Therefore, it is necessary to shift this equilibrium in one direction in order to make metathesis productive in preparative terms. The major ways to do this are depicted in Scheme 3 and the mechanism of one such application, the ring-closing metathesis (RCM) of a diene, is shown in Scheme 4. In this particular case, the forward process is entropically driven because RCM cuts one substrate molecule into two products. If one of them is volatile (ethene, propene, etc.) the desired cycloalkene will accumulate in the reaction mixture.

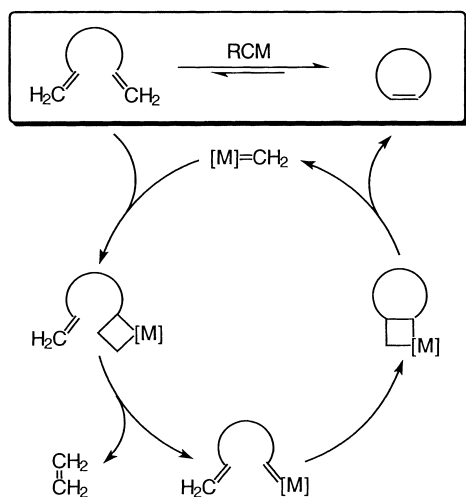


Scheme 3. Important types of metathesis reactions: RCM = ring-closing metathesis; ROM = ring-opening metathesis; ROMP = ring-opening metathesis polymerization; ADMET = acyclic diene metathesis polymerization; CM = cross-metathesis.

Another important factor for productive RCM is the sensitivity of most metathesis catalysts to the substitution pattern of the olefin as this constitutes a kinetic obstacle for the retroreaction. This argument, however, does not apply to strained cycloalkenes because the release of ring strain provides a formidable driving force for ring-opening metathesis (ROM) or ring-opening metathesis polymerization



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Scheme 4. Basic catalytic cycle of RCM. Although not specifically shown in the scheme, all individual steps involved and, as a consequence, the overall transformation, are reversible; see text.

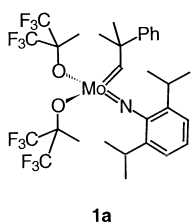
(ROMP).^[12] Finally, it should be mentioned that the intrinsic competition between RCM and acyclic diene metathesis polymerization (ADMET)^[12c] can be controlled to some extent by adjusting the dilution of the reaction mixture and is also strongly influenced by preexisting conformational constraints in the substrate.

As metathesis necessarily converts one alkene into a new one, this reaction is predisposed for sequential transformations. Particularly attractive are domino processes incorporating different metathesis events (for example, RCM/ROM/CM or RCM/ROM/RCM) as they provide a tremendous increase in molecular complexity in a single catalytic and atom-economical step. Likewise, combinations of metathesis and other transformations of alkenes, such as Diels–Alder, Heck, Cope, or ene reactions, can be envisaged and hold an as yet largely unexplored, preparative potential. Some pioneering studies along these lines which feature the strategic advantages of such “orchestrated” maneuvers are included in different sections of this article.

3. Recent Advances in Catalyst Design

3.1. Molybdenum-Based Catalysts and Asymmetric Metathesis Reactions

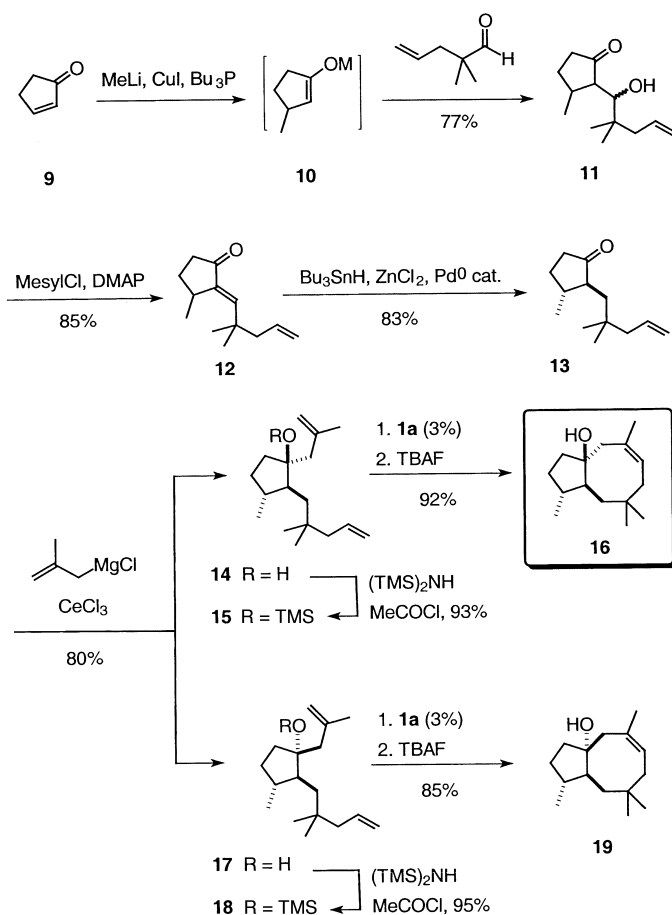
Schrock's tetracoordinated alkylidene species of the general formula $[M(=CHCMe_2Ph)(=NAr)(OR)_2]$ ($M = Mo, W$) with bulky substituents Ar and R on the imido and alkoxide ligands constitute potent and well behaved metathesis (pre)-catalysts which have been thoroughly studied from the mechanistic point of view.^[7] Among them, compound **1a** turned out to be particularly active and is now commercially available. This and related complexes are quite sensitive toward oxygen and moisture and must be handled in rigorously dried solvents using Schlenk techniques. Their superb reactivity,



1a

however, compensates for this inconvenience and renders **1a** a calibration point for all studies on metathesis reactions.

As the applications of **1a** in organic synthesis and polymer chemistry have recently been authoritatively reviewed,^[13] a single example may suffice to illustrate this point. For a long time **1a** has been the only metathesis catalyst allowing the formation of tri- and even tetrasubstituted double bonds by RCM (only recently, a ruthenium-based system has been launched which allows to access these products as well; this development is summarized in Section 3.2.2).^[14] Scheme 5 depicts a total synthesis of the terpene natural product

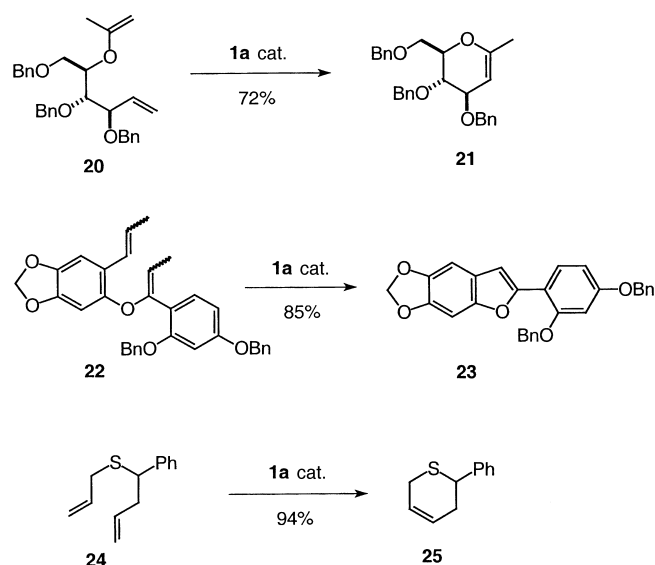


Scheme 5. Synthesis of dactylol (**16**) with RCM as the key step.

dactylol (**16**) based on RCM as the key step.^[15] A three-component coupling triggered by addition of “MeCu” to cyclopentenone and subsequent alkylation of the resulting enolate **10** with 2,2-dimethyl-4-pentenal affords aldol **11** which is elaborated into the *trans*-disubstituted ketone **13** by standard means. Addition of methallylcerium dichloride provides a mixture of tertiary alcohols **14** and **17** which, after separation and O silylation, readily cyclize on exposure to catalyst **1a** and provide the targeted terpene **16** and its epimer **19**, respectively, upon desilylation of the crude product. Attempted ring closure of diene **15** with the ruthenium carbene **2** failed completely. This comparison illustrates the superior activity of **1a** in the preparation of tri- or tetrasubstituted products and shows that RCM can even be used for the synthesis of rather strained medium-sized rings if the

precursor is conformationally predisposed for ring closure (see also Ref. [161]). All previous syntheses of dactyol following more conventional routes are lengthy and very low yielding (9–20 steps with overall yields <0.1%). Therefore, this application (6 synthetic operations, 17% yield overall for **16** and 19% for epimer **19**)^[15] gives a first impression of the advantages of metathesis in advanced organic synthesis.

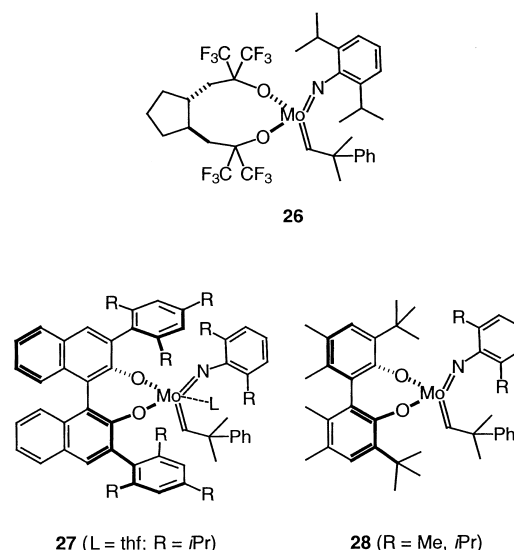
Additional advantages of the molybdenum complex **1a** reside in its tolerance towards certain functional groups which inhibit ruthenium-based metathesis catalysts. A mismatch of the “hard” Mo^{VI} center with “soft” sulfur or phosphine functionalities can explain why substrates such as **24** (Scheme 6) cyclize smoothly in the presence of **1a** but usually



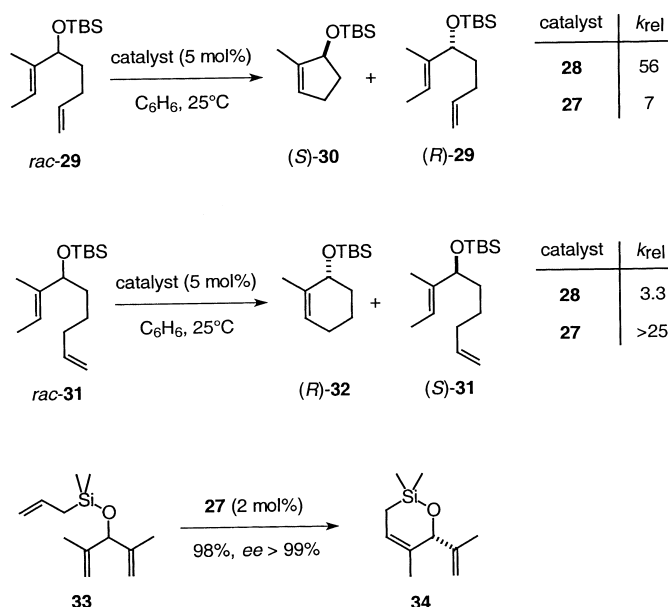
Scheme 6. RCM reactions which are only successful with the Schrock catalyst **1a**.

fail to react in the presence of the ruthenium carbene **2**.^[16] Furthermore, catalyst **1a** is hardly affected by the electronic properties of the olefinic substrates and reacts even with electron-rich olefins (for example, enol ethers) and electron-poor olefins (such as acrylates, acrylonitrile).^[17] Some examples are depicted in Scheme 6. Once again, the parent ruthenium carbene **2** is mostly unreactive towards these substrates,^[18] but newly developed heteroleptic ruthenium species are now starting to compete with Schrock's complex **1a** in this particular field of applications as well (see Section 3.2.2).

In addition to the pronounced reactivity, the modular character of Schrock's molybdenum alkylidene complexes constitutes a particularly favorable feature. Specifically, various alkoxides can be readily introduced into the ligand sphere of the Mo center, including chiral scaffolds. This paves the way for different kinds of asymmetric metathesis reactions, such as asymmetric ring-closing metathesis (ARCM) or asymmetric ring-opening/cross metathesis (CM) cascades. Proof of principle has been achieved with catalyst **26** which contains an elaborate cyclic mimic of the OCMc(CF₃)₂ units of the parent compound **1a**.^[19] Only recently, however,

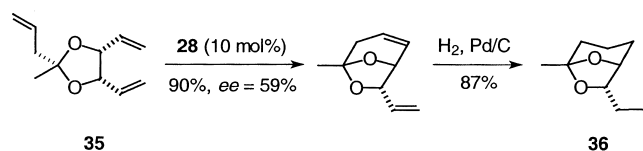


excellent levels of asymmetric induction have been reached with molybdenum complexes **27** and **28** carrying substituted BINOL- or BIPHEN-derived ligands.^[20] Although it is too early to draw a fully comprehensive picture, the data presently available indicate that BIPHEN-derived species **28** are the catalysts of choice for the asymmetric formation of five-membered rings by ARCM, whereas the BINOL-derived analogues **27** give superior results in case of six-membered products.^[20] However, the substitution pattern of the substrate must also be taken into account when choosing the best catalyst. Representative examples are compiled in Scheme 7.



Scheme 7. Examples for the application of **27** and **28**.

A synthesis of (+)-*endo*-brevicomine (**36**) based upon the desymmetrization of *meso*-triene **35** by means of complex **28** constitutes the first incorporation of this emerging new technology into target-oriented synthesis (Scheme 8).^[21]



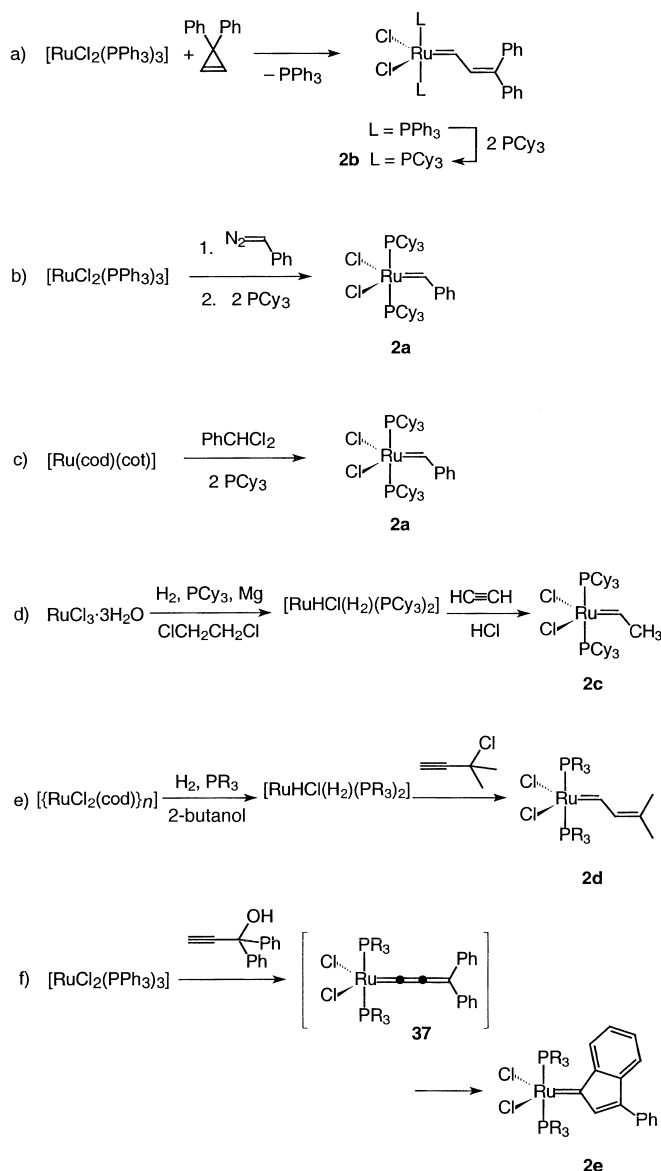
Scheme 8. Desymmetrization of the *meso* triene **35** through RCM in the synthesis of (+)-*endo*-brevicomine (**36**).

3.2. Ruthenium-Based Catalysts

3.2.1. Variations on a Theme by Grubbs

The disclosure of Grubbs that ruthenium carbene complexes of the general type **2** are highly active single component (pre)catalysts for all types of alkene metathesis reactions denoted a real breakthrough and has triggered an avalanche of interest in this transformation.^[8] Although their activity is usually lower than that of Schrock's molybdenum alkylidene **1a**, the spectacular tolerance of these "late" transition metal compounds toward an array of functional groups and the ease of handling caused by a reasonable stability against oxygen, water, and minor impurities in the solvents render them exceedingly practical tools and explain their unrivaled popularity in the organic and polymer chemists communities. Many elegant applications to the synthesis of complex target molecules highlight their truly remarkable scope. A wealth of information has accumulated which is summarized in several review articles and will therefore not be duplicated here.^[9–11]

The rather cumbersome preparation of the diphenylvinylcarbene complex **2b** ($R = CH=CPh_2$), the first compound of this series historically, constituted a certain handicap. This catalyst is formed by an Ru^{II} -induced rearrangement of diphenylcyclopropene (Scheme 9a).^[8a,b] Since the substituent R at the carbene unit of **2** itself, however, is irrelevant in many catalytic cycles,^[22, 23] several carbene sources other than diphenylcyclopropene have been investigated in order to develop more convenient entries into this family of ruthenium compounds. The most important procedures described so far are summarized in Scheme 9. A significant improvement was achieved by the use of diazoalkenes instead of diphenylcyclopropene,^[8c,e] although the hazardous nature of these reagents must be taken into account (Scheme 9b). Complex **2a** ($R = Ph$) thus obtained is now commercially available and has, therefore, found numerous applications. Other practical preparations that can be performed on industrially relevant scales either use *gem*-dihalides (Scheme 9c),^[24] alkynes (Scheme 9d),^[25] or propargyl chlorides (Scheme 9e)^[26] as the actual carbene sources. Yet another convenient method is the treatment of commercially available $[RuCl_2(PPh_3)_3]$ with diphenylpropargyl alcohol (Scheme 9f) to deliver phenylindenyl complex **2e** (rather than the allenylidene species **37** which was originally assumed to be the stable product of this reaction).^[27] A detailed assessment of **2e** has shown that this complex is as good as or even slightly better than the

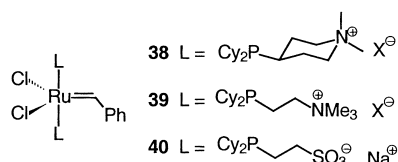


Scheme 9. Synthesis of some Grubbs-type catalysts.

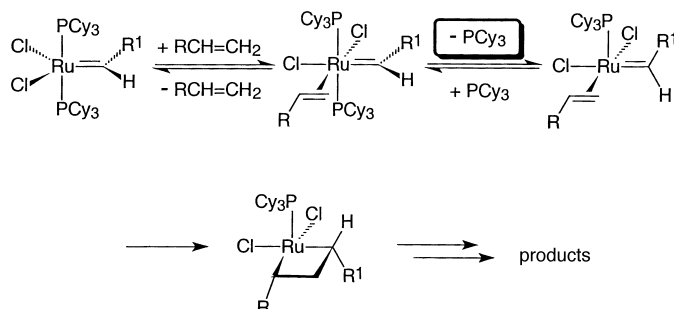
"standard" phenylcarbene **2a** in RCM (for an example, see Section 5.6).^[28]

The influence of the ligands on the catalytic activity of 5-coordinate, 16-electron ruthenium complexes of type **2** has been systematically studied.^[8, 29] With regard to the anionic substituent X , chloride seems to be optimal. The effect of this rather electron-withdrawing group, however, must be counterbalanced by electron-donating phosphines with large cone angles, such as PCy_3 or $P(cyclopentyl)_3$. Only a few other "designer" phosphines give similarly high activities.^[30] Among them, bulky aliphatic phosphines incorporating either a quarternary ammonium or a sulfonate group are most remarkable.^[31] The resulting catalysts **38–40** allow RCM of unprotected substrates in water or MeOH and enable emulsion polymerizations of appropriate substrates by ROMP in biphasic reaction media.

Detailed mechanistic studies allow the rationalization of the effect of the phosphines on the catalytic activity. It has been shown that the dominant pathway for productive



metathesis, which accounts for approximately 95% of the catalyst turnover in a typical RCM reaction, involves the dissociation of one of the two PCy_3 ligands from **2a**,^[32] although it is not clear whether this dissociation occurs before or after the coordination of the alkene substrate (Scheme 10). The bulky and electron-donating character of the residual

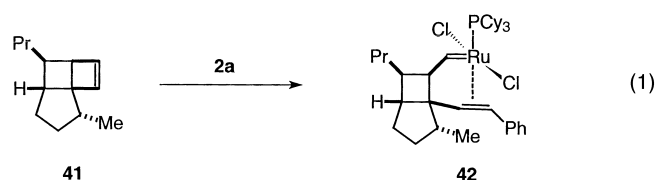


Scheme 10. Proposed dissociative mechanism for Grubbs-type catalysts.^[32]

phosphine is decisive for stabilizing the reactive intermediates formed during this dissociative step. The alternative path in which both phosphines remain attached to the Ru center, though operative, is much less efficient. The olefin binding site is presumably *cis* to the carbene and *trans* to one of the chlorides and the subsequent formation of the metallacycle is believed to be rate determining.^[32]

Recent investigations corroborate this picture, which was originally mainly based on the analysis of kinetic data. Supportive evidence comes from the inspection of reactive intermediates by electrospray-tandem-MS,^[33] from theoretical studies of the reaction coordinate,^[34] as well as from the following preparative results:

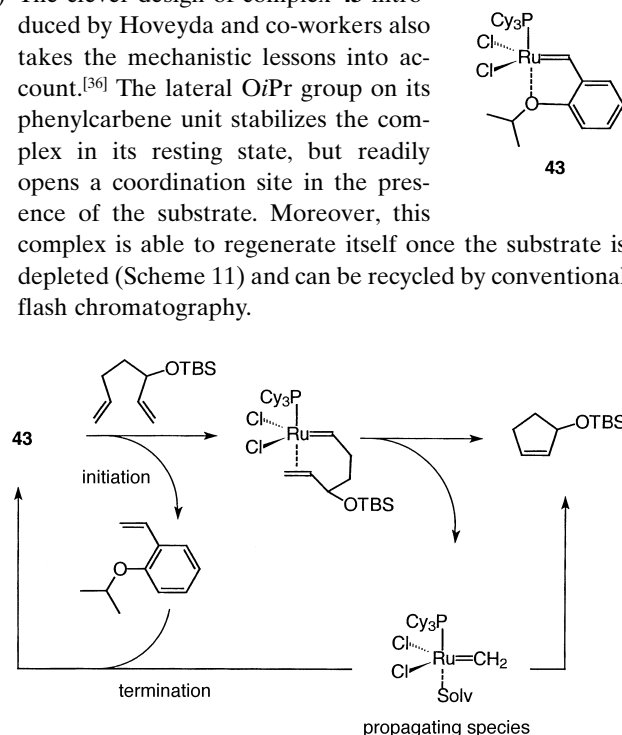
- 1) Snapper and co-workers were able to isolate and characterize a ruthenium catalyst “caught in the act”.^[35] Treatment of carbene **2a** with the strained olefin **41** provides complex **42** in which the tethered alkene moiety has replaced one of the PCy_3 groups. The authors have shown beyond doubt that **42** is a catalytically relevant species rather than a dead-end by-product [Eq. (1)].



- 2) Additives, such as HCl (DCl) or Cu^I salts, that are able to abstract PCy_3 from the precatalyst or to intercept free PCy_3 in solution greatly enhance the reaction rate at the expense of the lifetime of the active species because they

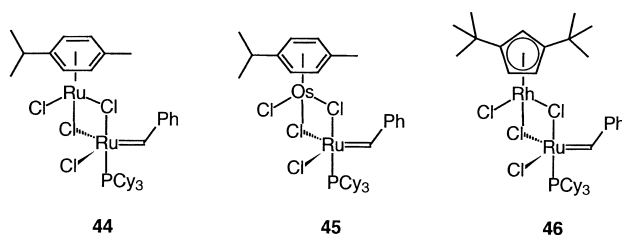
increase the concentration of reactive, but fragile, mono-phosphine metal fragments in solution.^[32]

- 3) The clever design of complex **43** introduced by Hoveyda and co-workers also takes the mechanistic lessons into account.^[36] The lateral *OiPr* group on its phenylcarbene unit stabilizes the complex in its resting state, but readily opens a coordination site in the presence of the substrate. Moreover, this complex is able to regenerate itself once the substrate is depleted (Scheme 11) and can be recycled by conventional flash chromatography.

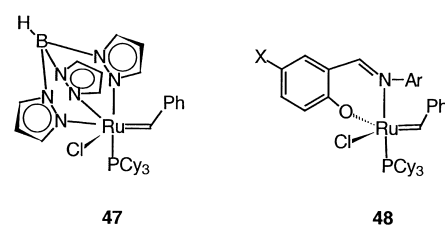


Scheme 11. Example of an RCM reaction catalyzed by complex **43**. Once the substrate is depleted, the active species is recaptured by $i\text{PrOC}_6\text{H}_4\text{CH}=\text{CH}_2$ and precatalyst **43** is, thereby, regenerated.

- 4) Similarly, replacement of one PCy_3 unit in the parent complex **2a** by a coordinatively labile metal fragment results in enhanced activity. This holds true, for example, for the dinuclear complexes **44–46**.^[37] Once again, however, the lifespan of the catalyst in solution is relatively small because the metal fragment does not reassociate and, therefore, cannot protect the intermediates against decomposition.



- 5) In contrast, all strongly chelating entities that dissociate reluctantly from the Ru center enhance thermal stability but lower the performance. This holds true for complexes **47** and **48** which bear either tris(pyrazolyl)borate or



tethered Schiff base ligands.^[38] While **48** shows appreciable activity at elevated temperatures, compound **47** is kinetically inert and cannot serve as a metathesis catalyst.

Further mechanistic studies have revealed the major decomposition pathways of complexes of type **2**.^[39] Thus, substituted carbenes ($R \neq H$) mainly decompose through bimolecular processes, whereas the methylidene complex ($R = H$)^[22] that is responsible for 95 % of the turnover in RCM experiments decays unimolecularly. This dichotomy explains firstly, why high catalyst loadings are necessary for productive RCM of substrates with low intrinsic bias towards ring closure, and secondly, why ligands that accelerate ring closure as well as decomposition have no major preparative advantages.

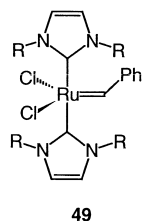
Despite these important mechanistic lessons, not all intimate details of metathesis reactions catalyzed by **2** are clear yet. This refers, for example, to a recent publication showing that structurally unknown radical intermediates may intervene at some stage during the catalytic cycle.^[40] The propensity of complex **2** to trigger radical processes such as Kharasch additions of chloroform to alkenes or the radical polymerization of methyl methacrylate is well documented in the literature.^[41]

3.2.2. Ruthenium Complexes Containing N-Heterocyclic Carbene Ligands: Catalysts of Extended Scope and Increased Reactivity

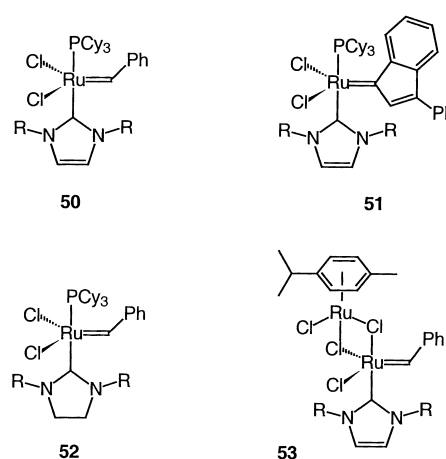
As outlined above, the steric and electronic properties of the residual neutral ligand in the catalytically relevant intermediates formed by the dissociative mechanism shown in Scheme 10 are decisive for the performance of the catalyst. They should be even more basic and sterically demanding than PCy_3 in order to increase its lifetime and reactivity. “Stable” N-heterocyclic carbenes (NHC) fall into this category.^[42]

Herrmann and co-workers were the first to report on their use in the context of metathesis.^[43] Complex **49**, in which both PCy_3 units of **2a** have been replaced by *N,N'*-disubstituted 2,3-dihydro-1*H*-imidazol-2-ylidene units, however, is rather stable but does not show an improved activity profile. This is mechanistically reassuring since the “sticky” NHC ligands render the dissociative pathway less likely and, therefore, result in a low concentration of the catalytically active ruthenium template in solution.

The use of one kinetically inert, electron-donating NHC ligand in combination with a coordinatively labile ligand on the other hand, however, should result in the desired synergetic effect. This idea was independently pursued by three different research groups which have almost simultaneously reported on the preparation and the catalytic properties of heteroleptic complexes such as **50–53**.^[44–46] They differ 1) in the particular NHC ligand chosen, which can either be “unsaturated” or “saturated” (compare **50** with **52**), 2) in the substituents *R* on the *N* atoms, 3) in the complementary labile ligand, and 4) in the alkylidene fragment which does not necessarily have to be $=CHPh$ (see compound **51**).



49



50

51

52

53

The results of solution calorimetry indicate that the NHC ligand with *R* = mesityl has a significantly stronger bonding (by approximately 5 kcal mol^{−1}) to ruthenium than PCy_3 .^[44] Furthermore, X-ray analysis shows that by virtue of the short metal–NHC bond length the bulky ligand is brought close to the metal center which is, therefore, effectively shielded against bimolecular decomposition. The Ru–NHC bond is formally best represented as a “single” bond since the ligand merely acts as a strong σ donor and has almost no π acceptor properties; rotation, however, is largely restricted for steric reasons.^[43, 44, 46b]

Although it is too early to give a fully comprehensive analysis of the catalytic properties of these “second-generation” ruthenium carbene complexes, the data presently available make clear that they open new vistas for preparative chemistry. Most importantly, their activity is significantly higher than that of the parent Grubbs carbene **2a** and comes close to or even surpasses that of Schrock’s molybdenum alkylidene complex **1a** discussed in Section 3.1. In combination with the exceptional thermal stability and the resistance toward oxygen and moisture, as well as the compatibility with many functional groups, these complexes are exceedingly useful tools for the practitioner and may well define new standards in this field.

The following examples from the recent literature substantiate this claim. One of the most notable features of these heteroleptic complexes is the ease of formation of tri- and even tetrasubstituted double bonds in RCM and CM reactions.^[45, 46a, 47] Representative examples, including bicyclic compounds formed by annulation reactions, are displayed in Table 1. Note that products of this type have been beyond reach of **2a** and could previously only be made using molybdenum alkylidene **1a**. Similarly, acrylates constitute problematic substrates for the parent ruthenium carbene **2a** but were found to cyclize smoothly in the presence of complex **50**, even if ring closure was additionally hampered by a high degree of substitution.^[45d, 47]

Another informative example is the formation of the conduritol derivative **55** shown in [Eq. (2)] and Table 2.^[48] Attempted cyclization of diene **54** derived from D-glucose with the parent Grubbs carbene **2a** turned out to be rather difficult; high concentrations of the catalyst and unacceptably long reaction times were required in order to reach complete

Table 1. RCM reactions catalyzed by ruthenium complexes with NHC ligands.^[a]

Product	Cat.	R	Yield [%]	Ref.
	50	Mesityl	96	[47]
	50	CHMePh	80	[46b]
	51	Mesityl	97	[47]
	53	C ₆ H ₁₁	63	[46b]
	50	CHMePh	96	[46b]
	51	Mesityl	89	[47]
	53	C ₆ H ₁₁	88	[46b]
	50	Mesityl	98	[47]
	51	Mesityl	71	[47]
	53	C ₆ H ₁₁	65	[46b]
	50	Mesityl	95	[47]
	50	Mesityl	92	[47]
	50	Mesityl	63	[47]
	50	Mesityl	89	[47]

[a] (E = COOEt).

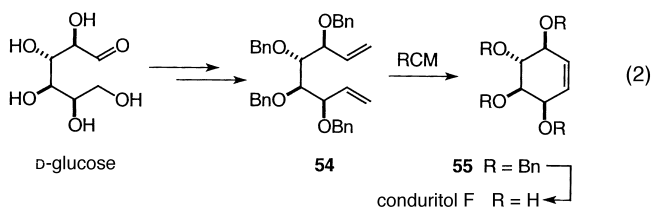


Table 2. Comparison of the reactivity of different metathesis catalysts in the RCM reaction shown in Equation (2).

Catalyst	Reaction time [h]	Yield of 55 [%]
2a	60	32 (GC)
1a	1	92
50 (R = mesityl)	2	89

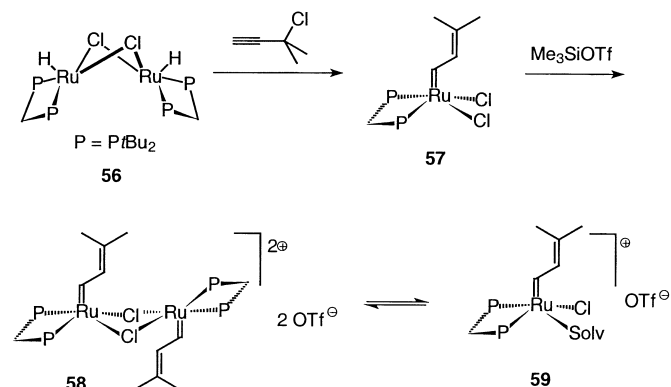
conversion.^[48, 49] The second entry shows the significantly higher reactivity of Schrock's catalyst **1a** which depletes the substrate within one hour and gives the desired cyclohexene derivative in excellent yield.^[48] As can be seen from the third entry, however, the fairly air-stable ruthenium complex **50** is similarly effective. This outcome illustrates the big increase in reactivity engendered by the NHC ligand and pinpoints the notion that these new heteroleptic complexes are most practical alternatives to the sensitive molybdenum catalyst **1a**.

Finally, it should be pointed out that the reactivity of **50** can be tuned to some extent by the proper choice of the

substituents R on nitrogen as well as by the reaction medium,^[47] although this cooperative effect is not yet well understood. Moreover, complexes such as **52** containing “saturated” Wanzlick-type HNC ligands seem to be even more reactive than their “unsaturated” Arduengo-type analogues **50**.^[45b,c] This can be explained by an increased basicity of the former, in which the carbene center is less stabilized. These ligands also allow incorporation of chirality in their carbon backbone as well as in the *N*-substituents and may, therefore, become useful for asymmetric metathesis reactions as well.

3.2.3. A Cationic Scenario: Ruthenium Carbene Complexes Activated by Chloride rather than Phosphine Abstraction

As summarized above, dissociation of one of the two neutral ligands from precatalysts of the Grubbs-type is believed to open the productive pathway for metathesis. Recent studies from Hofmann and co-workers show, however, that other options can also be pursued. Thus, exposure of $[\{\text{Ru}(\text{cod})\text{Cl}_2\}_n]$ to bis(di-*tert*-butylphosphino)methane (dtbpm) and Et_3N under an atmosphere of H_2 delivers the dinuclear hydridocomplex **56** which reacts with dimethylpropargyl chloride to give the monomeric carbene complex **57** in which the phosphine ligands are *cis* rather than *trans* to one another (Scheme 12).^[50] Although these neutral complexes

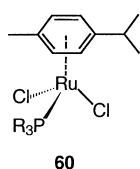


Scheme 12. Formation of the neutral carbene complex **57**, which can be further transformed into the cationic complex **59**.

themselves initiate ROMP of suitable cycloalkene substrates, they convert into much more active species upon treatment with TMSOTf.^[51] The irreversible formation of TMSCl forces the generation of cationic intermediates **58**. The latter are dimeric in the solid state, but the association is readily reversible in solution as shown by NMR spectroscopy (Scheme 12). The resulting monomeric and cationic carbenes **59**, which bear a bulky neutral chelate ligand but only one chloride in the coordination sphere of Ru, are very active catalysts for ROMP and effect RCM of 1,7-octadiene even at -40°C .^[51] A full assessment of their preparative potential, however, is still pending.

3.2.4. Other Ruthenium-Based Metathesis Catalysts

Despite the tremendous success of the Grubbs carbenes and derivatives thereof, the recent literature also documents a



search for alternative metathesis catalysts of comparable performance and scope.^[52] Among them, the mononuclear 18-electron complex $[(\eta^6\text{-cymene})\text{RuCl}_2(\text{PR}_3)]$ (**60**) deserves particular mention. Although this complex per se exhibits only moderate metathesis activity ($\text{R} = \text{Cy} > i\text{Pr} \gg \text{Ph}$), it constitutes a versatile and convenient precatalyst that can be activated by various means:

- 1) In the presence of catalytic amounts of trimethylsilyldiazomethane or diazoacetate, complex **60** efficiently catalyzes ROMP of cyclooctene and other cyclic monomers. The polymers formed have high molecular weight and low polydispersities.^[53] Esters, ethers, epoxides, and alkyl bromides turned out to be compatible with this system. Applications to RCM, however, have not yet been reported.
- 2) Compound **60**, as well as its osmium analogue, respond to photochemical activation. Irradiation with a UV lamp (200 W Hg lamp) leads to very active systems for the technically important polymerization of dicyclopentadiene ("Photo-ROMP"), most likely with decoordination of the arene ligand as the triggering event.^[54] Since then, it has been found that no special photochemical equipment is necessary for the activation of **60**. Thus, heating of a solution of this complex and a suitable diene substrate results in clean and high yielding RCM provided that the reaction is exposed to neon light (common neon tubes) or strong daylight.^[55] Although cyclization proceeds more slowly than with the Grubbs carbene **2a**, the yields obtained and the tolerance towards functional groups are comparable, as can be seen from the results compiled in Table 3. Since **60** can be conveniently formed in situ from the commercially available dimer $[(\eta^6\text{-cymene})\text{RuCl}_2]_2$ and PCy_3 , this "low-tech" procedure is highly user-friendly and deserves consideration in advanced organic synthesis.
- 3) Reaction of **60** with propargyl alcohols in the presence of a suitable chloride trap results in the formation of cationic ruthenium allenylidene complexes such as **61** or **63**, which are active metathesis catalysts (Scheme 13).^[56] Systematic variations of their basic structural motif have shown that the phosphine plays the most important role ($\text{PCy}_3 > \text{P}i\text{Pr}_3 \gg \text{PPh}_3$), but the escorting counterion X is also

involved in determining the rate and selectivity of the reaction, with $\text{X} = \text{OTf}$ being the most appropriate choice. Prototype cyclizations effected by **61** and a comparison with other metathesis catalysts are given in Table 3, which illustrate the wide scope and excellent overall application profile of these allenylidene catalysts.

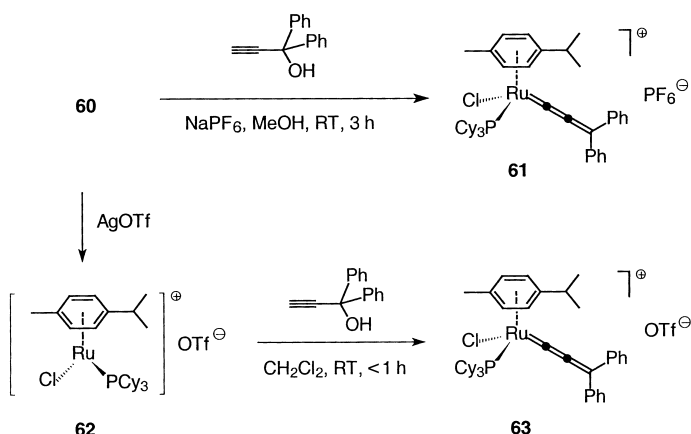
Table 3. Comparison of the reactivity of different ruthenium-based metathesis catalysts in typical RCM reactions.

Product	Yield [%]		
	2a	60/hv	61
	93	90	83
	–	78	86
	68	77	75
	76	72	66
	86	65	40
	79	80	90
	77	70	85

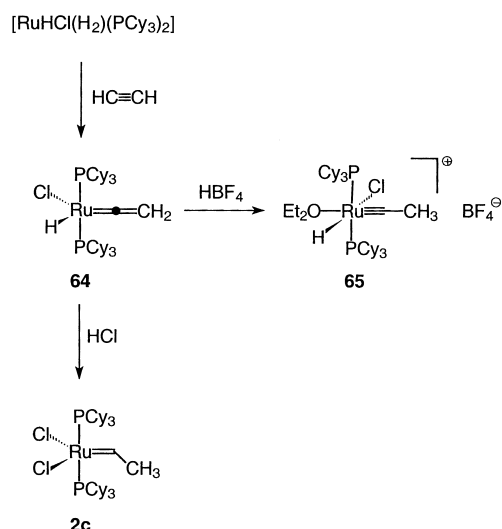
Another interesting system is the cationic ruthenium alkylidene complex **65** described by Werner and co-workers.^[57] This rather unstable complex is formed by protonation of the hydridovinylidene species **64** with HBF_4 and was found to effect ROMP of cyclooctene approximately 20 times faster than the standard Grubbs carbene **2a** does. (Note that protonation of **64** with HCl instead of HBF_4 leads to standard Grubbs-type carbene complex **2c**, see Scheme 14.) Although electron deficient alkenes in general are poor substrates for ruthenium-based metathesis catalysts (for exceptions, see Section 3.2.2.), **65** allows efficient ROM/CM between cyclopentene and methyl acrylate to form acyclic, unsaturated esters.^[57]

3.3. Development of New Metathesis Catalysts Based on Other Transition Metals

The development of the Tebbe reagent **5** (Scheme 2) was an early landmark which shows the link between metathesis and

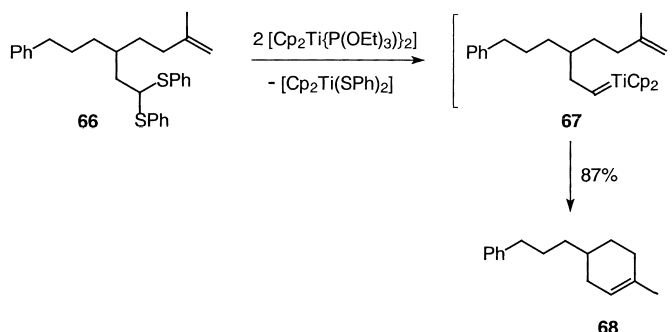


Scheme 13. Formation of the metathesis catalysts **61** and **63**.



Scheme 14. The choice of the proton source is important for the synthesis of **65**.

carbonyl olefination.^[6c, 58] This reagent has seen a renaissance in preparative organic chemistry in recent years (for an example, see Section 5.3). Quite interestingly, a second titanium-based procedure for metathesis has been disclosed which generates metal carbene intermediates from dithioacetals and low-valent titanium reagents $[\text{Ti}]$, formed in situ from $[\text{Cp}_2\text{TiCl}_2]$, $\text{P}(\text{OEt})_3$, and Mg (Scheme 15).^[59] Although this methodology requires (over)stoichiometric amounts of $[\text{Ti}]$, it widens the set of substrates amenable to metathesis beyond simple alkenes.



Scheme 15. Olefin metathesis with a titanium reagent.

4. General Preparative Aspects

4.1. Purification of the Reaction Mixtures

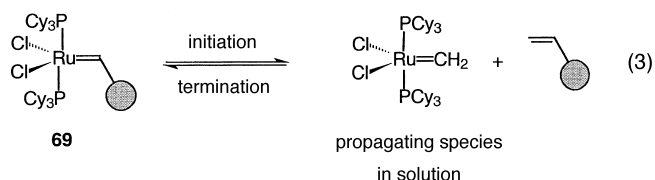
Standard work-up of a metathesis reaction followed by purification of the crude material with flash chromatography, recrystallization, distillation, etc. usually affords pure compounds which may, however, still contain traces of metal salts derived from the catalyst. The latter are problematic in subsequent transformations or in pharmacological studies of the products. Therefore, Grubbs has recently developed an improved work-up procedure which is particularly useful for reactions on a larger scale.^[60] It comprises the addition of excess tris(hydroxymethyl)phosphine to the crude mixture.

By virtue of the polar character of this commercially available ligand, the resulting ruthenium complexes can either be extracted during a standard aqueous work-up, or can be deposited on silica and then filtered off. This simple procedure reduces the ruthenium impurities in the products by factors of 10–100.^[60]

4.2. Recovery and Immobilization of the Catalyst

Several attempts have been made to immobilize Grubbs-type catalysts on solid supports to facilitate the work-up and to make this reaction even more attractive for industrial applications. An obvious possibility uses moderately cross-linked polystyrene that is functionalized with PCy_2 units. Stirring of a solution of **2a** with such a polymer leads to its immobilization through ligand exchange.^[61] The loaded polymer, however, was found to be at least two orders of magnitude less active than homogeneous solutions of **2a** and leaching of the active metal template from the polymer matrix could not be suppressed.

Another innovative possibility was explored by Barrett and co-workers who used the carbene rather than the phosphine as the anchor group [Eq. (3)].^[62] The carbene substituent of



the immobilized precatalyst **69** is cleaved off during the first turn of the catalytic cycle and the ruthenium template is, thereby, released from the polymer and acts as a homogeneous catalyst; it is, however, recaptured by the resin once the substrate in solution has been consumed. The authors have coined the expression “boomerang catalyst” which nicely describes this overall behavior.^[62] Since unimolecular decomposition of the active methyldene complex **2** in solution cannot be avoided, however, the activity of the polymer catalyst becomes insufficient after a few runs. It is obvious that replacement of the “standard” Grubbs carbene entity by more highly stabilized ruthenium catalysts (for example, the new generation of NHC-containing complexes) may significantly upgrade this creative design in the future.

4.3. Unconventional Media: Metathesis in Supercritical CO_2

Yet another way to achieve easy separation of the products from the catalyst is the use of supercritical CO_2 (scCO_2) as the reaction medium. Both, the molybdenum alkylidene complex **1a** and the ruthenium carbenes **2** turned out to catalyze RCM, ADMET, or ROMP in this environmentally benign medium.^[63] It is interesting to note that the course of the reaction strongly depends on the density of scCO_2 . Thus, it was found

that diene **70** cyclizes selectively to the musk-odored lactone **71** at densities of $\rho \geq 0.65 \text{ g cm}^{-3}$, whereas ADMET dominates below this threshold ([Eq. (4)] and Figure 1). In addition, product **71** is removed when the autoclave is vented, by virtue

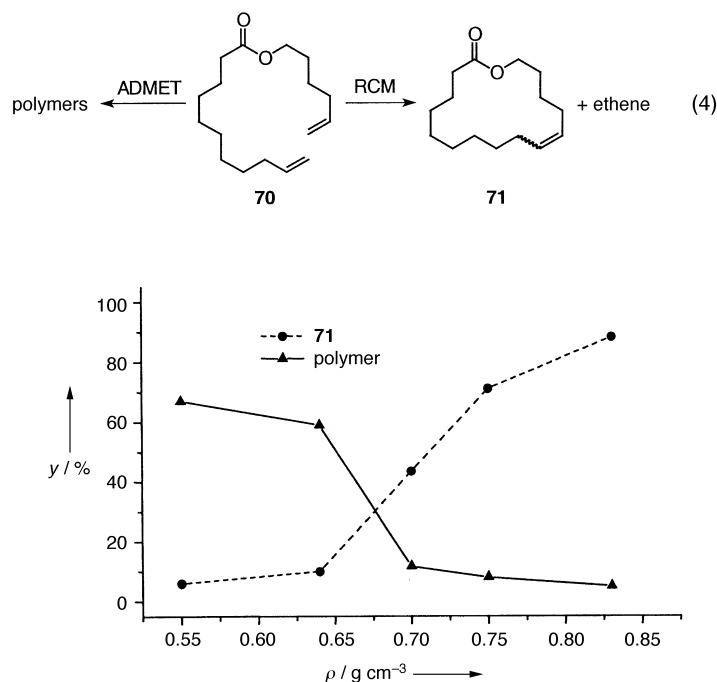


Figure 1. Effect of the density (ρ) of scCO_2 on the outcome of the reaction depicted in Equation (4).

of the extractive properties of scCO_2 . The product can be collected in an appropriate trap; the metal residue remaining in the reactor was found to be still active and can be directly used in the next run.^[63] This easy separation technique may upgrade the industrial application profile of metathesis and, hence, render this transformation useful for fine chemical production.

Finally, it should be mentioned that scCO_2 can also be used as a “protective medium” for certain functional groups.^[63] Thus, unprotected amines can be used as substrates due to the reversible formation of the corresponding carbaminic acid, whereas they must be protected if RCM is carried out in conventional organic solvents in order to avoid deactivation of the ruthenium catalyst.

4.4. Parallel Screening of Metathesis Catalysts

The impact of combinatorial chemistry on the advancement of life sciences inspires many attempts to exploit this technique in the context of catalysis as well. The successful examples reported to date highlight the crucial role of analyt-

ical techniques that allow rapid and parallel screening of potential catalysts. In view of the large number of metathesis-active complexes published during the last few years and the prospect that many still remain to be discovered, methods allowing a semiquantitative assessment in a parallel mode are called for.

An exploratory study has recently shown that high precision IR-thermography is a useful tool in this context.^[64] Catalytic activity is identified by heat uptake from the medium as monitored by the appearance of “cold spots”. It is likely that the heat of vaporization of the gaseous by-product ethylene (or other volatile alkenes) formed upon productive RCM plays a pivotal role, since RCM itself is only slightly endothermic or even thermoneutral. Control experiments and comparison with preparative data have unequivocally shown that this method constitutes a rapid, convenient, and reliable way to 1) assess the relative rate of initiation of RCM by different precatalysts, and 2) determine the inherent reactivity of various substrates towards a given metal complex. Figure 2 depicts a typical example of the time-resolved imaging of RCM of 1,7-octadiene catalyzed by four different ruthenium complexes.^[64]

4.5. Metathesis on Solid Supports and Applications to Combinatorial Chemistry

Organic chemistry on solid supports has gained tremendous importance during the last decade, mainly driven by the needs of pharmaceutical sciences. Metathesis was soon recognized as a useful technique in this context because of the robust and tolerant nature of the available catalysts.

The conceptually most simple application makes use of alkenes as covalent linkers for solid-phase synthesis. An illustrative example (Scheme 16) deals with oligosaccharide synthesis in which the first sugar unit is attached to the

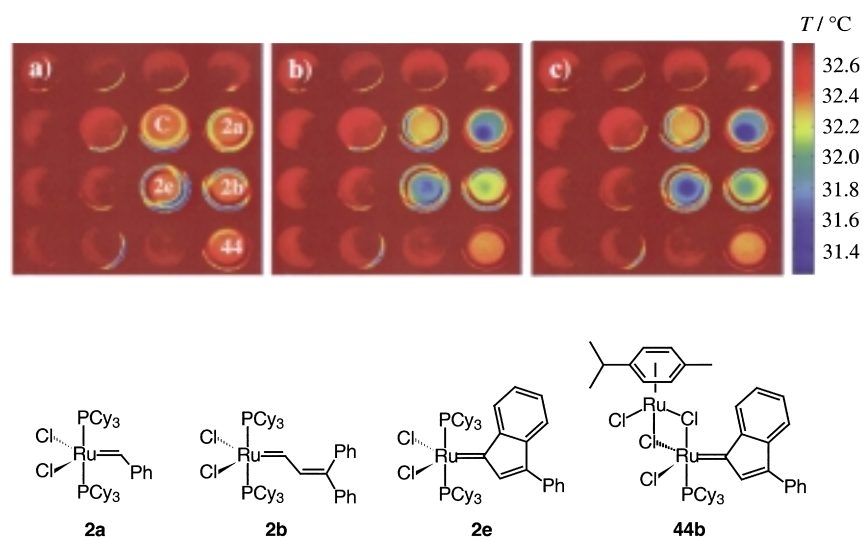
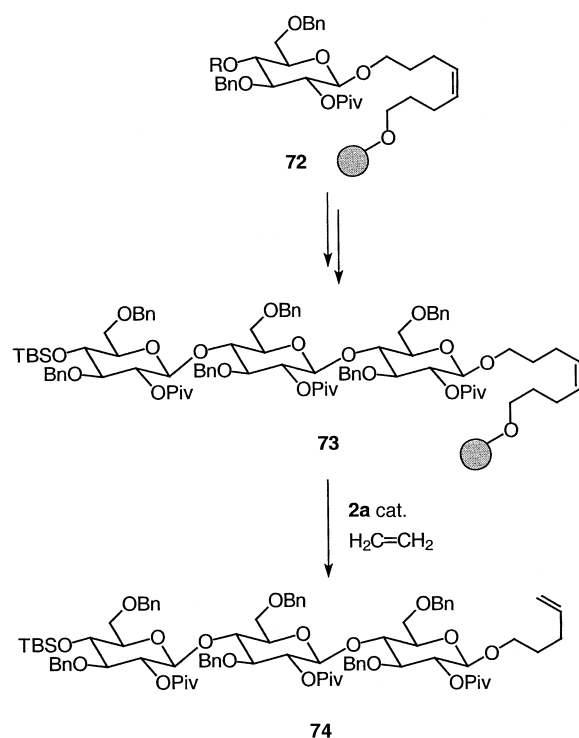


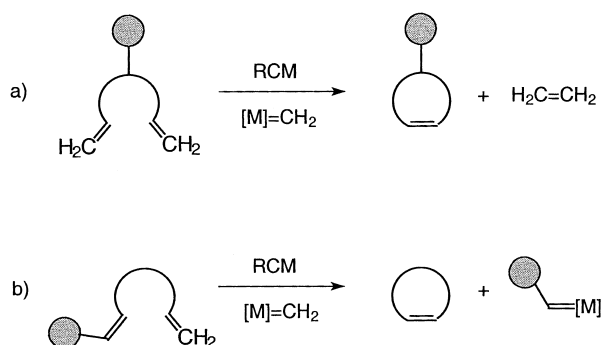
Figure 2. Parallel screening of four different ruthenium catalysts with IR-thermography as the detection method.^[64] The test reaction is the cyclization of 1,7-octadiene to cyclohexene. The tube marked “C” shows a control experiment in which catalyst **2a** is added to octane instead of the octadiene. a) Before addition of the catalysts; b) after 1 min of reaction time; c) after 2 min of reaction time.



Scheme 16. Alkene groups as covalent linkers in the solid-phase synthesis of oligosaccharides.

support through an unsaturated linkage.^[65] After a series of subsequent glycosylation reactions, the product is cleaved off the resin by cross metathesis with ethylene.

Metathesis can also be employed as a tool for the elaboration of immobilized compounds.^[66] Thus, a suitable alkene (diene, enyne, etc.) is covalently attached to the resin, metathesis is carried out, and the products formed are cleaved off by conventional methodology (Scheme 17a). Alternatively, the metathesis event itself can be used to form the product

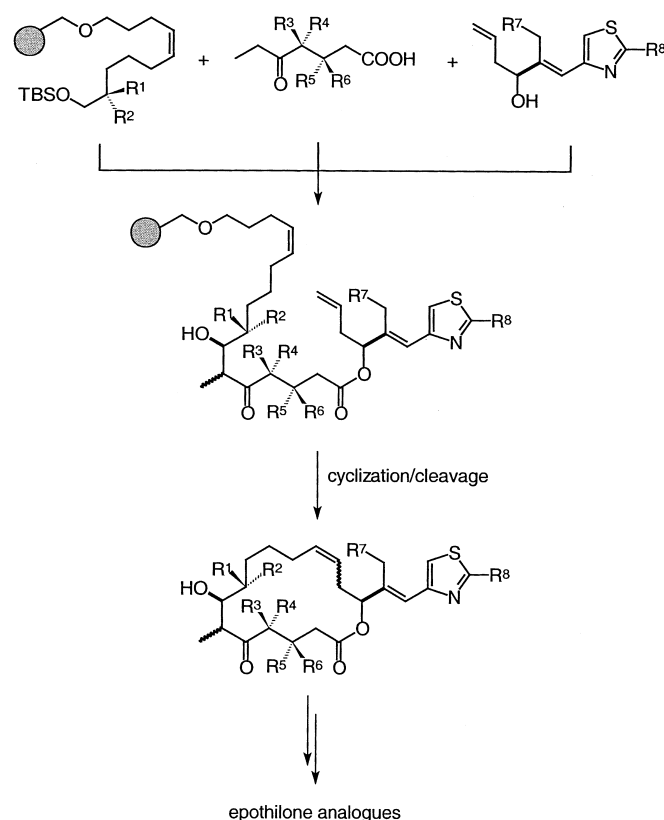


Scheme 17. Typical applications of RCM to polymer-bound substrates: a) The product is cleaved from the resin after the metathesis reaction; b) the product is released by the metathesis reaction.

and to release it simultaneously from the solid support (Scheme 17b). This strategy has the inherent advantage that only compounds with the correct functionality will be liberated, whereas any unwanted by-products remain bound to the polymer. Note, however, that the catalyst is captured by the polymer matrix during each productive “cyclization/

cleavage” event. Therefore, one has to use either very high catalyst loadings or “ancillary” terminal alkenes which liberate the catalyst from the bead by subsequent cross metathesis and reconver it into a soluble species.

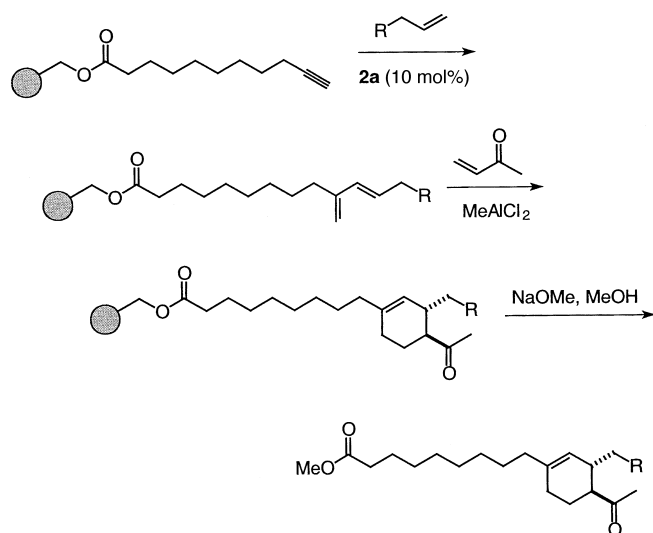
A typical application of the “cyclization/cleavage” technique is the synthesis of a library of epothilone analogues which enabled a synthesis-driven mapping of the pharmacophore of this promising anticancer agent (Scheme 18).^[67] Other applications follow a similar rationale.^[68]



Scheme 18. Outline of an epothilone-library synthesis by the cyclization/cleavage strategy.^[67]

Solid-phase synthesis also provides advantages in cross-metathesis reactions. Due to the compartmentalization of the reaction partners it is possible to drive the conversion by using an excess of the soluble component. Various successful applications illustrate this idea, among which the synthesis of a muscone library^[69] and the elegant use of intermolecular cross metathesis or crossed enyne metathesis reactions are most noteworthy.^[70, 71] In the latter case, the 1,3-diene formed by enyne metathesis is amenable to post-metathesis elaboration, for example, by Diels–Alder cycloadditions (Scheme 19).

In addition to these metathesis reactions using polymer-bound substrates, some examples of solution-phase combinatorial chemistry have also been reported. They span a wide range of applications including the preparation of “alkene libraries” by simple scrambling of a set of olefinic substrates^[72] and rather sophisticated studies into biased macrocycle synthesis,^[73] just to mention a few typical cases.



Scheme 19. Enyne cross metathesis and post-metathesis functionalization of polymer-bound substrates.^[70]

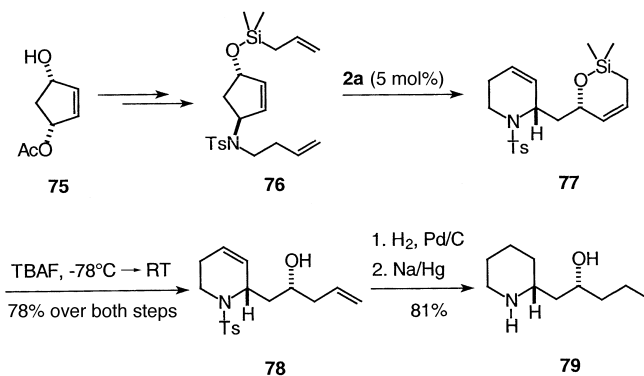
5. Selected Applications to Natural Product Synthesis

Once it became clear that the new catalysts tolerate a wide spectrum of polar functional groups due to their remarkable chemoselectivity profile, metathesis rapidly evolved into a relevant and mature tool for target-oriented synthesis. A proliferating number of applications substantiates this notion and makes the following treatise necessarily selective rather than comprehensive. The examples described below are meant to demonstrate some of the “strategic” advantages of this transformation such as high overall efficiency, inherent flexibility, practicality, atom economy, and an unrivaled “economy of steps” even in multiplex synthesis manoeuvres.

5.1. Ring Shuffling by Metathesis Cascades: Synthesis of (–)-Halosaline

Ring-opening metathesis is usually driven by the release of ring strain and has mainly been applied to the formation of speciality polymers by ROMP. However, it is possible to avoid polymerization by combining ROM with CM or RCM into suitable reaction dominos.^[74] This constitutes an elegant way, for example, to transfer stereochemical information from one ring to another, as illustrated by the total synthesis of the piperidine alkaloid (–)-halosaline (**79**) summarized in Scheme 20.^[75]

Acetate **75** is available in excellent optical purity (*ee* > 99%) by enzyme-catalyzed desymmetrization of the *meso*-diacetate precursor. Protection of the OH function with allyldimethylchlorosilane and cleavage of the remaining OAc group followed by introduction of a *N*-butenyl side chain by a Mitsunobu reaction delivers triene **76** as the key precursor for the subsequent domino event. Exposure of this substrate to catalytic amounts of the ruthenium carbene **2a** reorganizes the ring system and provides compound **78** in good yield after



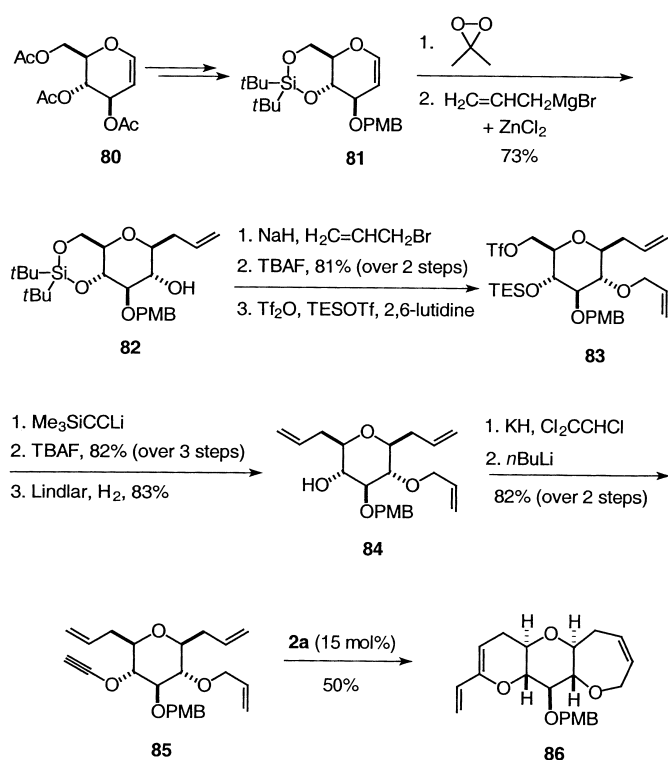
Scheme 20. A ROM/RCM domino reaction is employed in the synthesis of (–)-halosaline (**79**).

desilylation of the crude bicyclic product **77**. Note that the outcome of the crucial metathetic ring shuffling is independent of the site of initiation; it can be rationalized in terms of initial formation of a ruthenium carbene at one of the unsaturated appendages which opens the preexisting cyclopentene ring and, thereby, translates the carbene functionality such that it undergoes RCM with the other side chain. Hydrogenation of diene **78** under standard conditions and cleavage of the *N*-Ts group with Na/Hg in methanolic phosphate buffer delivers enantiomerically pure (–)-halosaline (**79**) in excellent overall yield.^[75] The enormous potential of such atom economical skeletal reorganizations has yet to be explored in detail and further elegant applications of this principle can be expected.

5.2. Two-Directional Metathesis: Polyether Syntheses

The structural complexity of brevetoxin or ciguatoxin and related polyether natural products constitutes an ideal forum to scrutinize the preparative potential of RCM. Many studies have been devoted to these targets;^[76] among them, two independent reports on two-directional strategies for the construction of the ABC ring framework of ciguatoxin are noteworthy from the conceptual standpoint.^[77, 78]

Both syntheses start from easily accessible tri-*O*-acetyl-D-glucal (**80**) and pursue similar routes. One of them is depicted in Scheme 21.^[77] Thus, epoxidation of the electron-rich double bond of **81** using dimethyldioxirane, followed by opening of the resulting epoxide with allylmagnesium bromide in the presence of ZnCl₂, provides alcohol **82** in a highly diastereoselective manner. Allylation followed by routine protecting-group manipulations lead to primary triflate **83** which allows chain extension upon treatment with lithium trimethylsilylacetylide. Desilylation and subsequent Lindlar reduction delivers alcohol **84** which is readily converted into alkynyl ether **85** according to a literature method. This sets the stage for the crucial two-directional ring closure. Exposure of this substrate to the Grubbs catalyst **2a** affords product **86** in respectable 50% yield which corresponds to the ABC ring system of ciguatoxin-3C. This transformation is noteworthy because it comprises a regular RCM on one side of the molecule and a simultaneous but independent enyne meta-



Scheme 21. Compound **85** is transformed into **86** in one step through RCM and enyne metathesis.

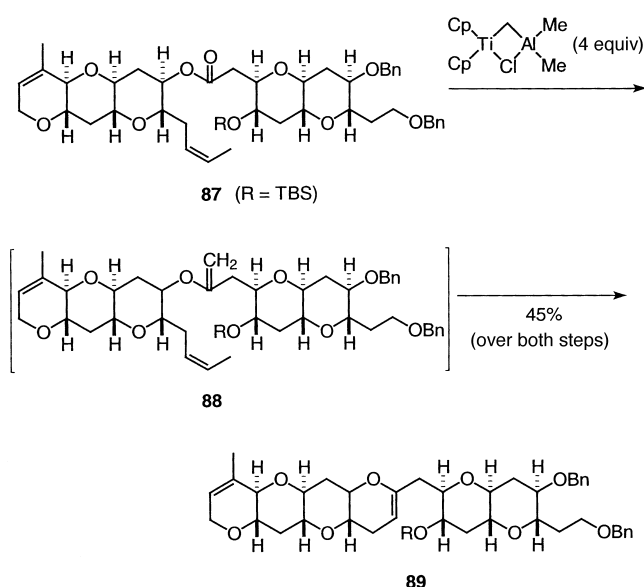
thesis on the other end which do not interfere with one another. This example is also one of the rare cases in which an electron-rich alkyne is subjected to ruthenium-catalyzed enyne metathesis, a yet largely unexplored arena.^[77]

5.3. Carbonyl Olefination/RCM Cascades: Polyether Syntheses

A complementary approach to the formation of cyclic ether skeletons takes advantage of the fact that the Tebbe reagent (or its more convenient surrogate $[\text{Cp}_2\text{TiMe}_2]$) is nucleophilic enough to effect Wittig-type olefination even of ester carbonyl groups and can also catalyze regular alkene metathesis.^[6c, 58] These two properties were cleverly combined into a domino process which allows the transformation of acyclic olefinic esters into cyclic enol ethers. The efficacy of this technique is illustrated by the preparation of various polyether domains of maitotoxin.^[79] A representative example is depicted in Scheme 22.

5.4. Carbocycles from Carbohydrates

The widespread occurrence and biological significance of polyoxygenated carbocycles provide constant impetus for the development of flexible methods for the synthesis of these compounds. RCM constitutes a valuable tool that supplements the existing arsenal. Therefore, many applications have been reported in which sugar-derived dienes are transformed into enantiomerically pure carbocycle derivatives by RCM.



Scheme 22. Methylation and subsequent RCM with the Tebbe reagent.

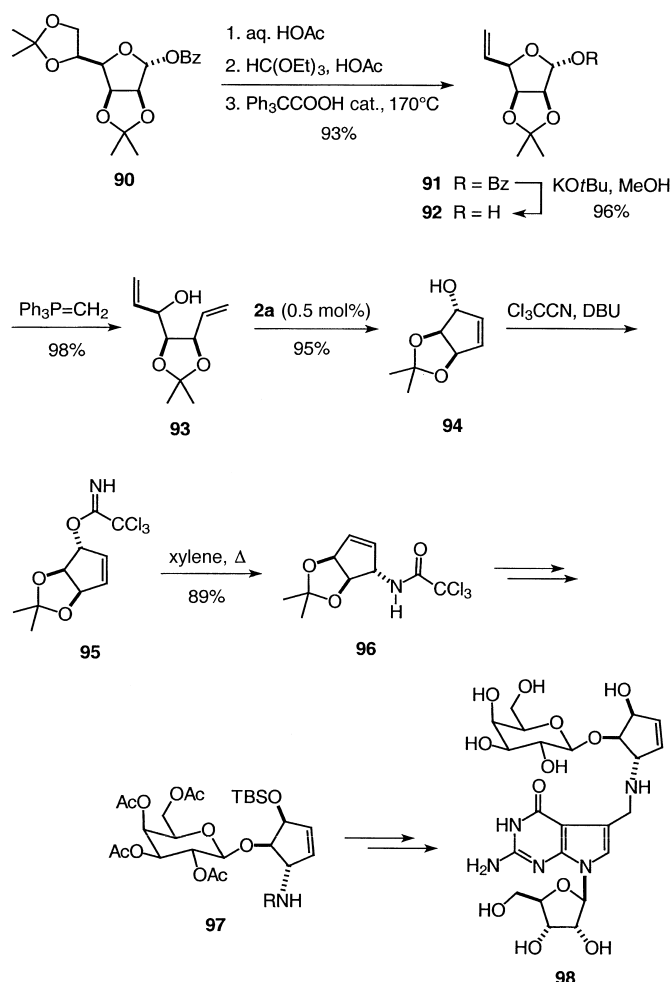
Most notable among them are syntheses of various conduritol derivatives (see also Chapter 3.2.2.),^[48, 49] (+)-valienamine,^[80] as well as various azasugars,^[81] carbasugars, and sugar mimics.^[82, 83]

A nice illustration is shown in Scheme 23, which summarizes the preparation of the unusual cyclopentene appendix of nucleoside **Q 98**.^[84] The mannofuranose derivative **90** is easily accessible on large scale from the parent sugar. Regioselective cleavage of the 5,6-*O*-isopropylidene acetal, treatment of the crude diol derivative with $\text{HC}(\text{OEt})_3$, and acid-catalyzed thermal rearrangement of the resulting orthoester deliver alkene **91** in excellent yield; this is converted into diene **93** by cleavage of the anomeric benzoyl group and subsequent Wittig olefination of the resulting hemiacetal **92**. RCM of **93** catalyzed by ruthenium carbene **2a** gives 95% of the desired cyclopentene derivative **94**. Introduction of the missing amino group is elegantly achieved by a [3,3]-sigmatropic Overman rearrangement of the trichloroacetimidate **95** derived thereof. Standard protecting-group manipulations of compound **96** thus obtained, followed by β -galactosylation, provide the desired product **97** suitable for incorporation into nucleoside **Q**.^[84]

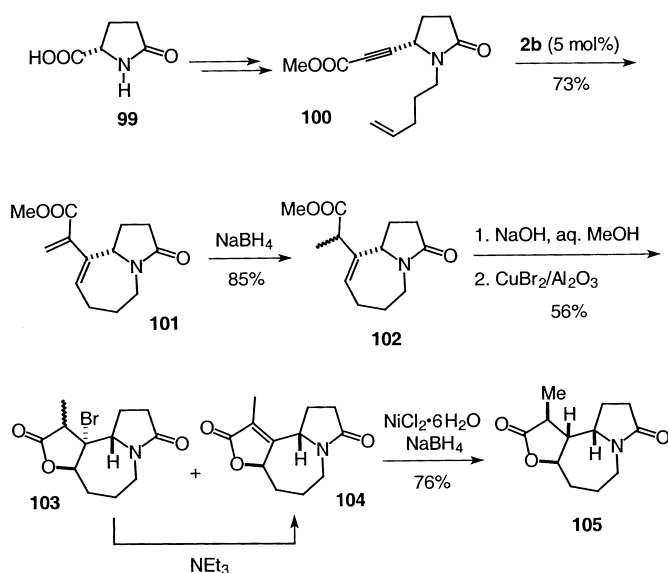
5.5. Enyne Metathesis: (–)-Stemoamide

Although many studies have demonstrated the preparative potential of inter- as well as intramolecular enyne metathesis in general,^[85, 86] applications to natural product synthesis are still relatively scarce. A nice exception is the synthesis of (–)-stemoamide (**105**) reported by Kinoshita and Mori (Scheme 24).^[87]

Pyroglutamic acid **99** is converted by conventional means into enyne **100** which undergoes a high yielding cyclization to the corresponding diene **101** on exposure to catalytic amounts of ruthenium carbene **2b**. Regioselective reduction of the conjugated double bond, followed by bromolactonization of



Scheme 23. RCM is the key step in the synthesis of the cyclopentene fragment of nucleoside **Q** (**98**).



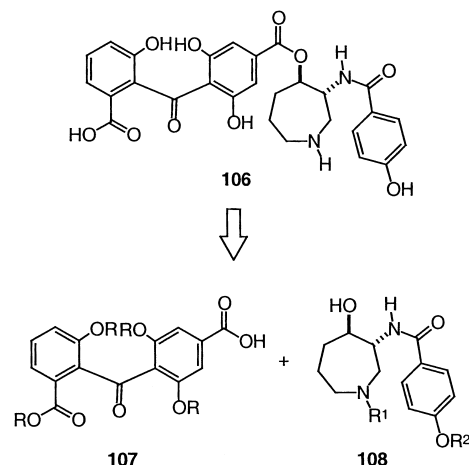
Scheme 24. An enyne metathesis in the early stages of a natural product synthesis.

102, delivers a mixture of bromide **103** and the elimination product thereof. Treatment with base drives the elimination process to completion and gives lactone **104** as the sole

product. This is finally reduced with NaBH₄ in the presence of NiCl₂·6H₂O to afford the natural product in good overall yield.^[87]

5.6. (–)-Balanol

The structurally rather unusual alkaloid balanol (**106**) represents an important new lead structure in the quest for selective inhibitors of protein kinase C. All syntheses of **106** reported to date assemble the product by esterification of the benzophenone carboxylic acid fragment **107** with the hexahydroazepine domain **108** (Scheme 25). Although the latter is a

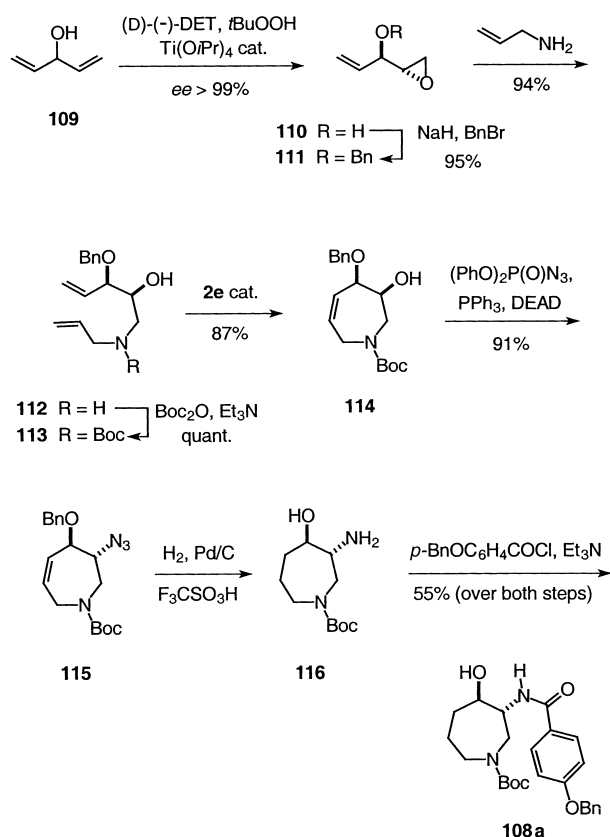


Scheme 25. Retrosynthesis of balanol (**106**).

relatively simple structure, all but one syntheses of enantiomerically pure **108** reported to date require more than 12 steps, independent of whether they rely upon 1) the exploitation of the chiral pool, 2) ligand-controlled enantioselective transformations, or 3) the resolution of racemates. Only recently, a much shorter route to this key intermediate has been described which is based on RCM as one of the key steps.^[88]

Sharpless epoxidation of divinylcarbinol **109** affords epoxide **110** in good chemical yield and excellent optical purity (Scheme 26). *O*-Benzoylation followed by regioselective opening of the oxirane ring of **111** with allylamine gives diene **112** which is protected with an *N*-Boc group and subsequently submitted to ring closure. The ruthenium indenylidene complex **2c**^[27, 28] was found to be the best choice, although the “standard” Grubbs carbene **2a** also provides respectable yields of the desired seven-membered ring **114**. Conversion of the free OH group into azide **115** under Mitsunobu conditions followed by hydrogenation and *N*-acylation of the crude amine **116** thus formed delivers compound **108a** which is amenable to (–)-balanol according to literature procedure.^[88]

It is worth mentioning that the order of azide formation and RCM can also be reversed. In this case, however, only Schrock’s molybdenum alkylidene **1a** allows productive RCM,^[88] whereas all ruthenium-based initiators, which require higher reaction temperatures, led to decomposition of the azide moiety.

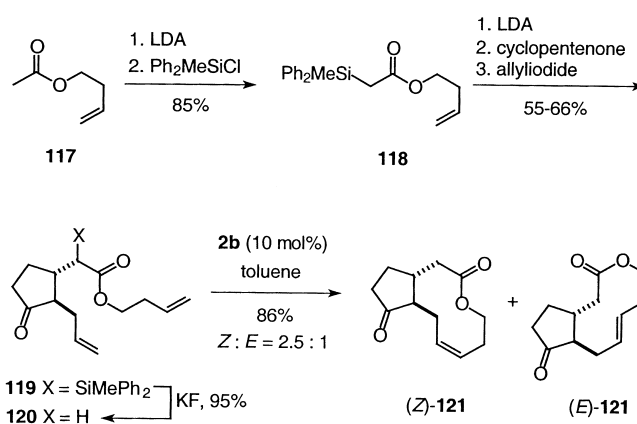
Scheme 26. Synthesis of the balanol precursor **108a**.

To date, this approach constitutes the shortest (eight steps) synthesis of the hexahydroazepine domain of (–)-**106** in enantiomerically pure form. All key operations of this sequence are catalytic in nature (epoxidation, RCM, hydrogenolysis, hydrogenation) and scale-up seems to be possible. Finally, the route can be adapted to the synthesis of the antipode of balanol simply by switching from (D)-(-)-DET to (L)-(+)-DET in the initial Sharpless epoxidation reaction and it provides ample opportunity for the preparation of analogues by deviating from the blueprint outlined above at different stages of the synthesis.

5.7. Three-Component Coupling/RCM: Synthesis of Jasmine Ketolactone

Due to the inherent strain of medium-sized rings, eight- to eleven-membered cycloalkenes are excellent substrates for ROMP but constitute particularly challenging targets for RCM. If preexisting conformational constraints, however, force the substrates to adopt a favorable conformation for ring closure, even this class of compounds is within reach, as witnessed by a rapidly growing number of successful applications.^[89] In this context, a recent report deserved particular emphasis in which an eight-membered ring incorporating an *E*-configured double bond has been formed by RCM.^[89b]

A typical example that illustrates the efficiency as well as the limitations of RCM in this arena is a synthesis of jasmine ketolactone ((*Z*)-**121**; Scheme 27).^[90, 91] A three-component

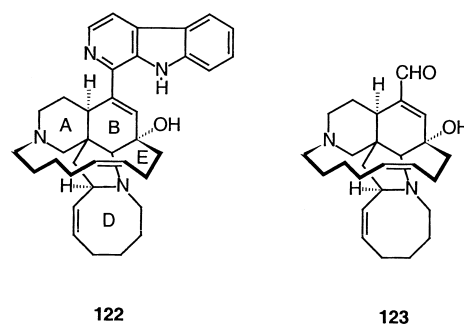
Scheme 27. Synthesis of (*Z*)-**121** and (*E*)-**121** through a three-component coupling with subsequent RCM.

coupling comprising 1,4-addition of the lithium enolate of butenyl (diphenylmethyl)silylacetate (**118**) to cyclopentenone, followed by alkylation of the resulting ketone enolate with allyl iodide, delivers compound **119**. Subsequent protodesilylation followed by RCM of the resulting diene **120** under high dilution conditions affords the targeted ten-membered lactone **121** in remarkable 86% yield. The efficiency of this five-step sequence, however, is compromised by the fact that both geometrical isomers of the product are formed, although the required *Z* isomer is slightly favored (*Z*:*E* = 2.5:1).

In general, it is not yet possible to predict or control the stereochemistry of the newly formed double bond of cycloalkenes with more than ten ring atoms. This important limitation is evident from many other syntheses as well, among which the epothilone case is certainly the most prominent one. Approaches to this anticancer agent based on RCM are not discussed in detail in this article, because several timely and comprehensive review articles are available.^[92, 93] However, these examples make clear that it is a prime strategic goal for future investigations to gain control over the *E*:*Z* ratio in metathesis reactions. As will be shown in Section 7, alkyne metathesis followed by partial reduction of the cycloalkynes thus formed is presently the only viable solution for this stereochemical problem.

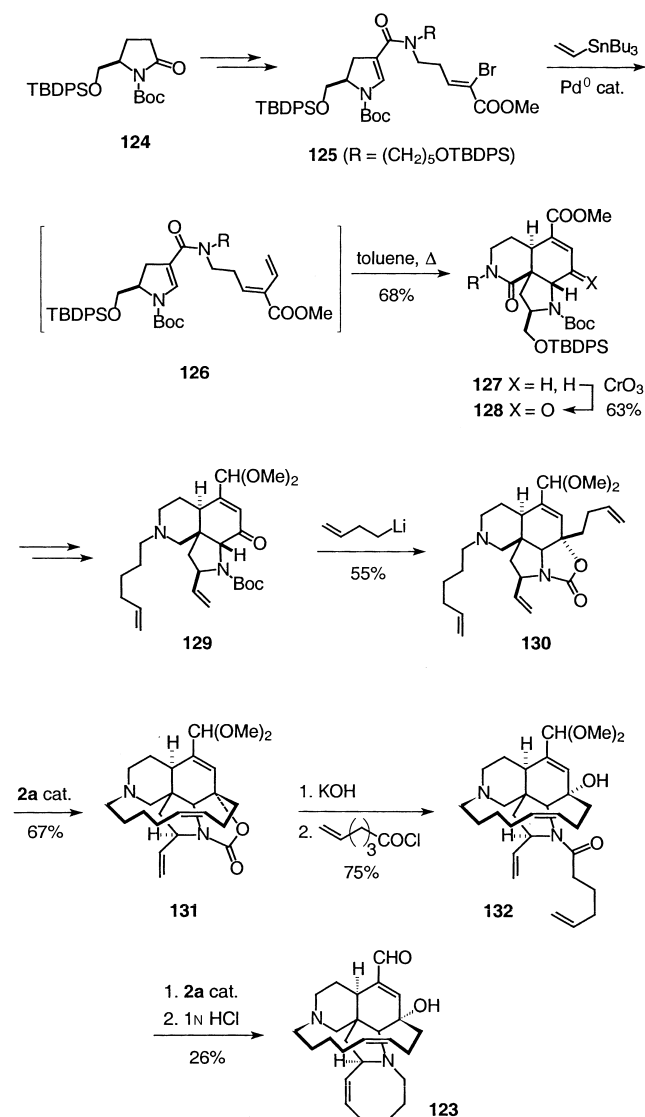
5.8. Ircinal A and Related Manzamine Alkaloids

The intricate polycyclic structure of the potent antitumor agent manzamine A (**122**) was soon recognized as an excellent testing ground for RCM. Several model studies have shown



that the macrocyclic E ring, as well as the eight-membered D ring, can be accessed by this technique.^[94] It was only recently, however, that a concise total synthesis of enantiomerically pure ircinal A (**123**), a biosynthetic precursor to manzamine A, has been completed which makes strategic use of this transformation.^[95]

Starting from the easily accessible imide **124**, a cleverly designed domino Stille/Diels–Alder strategy leads to the tricyclic core **127** which is elaborated into compound **130** by routine functionalizations of the carbon backbone (Scheme 28). A facile macrocyclization of **130** to product **131** takes place (67%, *Z:E*=8:1) on exposure to Grubbs



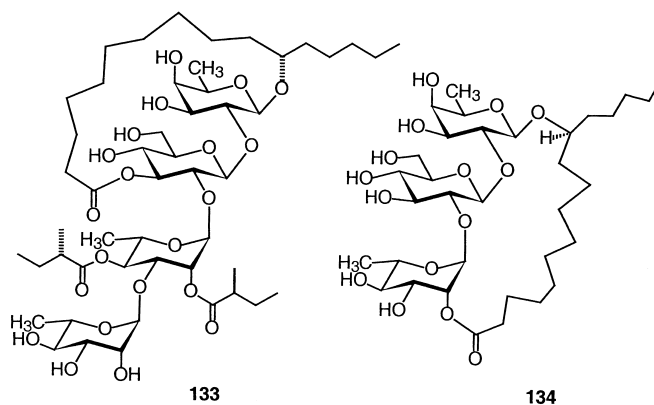
Scheme 28. Synthesis of ircinal A (**123**) with two RCM steps.

carbene **2a** under high dilution conditions even without protonation of the tertiary amine function. The regioselectivity of this RCM step may be interpreted in terms of different steric hindrance close to the alkene moieties of substrate **130**. While the two unhindered ones result in the formation of the thirteen-membered ring, the third olefin is sterically encumbered and, therefore, does not interfere. In turn, however, this

crowded situation renders the final formation of the eight-membered D ring (**132** → **123**) highly problematic and results in only 26 % isolated yield of the desired product. Despite this unfavorable outcome, the ircinal A synthesis summarized in Scheme 28 constitutes a nice example for the cooperative use of transition metal catalyzed reactions in advanced natural product synthesis.^[95]

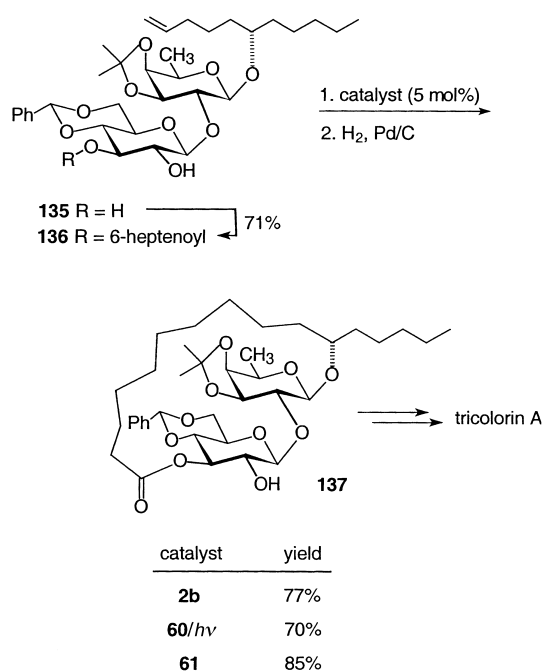
5.9. Inherent Flexibility: Total Synthesis of Tricolorin A and Related Glycolipids

A detailed analysis of the amazing structures of resin glycosides such as tricolorin A (**133**), tricolorin G (**134**), and other members of this series shows that all of them contain 11(*S*)-hydroxyhexadecanoic acid as the aglycon which forms a macrolactone ring by spanning two or more sugar units of the oligosaccharide backbone. Although these glycolipids exhibit promising biological activities including cytotoxicity against human cancer cell lines, systematic structure/activity correlation studies are difficult as long as each analogue requires a labour intense independent synthesis.

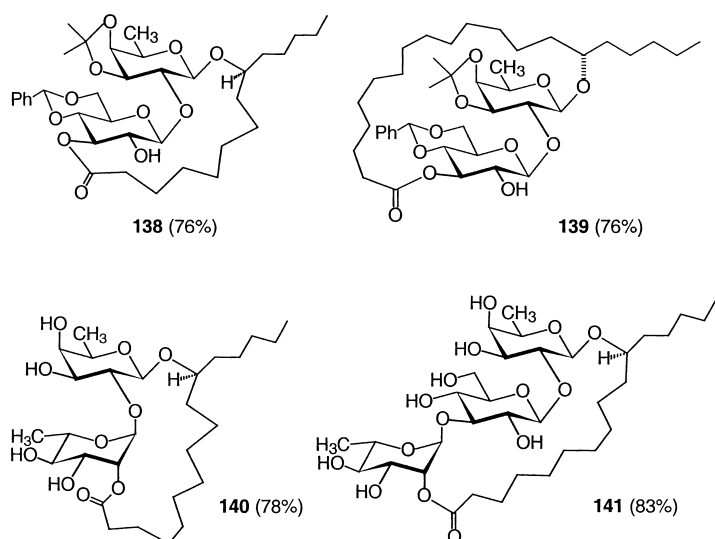


The inherently modular character of metathesis allows the problem to be circumvented.^[96] Specifically, esterification of the more reactive 3'-OH group of the disaccharide diol derivative **135** with 6-heptenoic acid followed by RCM of the resulting diene **136** and hydrogenation of the *E,Z* mixture thus formed gives macrolide **137** that can be elaborated into tricolorin A according to a literature procedure (Scheme 29). The cationic ruthenium allenylidene complex **61**^[56] and the photochemically driven procedure using [(η^6 -cymene)Ru-Cl₂(PCy₃)] (**60**)^[55] as a catalyst precursor perform similarly well as the “standard” Grubbs carbene **2b**^[8] which serves as the calibration point in this investigation.

More importantly, however, diol **135** can be used as a common platform for the preparation of many analogues and positional isomers simply 1) by incorporating unsaturated acids other than 6-heptanoic acid; 2) by attaching the unsaturated moieties to the 2'-OH instead of the 3'-OH group; 3) by replacing the ester linkage with, for example, ether, carbonate, or carbamate functionalities; or 4) by incorporating further carbohydrate residues into the back-

Scheme 29. Synthesis of the tricolorin A precursor **137** by RCM.

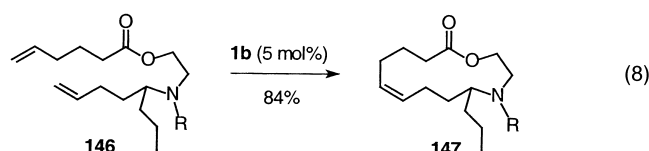
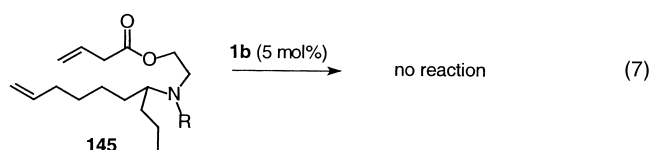
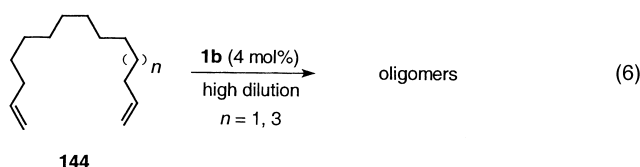
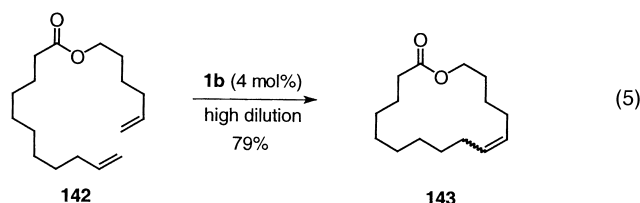
bone prior to ring closure. In fact, an array of products (for example, **138–141**) has been obtained in excellent yields by this strategy including tricolorin G (**134**) which had not been synthesized before.^[96]



5.10. Inherent Flexibility: The Roseophilin Case

From the large set of data available on macrocyclizations with RCM it was possible to deduce some basic rules which help to identify the proper site within a given target molecule where productive formation of a large ring can be expected.^[97] According to this rationale, a suitably biased ground state conformation is not required as can be seen, for example, from the successful preparation of the 16-membered ring **143** from

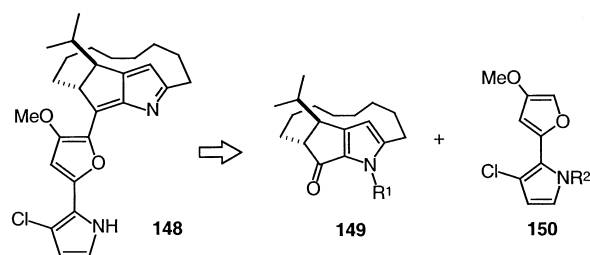
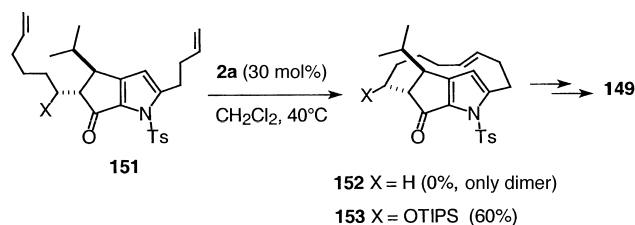
diene **142** [Eq. (5)].^[98] However, a well balanced interaction of Lewis-basic heteroatoms of the diene substrate with the emerging Lewis-acidic carbene intermediates is crucial for efficient macrocyclizations [see Eqs. (5)–(8)].^[97, 98] If such



chelation becomes too strong, the activity of the metal carbene is attenuated and RCM is likely to cease; this may explain why the attempted cyclization of diene **145** has failed.^[98b] Therefore, it is of utmost importance in retrosynthetic planning to assess the distance as well as the affinity of the polar groups towards the catalytically active metal species. One must keep in mind, however, that RCM-based macrocycle formations are mainly entropically driven and will hardly overcome large enthalpic barriers. The formation of highly strained products will, therefore, be problematic unless preexisting conformational constraints help to shape the substrate and, thereby, facilitate the ring-closing reaction.

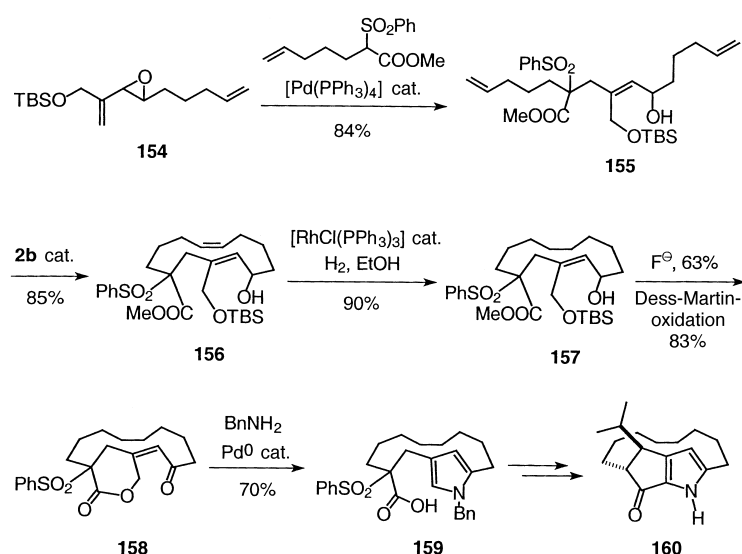
These notions are illustrated by three different approaches directed toward roseophilin (**148**), a structurally unique, cytotoxic alkaloid of marine origin. The only total synthesis of this deeply red-colored pigment reported so far assembles the azafulvene chromophore by combining the heteroaromatic side chain **150** with the rather strained macrotricyclic ketone derivative **149** (Scheme 30).^[99] The latter constitutes an obvious target for RCM; it has been made by three different RCM-based routes. A comparison of these strategies is quite informative.

Fuchs and co-workers form both five-membered rings prior to the cyclization of the ansa chain (Scheme 31).^[100] Attempted RCM of diene **151** (X=H), however, turned out to be unsuccessful even under very high dilution conditions (0.0005 M) because all the strain in the product must be built up during macrocyclization. Only after the introduction of an

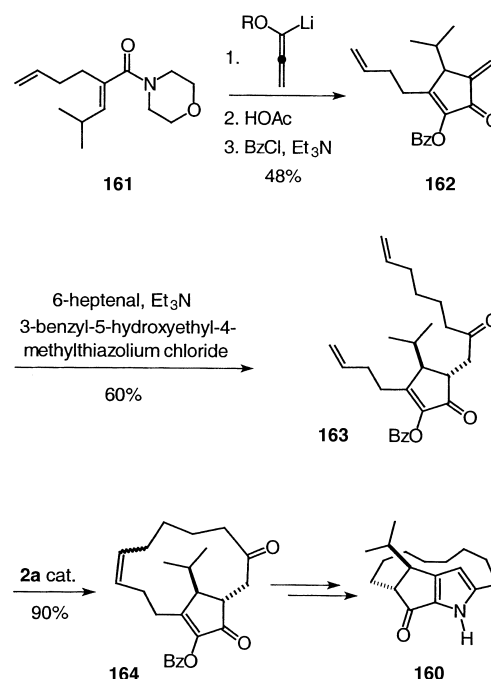
Scheme 30. Retrosynthesis of roseophilin (**148**).Scheme 31. On route to roseophilin: The ring closure to macrocycle **149** only occurs after the introduction of a group to aid the conformational preorganization.

ancillary group (X = triisopropylsiloxy) as a conformational control element which forces the unsaturated side chains into closer proximity was it possible to accomplish the reaction in 60% yield by using a rather high catalyst loading.^[100]

In contrast to this result, the large ring forms without difficulties if the order of events is inverted.^[101] Specifically, treatment of triene **155** with Grubbs carbene **2b** delivers, in 85% yield, the desired thirteen-membered ring **156** which is then converted into the ketopyrrole entity as shown in Scheme 32; note that the strain in the molecule is now introduced during the kinetically more favorable closure of the five-membered rings.^[101] This example also highlights the excellent compatibility of the ruthenium complex with various functional groups and shows that this catalyst rigorously distinguishes between the terminal and the trisubstituted alkene groups of substrate **155**. In line with this finding, macrocyclization also proceeds uneventfully with the mono-

Scheme 32. On route to roseophilin: It is advantageous to perform the RCM-based macrocyclization at an early stage of the synthesis of **160**.

cyclic diene **163** (Scheme 33).^[102] The resulting product **164** is then converted into the target by a conventional Paal–Knorr pyrrole synthesis.

Scheme 33. On route to roseophilin: An alternative route to **160**.

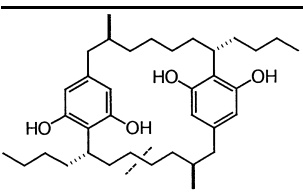
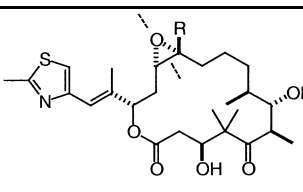
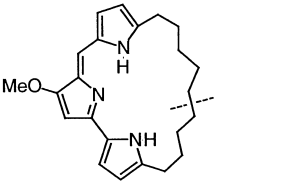
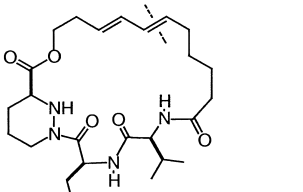
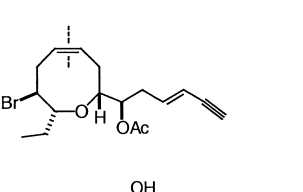
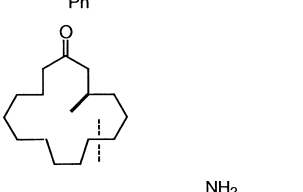
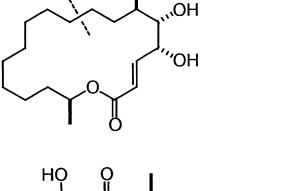
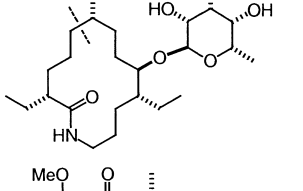
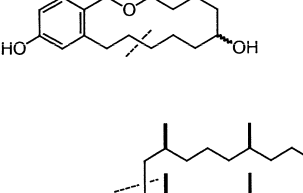
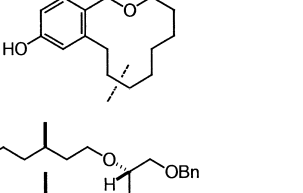
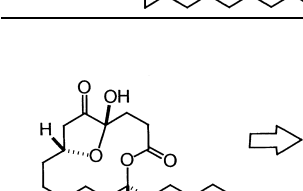
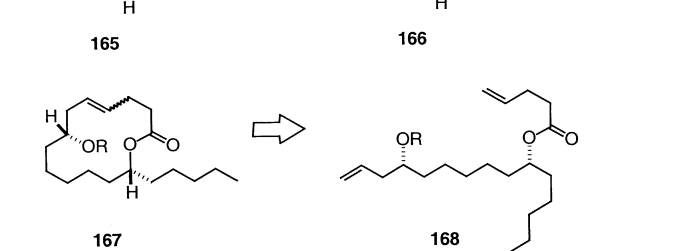
Irrespective of the individual features of each route, the roseophilin case exemplifies the flexibility and liberty in retrosynthetic planning that is made possible by the truly remarkable application profile of modern metathesis catalysts. More examples of RCM-based syntheses of macrocyclic natural products are compiled in Table 4.^[92, 93, 103–112]

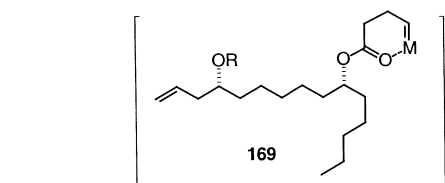
5.11. Binary Catalyst System: (–)-Gloeosporone

The retrosynthetic analysis for (–)-gloeosporone (**165**) depicted in Scheme 34 suggests that RCM may open an unprecedentedly short entry into this macrolide antibiotic, which has previously been prepared by lengthy lactonization strategies. Bearing in mind, however, that the distance and the affinity of the polar groups of a given substrate toward the emerging carbene entities during RCM play a decisive role for productive macrocyclization,^[97, 98] diene **168** may be a problematic substrate. If the catalyst attacks the 4-pentenolate entity, a fairly stable six-membered chelate **169** may evolve which can potentially intercept the catalyst in an unproductive form.

The required diene **168** is readily obtained in enantiomerically pure form starting from cycloheptene as shown in Scheme 35. In line with the rationale presented above, this substrate fails to afford any cyclized product on exposure to catalytic amounts of ruthenium carbene **2a**. This outcome, however, can be easily rectified upon addition of $\text{Ti}(\text{OiPr})_4$ to the reaction mixture. The binary system comprising catalytic amounts of **2a** and substoichiometric

Table 4. Macrocyclic natural products prepared by RCM. The site of ring closure is indicated by a dotted line.

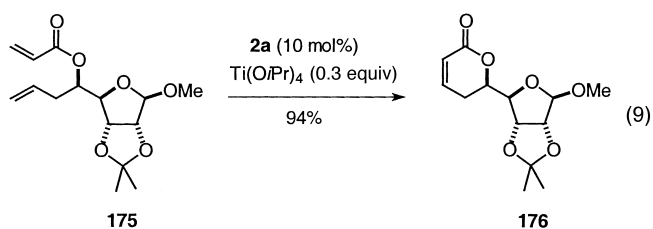
Product	Ref.	Product	Ref.
	[103]		[92, 93]
	[104]		[105]
	[106]		[111]
	[107]		[108]
	[109]		[109]
	[110]		
			

Scheme 34. Retrosynthesis of (–)-gloeosporone (**165**).

amounts of $\text{Ti}(\text{OiPr})_4$ results in a clean and high yielding formation of the desired macrocycle **167**, which is then converted into gloeosporone by oxidation of the alkene and cleavage of the silyl group during work-up.^[113]

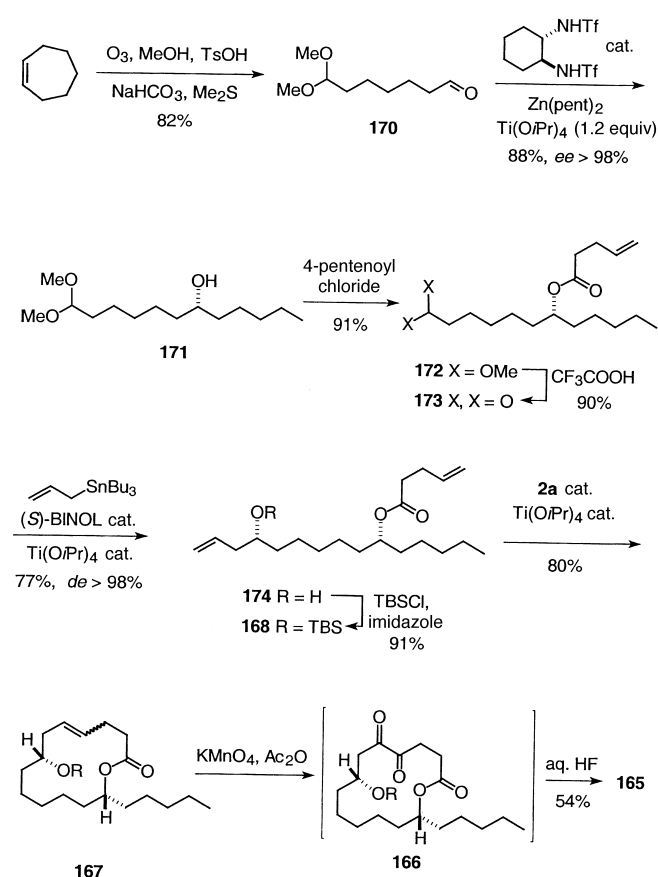
From the conceptual point of view, several aspects of this approach are noteworthy. It is significantly shorter than all previous syntheses of **165** reported in the literature due to the highly chemoselective character of metathesis allowing structurally unproductive protecting-group manipulations to be minimized.^[114] Specifically, enantiomerically pure gloeosporone is obtained in only eight steps with an overall yield of 18% starting from cycloheptene. Note that all C–C-bond formations are transition-metal catalyzed and that the chiral centers are concomitantly formed in a ligand-controlled manner. This gives maximum flexibility for the preparation of analogues of this bioactive target and illustrates the relevance of catalysis in general and of metathesis in particular for natural product chemistry.

Although the precise mode of action of $\text{Ti}(\text{OiPr})_4$ is not yet clear, one can envisage three different possibilities: 1) This weakly Lewis-acidic additive competes with the emerging ruthenium carbene for a kinetically labile coordination onto the ester and, thereby, avoids the formation of unreactive intermediates such as chelate **169**. 2) Alternatively, $\text{Ti}(\text{OiPr})_4$ may coordinate onto the ester and, hence, direct the initiation of RCM to the other olefinic site of the molecule. 3) Finally, one can envisage that the Lewis-acidic $\text{Ti}(\text{OiPr})_4$ abstracts one of the basic PCy_3 ligands from **2a** and, thereby, activates the catalyst. The data presently available do not allow one to decide which of these effects is operative.^[113] The beneficial influence of $\text{Ti}(\text{OiPr})_4$ on RCM, however, seems to be quite general as witnessed by a rapidly growing number of successful applications reported in the literature.^[115] Problematic substrates such as acrylates were shown to react efficiently if the binary system **2a**/ $\text{Ti}(\text{OiPr})_4$ is employed as a metathesis catalyst mixture. A typical example is shown in Equation (9).

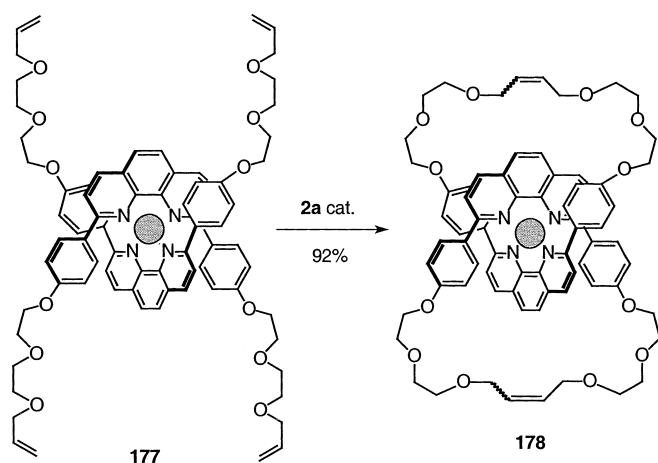


6. Highlights from the Nonnatural Product Series

Metathesis has also seen a prolific use in the nonnatural product series and this section must, therefore, be restricted to a few recent highlights. Among them, several reports on the efficient formation of catenanes by means of RCM have to be mentioned.^[116] A representative example is displayed in Scheme 36, which shows the self assembly of two 1,10-phenanthroline molecules bearing unsaturated appendages



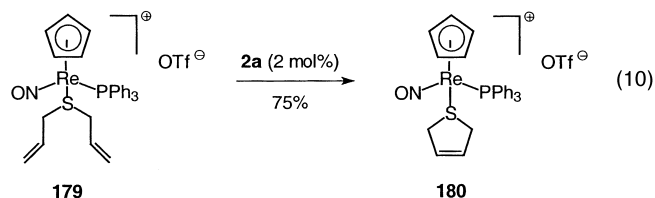
Scheme 35. Synthesis of (–)-gloeosporone (**165**) from cycloheptene with the use of a binary catalyst system in the metathesis step.



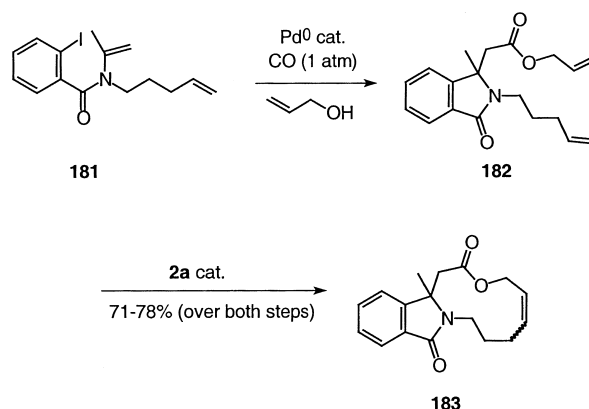
Scheme 36. Synthesis of a [2]catenane by double RCM.

on a Cu^{I} -template followed by RCM of the resulting tetraene **177**. This reaction provides the interlocked complex **178** in 92% yield as the only reaction product which can be quantitatively demetalated with KCN to afford the corresponding [2]catenand. The basic principle of this approach was later extended to the preparation of even more sophisticated structures such as molecular knots.^[117] In addition to the use of metal templates such as Cu^{I} , hydrogen bond directed assembly and templating π -acceptor/ π -donor interactions have also been exploited for the formation of complex catenane structures by RCM-based ring closure.^[118]

Superb examples for metathesis in metal coordination spheres have been reported by Gladysz and co-workers.^[119] These authors succeeded in cyclizing various unsaturated metal complexes such as the cationic rhenium compound **179** shown in Equation 10. In this particular case, the metal atom simultaneously acts as a “protecting group” for the sulfide, because RCM of free thioethers is known to be unproductive if the Grubbs catalyst **2a** is used.^[16]

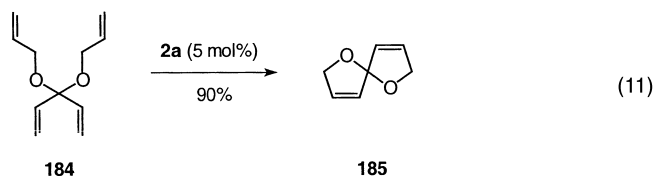


An illustration of how metathesis can be employed in concert with other transition metal catalyzed processes has been described by Grigg and co-workers.^[120] They invented a domino process resulting in the formation of four bonds, two rings, and one tetrasubstituted C atom by means of a palladium-catalyzed Heck reaction/carbonylation followed by RCM (Scheme 37).



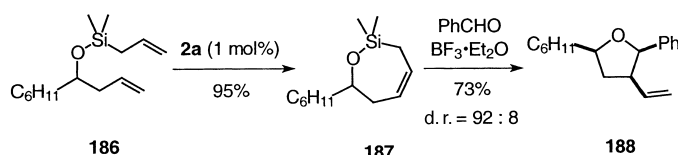
Scheme 37. RCM as the last step of a domino process.

Two or more simultaneous RCM events can be surprisingly selective as shown, for example, by the high yielding formation of spirocyclic arrays such as **185** from the tetraene precursor **184** [Eq. (11)].^[121] The observed selectivity in



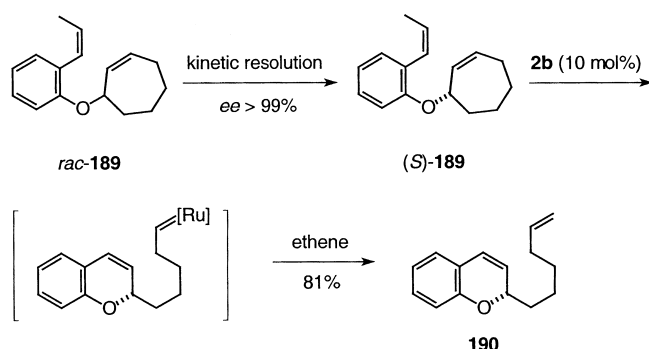
this particular case is probably the result of a kinetic preference for the formation of the five-membered rings in combination with a slow reversibility of the reaction which avoids undue scrambling. Similar ideas have been pursued by Lautens et al. en route to various bicyclodiene derivatives.^[122]

Yet another general synthesis method makes use of temporary linkers which enable directed cross metathesis of different olefins.^[123, 124] As has been independently shown by several research groups, silicon-based linkers are particularly useful in this context due to the ease of preparation and cleavage.^[125] The silicon moiety can even be exploited for subsequent transformations as exemplified by cleverly designed RCM/Sakurai reaction sequences (Scheme 38).^[126] Likewise, an efficient approach to D-altritol is based on temporary silicon-tethered RCM followed by dihydroxylation of the resulting alkene product.^[127]



Scheme 38. A sequence of RCM and a Sakurai reaction.

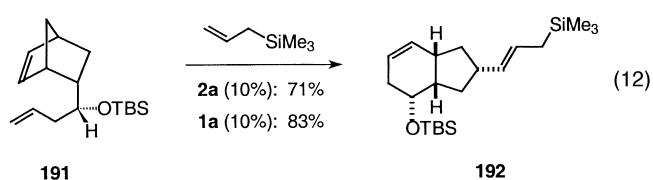
Hoveyda and co-workers have developed a Zr-catalyzed kinetic resolution protocol providing good access to cyclic diene derivatives such as (*S*)-**189**, which serve as starting materials for imaginative ROM/RCM/CM cascades.^[128] Upon treatment with a suitable metathesis catalyst they convert into 2-substituted chromenes (Scheme 39) and they have been



Scheme 39. An ROM/RCM/CM cascade which leads to a chromene derivative.

used for an efficient synthesis of the antihypertensive agent (*S,R,R,R*)-neбиволol.^[129] Depending on the level of substitution, the domino process may either be triggered by interaction of the catalyst with the styrenyl or with the carbocyclic alkene; the outcome of the reaction, however, is independent of the site of initiation. Note that an ethylene atmosphere is imperative for the typical transformation displayed in Scheme 39 as it terminates the reaction domino by cross metathesis and, thereby, avoids the formation of dimeric products.

Other variants of ROM/RCM/CM cascades have been reported which take advantage from the high reactivity of norbornene or 7-oxanorbornene substrates in metathesis reactions [Eq. (12)].^[130] Furthermore, multicyclization reactions of polyunsaturated precursors containing acetylenes or cyclic olefins as relays should also be mentioned in this context.^[131]

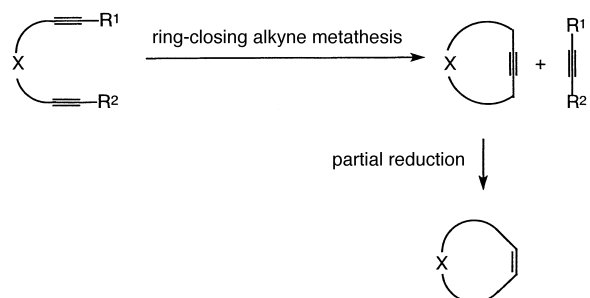


Metathesis has been used to capture and covalently stabilize certain conformations of more or less flexible backbones. Ramification of β -sheet peptide assemblies^[132] or the intramolecular cross-linking of the α -helix structure of peptides are highlights in this field.^[133] Furthermore, a cleverly designed “rolling loop scan” should also be mentioned which allows the preparation of small libraries of compounds which have the same peptide sequence but different active conformations locked in by RCM.^[134, 135] Moreover, an interesting application to medicinal chemistry deserves mentioning, in which taxol hybrids with restricted conformations are prepared by RCM.^[136] All of these applications are made possible by the excellent tolerance of Grubbs-type catalysts towards densely functionalized molecules.

7. Beyond Alkenes: Alkyne Metathesis and Ring-Closing Diyne Metathesis

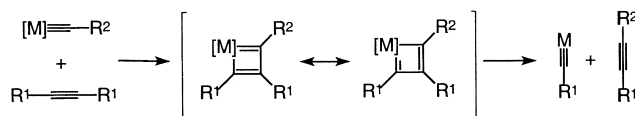
Among the few shortcomings that infringe upon the superb overall application profile of RCM, the lack of control over the configuration of the newly formed double bond is most noteworthy if the reaction is applied to the macrocyclic series. The products formed are usually obtained as mixtures of the *E* and *Z* isomers, with the former dominating in most of the recorded cases. This constitutes an obvious drawback in target-oriented synthesis, as can be seen from many examples reported in the literature.

In the long term, this fundamental problem calls for the development of stereoselective RCM catalysts. One may, however, contemplate that ring-closing alkyne metathesis followed by partial reduction of the resulting cycloalkyne also provides a viable solution (Scheme 40), in particular since several alkyne metathesis catalysts are known. They fall into two categories. Schrock-type alkylidyne complexes of various transition metals are particularly attractive because they are structurally well defined, show high catalytic activity, and have been thoroughly studied from the mechanistic point of



Scheme 40. Formation of stereochemically defined cycloalkenes by ring-closing alkyne metathesis and subsequent Lindlar hydrogenation.

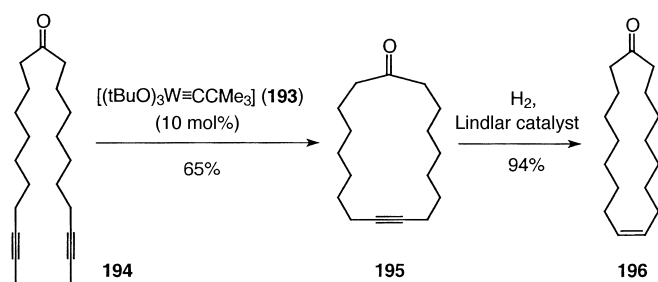
view.^[137] They react by a Chauvin-like mechanism as depicted in Scheme 41.^[138] The other class comprises multicomponent initiator systems in which a structurally unknown catalyst is formed in situ from different ingredients. For example, the combination of $[\text{Mo}(\text{CO})_6]$ and phenol additives ($p\text{-ClC}_6\text{H}_4\text{OH}$, $p\text{-(F}_3\text{C)C}_6\text{H}_4\text{OH}$, etc.) was introduced early on, although catalytic activity is observed only at rather elevated temperatures.^[139, 140]



Scheme 41. Mechanism of alkyne metathesis.

Surprisingly, however, applications of these alkyne metathesis catalysts have been very limited and were essentially confined to the preparation of a few speciality polymers^[141] and to the dimerization or cross-metathesis of simple acetylene derivatives.^[142]

The first application of alkyne metathesis to the formation of large rings has been reported in 1998 using Schrock tungsten alkylidyne complex $[(t\text{BuO})_3\text{W}\equiv\text{CCMe}_3]$ **193** as the catalyst.^[143, 144] This study made clear that: 1) rings with more than 12 atoms—even including very large rings—are readily accessible by this method in good to excellent yields; 2) ring-closing diyne metathesis proceeds even faster than RCM of the corresponding dienes; 3) the reaction tolerates ethers, esters, silyl ethers, amides, urethanes, ketones, enones, sulfones, furans, etc.; 4) the catalyst rigorously distinguishes between alkynes (reactive) and preexisting alkene groups in the substrate, which were found to be inert; and 5) this method provides an excellent entry into macrocyclic (*Z*)-alkenes if combined with a Lindlar reduction of the cycloalkyne products. This two-step strategy constitutes an indirect but convenient solution for the stereochemical shortcomings of RCM mentioned above.^[143, 144] A typical example, the stereoselective synthesis of the macrocyclic musk civetone (**196**) is displayed in Scheme 42.^[145]

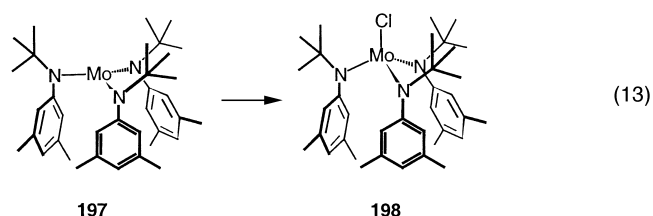


Scheme 42. Synthesis of civetone (**196**) by the alkyne metathesis/Lindlar strategy.

The $[\text{Mo}(\text{CO})_6]/p\text{-chlorophenol}$ initiator system has also been applied to various ring-closure reactions.^[144–146] Although this user-friendly “instant” system optimized by Bunz and co-workers,^[140] which requires no particular precautions as to handling and drying of the reagents and solvents used,

gives satisfactory results in many cases, its scope is somehow limited and the quite harsh conditions (130–150 °C in dichloro- or trichlorobenzene) prevent applications to sensitive substrates.

Very recently, yet another alkyne metathesis catalyst has been developed which is formed in situ from the trisamido-molybdenum complex **197** and halide sources such as CH_2Cl_2 or TMSCl [Eq. (13)].^[147, 148] Among various molybdenum



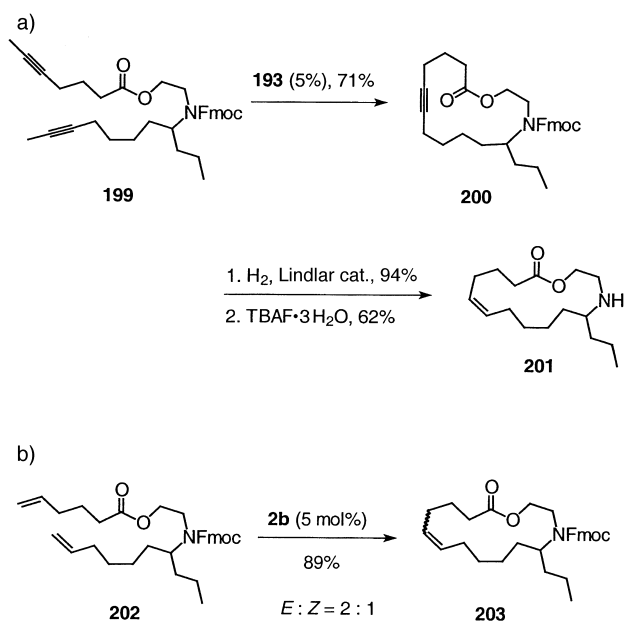
compounds formed during this activation phase, chloride **198** was identified as a catalytically relevant species although it is presently not clear how this compound interacts with the substrates and what the intermediates along the ensuing alkyne metathesis pathway look like. The preparative data, however, show the excellent performance of this novel initiator which tolerates even “soft” donors such as thioethers, crown ether segments, and pyridine rings that are incompatible with $[(t\text{BuO})_3\text{W}\equiv\text{CCMe}_3]$ (**193**).^[147] Table 5 provides a comparison of the three catalyst systems used so far for ring-closing alkyne metathesis.

With these tools in hand, several applications of alkyne metathesis/Lindlar reduction sequences to the synthesis of bioactive targets have been accomplished, including stereoselective syntheses of musk-odored perfume ingredients (civetone, ambrettolide, yuzu lactone),^[144, 145] as well as an application to the insect-repellant azamacrolides epilachnene

Table 5. Comparison of the yields in ring-closing alkyne metathesis reactions with three different catalysts.

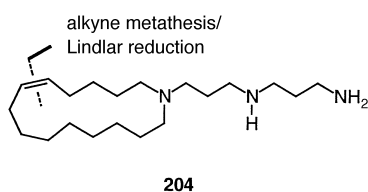
Product	193	$[\text{Mo}(\text{CO})_6] +$ $\text{ClC}_6\text{H}_4\text{OH}$	197 + CH_2Cl_2
	73	64	91
	0		84
	R = H: 62 R = Me: 72	0 64	0 72
	0		88
	55	(decomp)	74

and homoepilachnene.^[144] It is interesting to note that the latter compounds had previously been targeted by conventional RCM (Scheme 43 b).^[98b] Although the macrocyclization **202** → **203** proceeds uneventfully, a mixture of both stereoisomers is obtained, with the unnatural *E* alkene dominating.

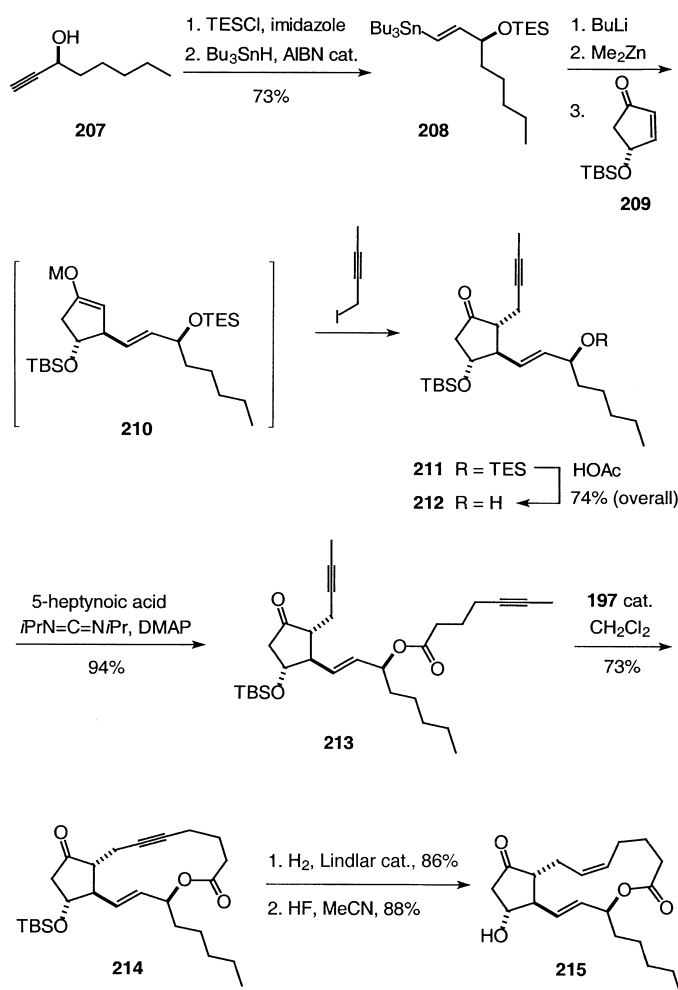


Scheme 43. a) Epilachnene (**201**) is obtained as a pure diastereomer through use of the alkyne metathesis/Lindlar strategy. b) When conventional RCM is employed in the synthesis, the precursor **203** is formed as a mixture of diastereomers.

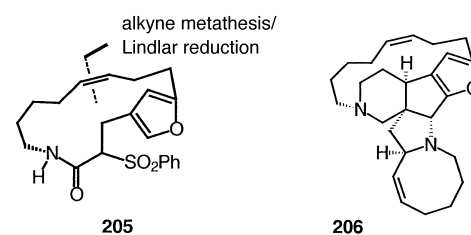
The use of the alkyne metathesis/Lindlar strategy solves this problem and delivers pure epilachnene (**201**) via cycloalkyne **200** as the key intermediate (Scheme 43 a).^[144] The same holds true for a synthesis of motuporamine C (**204**), a cytotoxic spermine-type alkaloid. While RCM affords a mixture of *E* and *Z* alkenes,^[149] ring-closing diyne metathesis followed by Lindlar reduction stereoselectively delivers this natural product in excellent overall yield.^[150]



The most advanced applications of alkyne metathesis reported to date are the preparation of the macrocyclic perimeter **205** of the polycyclic marine alkaloid nakadomarin A (**206**),^[144] as well as an efficient total synthesis of prostaglandin E₂-1,15-lactone (**215**; Scheme 44).^[151] The latter comprises a three-component coupling reaction for the assembly of compound **211**, ring-closing alkyne metathesis of diyne **213** derived thereof by means of the molybdenum-based catalyst **197**/CH₂Cl₂, followed by Lindlar reduction of the resulting cycloalkyne **214** and fluoride-mediated deprotection of the *Z* alkene thus formed. The fact that the labile



Scheme 44. Total synthesis of prostaglandin-E₂-1,15-lactone (**215**) with the alkyne metathesis/Lindlar strategy.



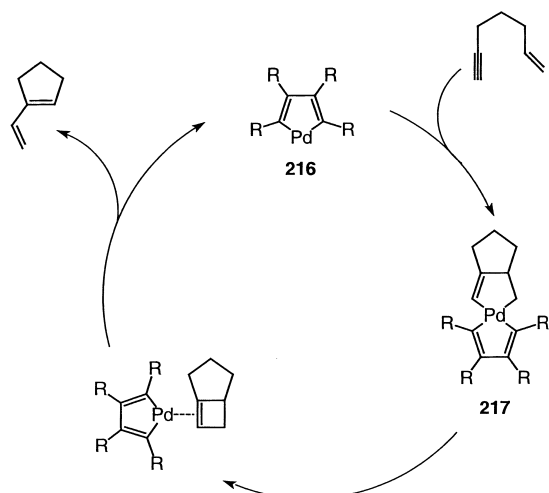
aldol substructure of **213** remains intact and that no epimerization or racemization was observed attest to the mildness of the ring-closing alkyne metathesis step and corroborate the notion that this transformation qualifies for applications to even more advanced targets.^[151]

8. Excursion into the “Non-Chauvin” World

As outlined in the Introduction, metathesis usually follows the mechanistic rationale originally proposed by Chauvin,^[5] who was the first to interpret this reaction as a sequence of formal [2+2] cycloaddition/cycloreversion steps of metal

carbene and metallacyclobutane intermediates. An analogous pathway has been proposed and experimentally corroborated for alkyne metathesis, comprising metal alkylidynes and metallacyclobutadiene derivatives as the relevant species.^[138]

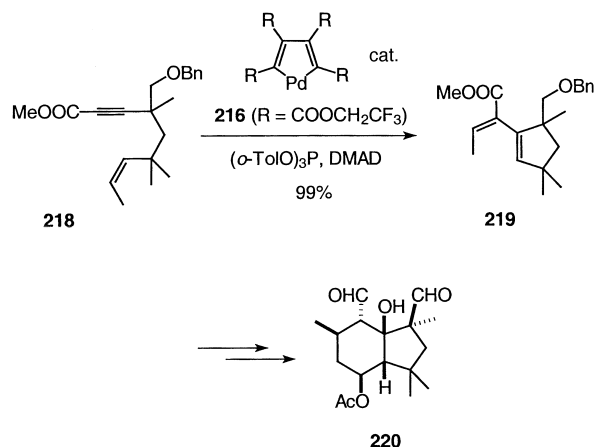
One can, however, envisage other scenarios resulting in formal metathesis events. Redox chemistry such as the Pd^{II}/Pd^{IV} system proposed by Trost and Krische leads to “metathesis” of 1,6- or 1,7-ene-yne substrates (Scheme 45).^[152] In this



Scheme 45. Formal enyne metathesis by a Pd^{II}/Pd^{IV} system.

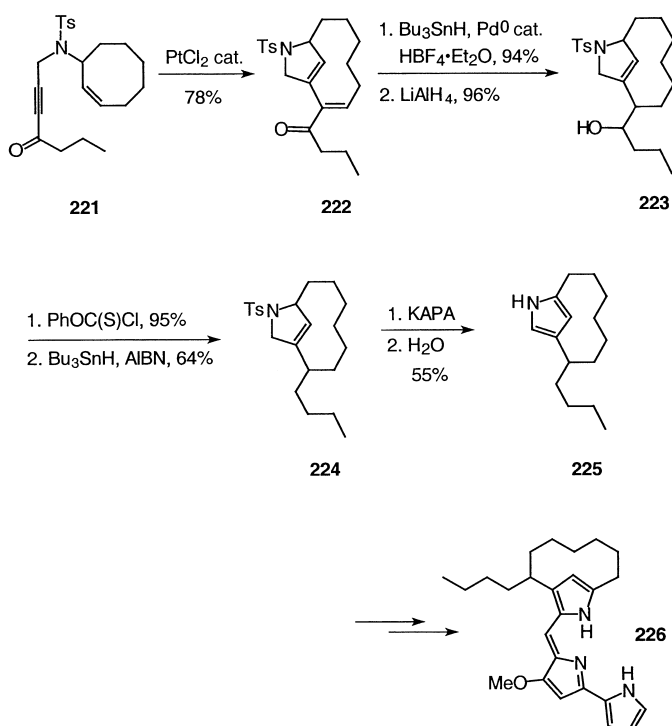
particular case, the metallacyclopentene derivative **217** initially formed undergoes reductive elimination with formation of a cyclobutene which delivers the expected product by electrocyclic ring opening. Evidence for this mechanism comes from the fact that cyclobutene derivatives are occasionally isolable.^[153] A multicomponent catalyst system comprising palladacycle **216** (R = COOCH₂CF₃), tris(*o*-tolyl)-phosphite, and dimethyl acetylenedicarboxylate (DMAD) effects this transformation, provided that the alkyne entity of the substrate bears an electron-withdrawing group.^[154] This reaction has been employed as a key step in an approach to the botrydiane natural product **220** shown in Scheme 46.^[155]

Murai and co-workers have found that PtCl₂ or [{RuCl₂(CO)₃}]₂ are even more practical and atom-economical catalysts for the same type of transformation.^[156] If substrates containing cyclic alkene entities are employed, the preexisting



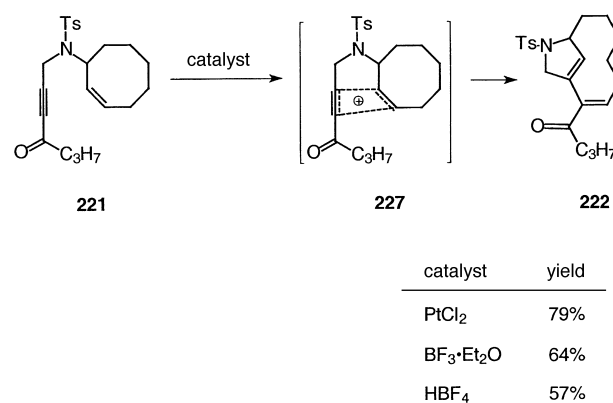
Scheme 46. Enyne metathesis in a natural product synthesis.

ring is expanded and bridged bicyclic products are formed. This reaction has been employed as a key step (**221** → **222**) in a recent total synthesis of the immunosuppressive alkaloid streptorubin B (**226**; Scheme 47).^[157] During the course of this



Scheme 47. Ring expansion by metathesis in the total synthesis of streptorubin B (**226**).

study, it was also possible to shed light onto the mechanism of this unusual transformation which formally cleaves a C=C bond, forms two new C=C bonds, and concomitantly installs a bridgehead alkene function. Thus, careful analysis of some very minor by-products suggests that a carbocation such as **227** is responsible for the formation of product **222**. The formation of this reactive intermediate, however, is not only triggered by coordination of PtCl₂ onto the π system of substrate **221**, but can also ensue from coordination of hard Lewis or Bronsted acids onto the adjacent carbonyl group. In fact, BF₃·Et₂O or even HBF₄ were found to be efficient catalysts for the conversion of substrate **221** into the bicyclic product **222** (Scheme 48). This result can be formally inter-



Scheme 48.

preted as the first example of a “proton-catalyzed enyne metathesis”, although the actual mechanism is a complex Wagner–Meerwein rearrangement.^[157] In any case, it shows that “metathesis” is by no means restricted to anionic systems.^[158] Totally different but largely unexplored scenarios can be envisaged that hold great promise for preparative chemistry.

9. Conclusions and Outlook

Tremendous progress in catalyst design has firmly established metathesis as a mature tool for advanced organic synthesis and polymer chemistry, but this development is not yet over. During the last year, new complexes have been introduced that allow applications which were previously either impossible or, at least, very difficult to achieve. Moreover, the logic of retrosynthetic planning is strongly affected by metathesis since this transformation can be used in a strategic manner for the design of unprecedentedly short and efficient synthesis routes. This is particularly true if it is cleverly combined with other transition metal catalyzed reactions for the formation of the required alkene, diene, or polyene substrates. Likewise, metathesis cascades are highly promising and will almost certainly gain great importance in the near future.

Although metathesis of alkenes will remain the core business, recent developments pinpoint the notion that metathesis of other π systems may also fertilize advanced organic synthesis. This refers in particular to enynes, alkynes, allenes,^[159] and, very probably, even to heterodouble and heterotriple bond systems.^[160] Investigations into this area are just starting. The same holds true for metathesis reactions deviating from the classical Chauvin pathway. In any case, it can be concluded that metathesis in general and olefin metathesis in particular are among the most important advancements in preparative chemistry seen in recent years, although many stimulating discoveries certainly still lay ahead.

Abbreviations

Ac	acetyl
ADMET	acyclic diene metathesis polymerisation
AIBN	2,2'-azobisisobutyronitrile
ARCM	asymmetric ring-closing metathesis
BINOL	2,2'-dihydroxy-1,1'-binaphthyl
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
Bz	benzoyl
CM	cross-metathesis
cod	cycloocta-1,5-diene
cot	cycloocta-1,3,5,7-tetraene
Cp	cyclopentadienyl
Cy	cyclohexyl
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DEAD	diethylazodicarboxylate
DET	diethyltartrate

DMAD	dimethylazodicarboxylate
DMAP	4-dimethylaminopyridine
dtbpm	bis(di- <i>tert</i> -butylphosphino)methane
Fmoc	9-fluorenylmethoxycarbonyl
KAPA	potassium 3-aminopropylamide
LDA	lithium diisopropylamide
NHC	<i>N</i> -heterocyclic carbene
Piv	pivaloyl
PMB	<i>para</i> -methoxybenzyl
RCM	ring-closing metathesis
ROM	ring-opening metathesis
ROMP	ring-opening metathesis polymerisation
solv	solvent
TBAF	tetrabutylammonium fluoride
TBDPS	<i>tert</i> -butyldiphenylsilyl
TBS	<i>tert</i> -butyldimethylsilyl
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TIPS	triisopropylsilyl
TMS	trimethylsilyl
Tol	tolyl
Ts	tosyl (4-toluenesulfonyl)

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Kekulé Escapes, Popper Notwithstanding**

Jerome A. Berson*

1. Does the Philosophy of Science Teach Us How to Do Science?

In ancient Greece, during the age of Aristotle (384–322 B.C.) and Plato (427–347 B.C.), the disciplines of science and philosophy overlapped, often in the work of the same individual. Beginning at the time of Francis Bacon (1561–1626), if not earlier, some philosophers of science have pressed upon us sets of guidelines on how science should be done, and in some cases, have attempted to show that the events, discoveries, theories, and controversies of science—in short, the components of the history of science—correspond to such guidelines. Some authors of elementary science textbooks, often not working scientists themselves, have codified various versions of these guidelines into what they teach as “the scientific method.”

At least partly in response to the imperatives of specialization, the fields separated over the centuries, and scientists, despite such notable exceptions as Descartes, Mach, Bohr, and Einstein, became increasingly ignorant of philosophy. Relationships between the practitioners have now become remote. Indeed, some highly visible and influential scientists^[1, 2] express active hostility toward the enterprise of philosophy, especially toward the philosophy of science. Even those working scientists who do not reject philosophical contributions as a matter of principle, nevertheless give little thought to an activity that once commanded the deep concern of our remote predecessors.

I would argue that, in spite of Weinberg, Feynman, and other skeptics of philosophy, those of us in the community who recognize our own ignorance might want to learn enough about that field to make reasoned decisions about what modern philosophers of science tell us, and to make up our own minds on its relevance to the work of science itself. Perhaps we will conclude that the philosophers are wasting our time and their own, but on the other hand, perhaps we will learn something that may be helpful in our own work. This will require that we familiarize ourselves, at least to some extent, with the major themes in the philosophy of science.

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The present article, necessarily limited in scope, cannot claim to provide a broad view. Instead, it should be seen as a preliminary scrutiny of the interaction of one philosophically derived guideline with the events of actual scientific discovery.

2. Popper Prohibits Ad-Hoc Rescue of Refuted Theories.

I daresay that of the modern philosophers of science, Sir Karl Popper is among the very few whose names are at all familiar to most chemists. In a famous book, *The Logic of Scientific Discovery*, first published in 1934^[3] and followed by a later companion volume,^[4] Popper developed a set of rules for close logical analysis of criteria for the acceptability of theories and of the steps scientists should take to test their validity. The dominant theme of Popper's scientific philosophy is that progress can be made only by a series of *conjectures and refutations*. According to Popper, because no theory can ever be *proven* correct, the major work of the experimentalist is to *falsify*, that is, to prove incorrect, the conjectures of the theorist. We must defer even a cursory examination of these highly influential and highly controversial ideas to a later study, but it may be instructive to examine here one corollary of the method of falsification.

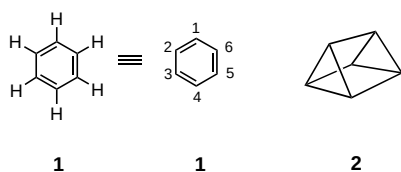
Popper^[3] set down the restriction that, once promulgated, a theory could not be changed in response to experiments that seemed to disagree with it. As a direct consequence of the falsification strategy, this prohibition can be seen simply as fair play, for one cannot hope to land a telling blow on a target that will not stand still. He scorned the “strategems”, especially the *auxiliary hypotheses*, that theoreticians sometimes use to save a theory that appears to be threatened by a seemingly contradictory finding.

“As regards auxiliary hypotheses, we propose to lay down the rule that only those are acceptable whose introduction does not diminish the degree of falsifiability or testability of the system in question, but, on the contrary, increases it.... An example of an unsatisfactory hypothesis would be [one] which had no falsifiable consequences but merely served to restore the agreement between theory and experiment.”

We examine now a famous (and famously fruitful) instance of the rescue of a theory. As we shall see, the intellectual construct put forward for this purpose violated this stricture on auxiliary hypotheses.

3. Kekulé's Benzene.

In 1865, August Kekulé proposed the cyclohexatriene structure for benzene (molecular formula C_6H_6).^[5, 6] In his original publications Kekulé did not actually write down the formula shown as **1**,^[7, 8] but there is no doubt that is what he



had in mind. In his verbal description,^[6] the structure was conceptually generated by cyclization of an open-chain 1,3,5-hexatriene to a symmetrical six-membered carbon ring with each carbon atom attached to a hydrogen atom. Additionally, the hydrogen atoms were explicitly assumed to be equivalent.

Our inquiry focuses on one of the main lines of argument used then to test the theory, namely the concordance of the number of known distinguishable isomers of multiply substituted benzene with the number predicted by the theory.

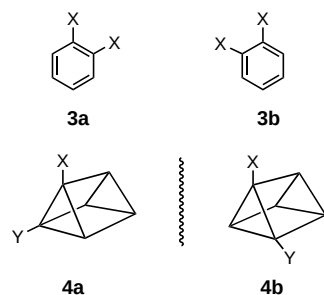
4. Ladenburg's Objection.

Although the initial reaction of the chemical community to Kekulé's theory was quite favorable, by 1869 a serious criticism of the Kekulé's formula had emerged in a discussion by Albert Ladenburg, who had been a laboratory assistant to Kekulé at Ghent. This criticism appeared in the paper^[9] in which Ladenburg proposed the prismatic structure **2** for benzene as an alternative to Kekulé's cyclohexatriene structure. The opening passage of his paper succinctly summarizes the problem:

"Several years ago I already had the opportunity to point out to Mr. Kekulé that the ... graphical formula which he had assigned to benzene does not suffice for the hypothesis, since in it [the] 1.2 and 1.6 [bonds] are not equivalent..."

Thus, the Kekulé structure leads to a difficulty when the molecule is *ortho*-substituted with groups other than H, since now there should be two structural isomers (**3a** and **3b**). Only one *ortho* isomer had ever been observed, regardless of the method of synthesis.

Ladenburg's prismatic structure **2** does not suffer this deficiency. Today we notice, however, that the prismatic structure with two *nonequivalent ortho* substituents, would lead again to two isomers (**4a** and **4b**), but these are



enantiomers (nonsuperimposable mirror images) rather than structural isomers. The nature of enantiomerism was not recognized until five years later, with the advent of Van't Hoff and Le Bel's theory of stereochemistry. Indeed, Van't Hoff himself^[10] pointed out the flaw in Ladenburg's theory soon after.

Even though the prismatic structure thus had its own difficulties, Ladenburg's objection to Kekulé's structure did not depend upon whether the prismatic structure was correct. He had pointed out a very serious obstacle to Kekulé's theory. In fact, his arguments potentially represented a refutation of it.

5. Kekulé's Auxiliary Hypothesis.

It took three years for Kekulé to respond to this challenge.^[11] Significantly, he did not bring into play a number of escape mechanisms he might have used. These included the possibility that the properties of the two isomers called for by his original theory were so similar that the compounds were experimentally indistinguishable. Also, the whole method of isomer numbers, which organic chemists often used for want of a better procedure, was logically vulnerable because the apparent nonexistence of isomers in a number greater than that predicted by the theory is a negative criterion, that is, one could not be sure that further isomers might not be found sometime in the future.

Instead, Kekulé put forward a completely new and unprecedented hypothesis which significantly modified his theory. This hypothesis was later formulated by others as a fast oscillation of the bonds in benzene, so that each double bond became single, and each single bond became double. It is of great interest, as Gero^[12] has pointed out, that Kekulé's proposal in the new modification of his cyclohexatriene theory was not really an oscillation of bonds. We can describe Kekulé's modifying argument only briefly here. It put forward a new theory of the actual bonding forces in benzene, which he believed to be controlled by the rates of collisions of the atoms within the molecule with each other, occurring not randomly, but according to an arbitrary and invariant assigned sequence. He advanced this crucial idea without derivation from existing theory or experiment. The proposal was a breathtakingly daring (but of course erroneous) rationale for the entire physical basis of valence. Its operative consequences *in terms of the experimental tests available in 1872* were the same as that of the bond-switching formulation to which it was later simplified.

Kekulé's new argument amounts to the assertion that he wrote double and single bonds in benzene to keep track of the tetravalence of carbon, not to represent their actual nature. The actual bonds in benzene are assumed not to be like the familiar single and double bonds of the aliphatic compounds. Instead, all the C–C bonds of benzene are *equivalent*. He suggested that the best explanation at the time for this new type of bonding was given by his collision theory.^[13]

The revised theory thus consists of three parts: 1) benzene is cyclohexatriene; 2) despite that, the molecule is regularly hexagonal, with all C–C bonds equal and specifically with $C_1-C_2=C_1-C_6$; 3) the *explanation* of the apparent incompat-

ibility of points 1 and 2 is given by the auxiliary hypothesis of the collision theory. It will be noted that the second hypothesis is itself more vulnerable to the method of isomer numbers than the first, since an experimental finding of two *ortho* isomers would disprove the second hypothesis but merely confirm the first. However, the vulnerability of the second hypothesis is inherent and is not increased by the merely explanatory rescuing hypothesis of the collision theory. The revision of Kekulé's benzene theory thus violates Popper's requirement that "only those [auxiliary hypotheses] are acceptable whose introduction does not diminish the degree of falsifiability or testability of the system in question, but, on the contrary, increases it."

Kekulé's brilliant strategem not only had no theoretical justification at the time but would not find any for another sixty years, until the advent of the quantum mechanical concept of delocalization in the 1930s. It was simply a device for salvaging the cyclohexatriene structure. Walter Hückel^[14] called it a "hypothesis of embarrassment."

The history of organic chemistry shows that even though this theory was not really understood by most organic chemists of the 19th century, it was applied nearly everywhere. The tremendous flowering of synthesis and the discovery of an abundance of new reactions and structures during that time all took place in an atmosphere of growing conviction that, *for whatever reason*, the C₁–C₂ and C₁–C₆ bonds of benzene were structurally equivalent, as Kekulé had said. Even Ladenburg eventually agreed that the Kekulé structure probably was superior to the prism or other alternatives that had been proposed.

6. Theory Confronts Experiment in a Specific Time Period.

In Kekulé's time such techniques as X-ray crystal structure analysis, electron diffraction, vibrational (IR and Raman) spectroscopy, and NMR spectroscopy, which today provide direct information about molecular symmetry and actual bond lengths and convince the modern chemist that the hypothesis of equivalent C–C bonds in benzene is true, lay decades in the future. By Popper's rules, Kekulé's benzene theory should have been abandoned forthwith as invalid on the grounds of its unfalsifiability.

Popper's demand for expanded testability of any theory rescued by an ad-hoc hypothesis does not take into account the historical context of the moment when the hypothesis was put forward. How soon should we discard such a theory? Is it not possible that at a future time, as happened in the benzene

case, predictions made or implied by the theory may become testable?

7. Conclusions

The issue of whether a theory is logically testable can hardly be of great concern to the working chemist. In our daily encounters with nature we are guided by all kinds of theories, some tested by experiment, some logically testable but not yet tested, *and some not testable by any procedure known or conceivable at the time*. The progress of chemical research cannot be reasonably expected to stand still until enough time has passed for the means finally to emerge that can test a theory by the falsification protocol. Thus, our decision on whether to continue to use the theory must be based upon pragmatic considerations: Does it predict new phenomena or rationalize a body of information?

That Kekulé's theory eventually was shown to be testable was of no help to the chemists of the late 19th century. In fact, had the organic chemical community applied Popper's requirement and abandoned Kekulé's benzene formula, much of the development of the subject in that period would have been retarded. Because the "no ad-hoc rescues" rule is central to the falsification strategy, such cases reveal reasons why we should resist attempts to force our research efforts onto this Procrustean bed.

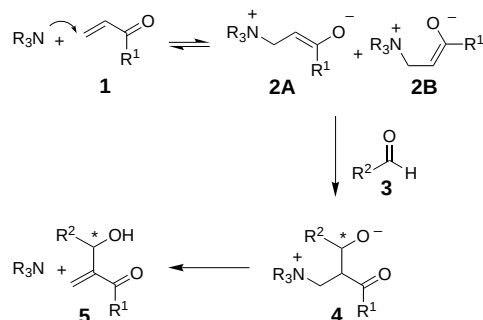
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New Strategies for the Development of an Asymmetric Version of the Baylis–Hillman Reaction**

Peter Langer*

1. Variations, Preparative Scope and Mechanism of the Baylis–Hillman Reaction

The stereoselective formation of carbon–carbon bonds is an important challenge in organic chemistry. The Baylis–Hillman reaction allows the direct preparation of α -methylene- β -hydroxycarbonyl compounds by base-catalyzed reaction of α,β -unsaturated carbonyl compounds with aldehydes.^[1–3] The first step of this reaction involves nucleophilic attack of the catalyst on the Michael acceptor **1** leading to the formation of the zwitterionic intermediate **2**. Subsequently, this intermediate reacts in the rate-determining step of the Baylis–Hillman reaction with the aldehyde **3** to give the alcoholate **4** (Scheme 1). The product **5** is finally formed by a



Scheme 1. The mechanism of the Baylis–Hillman reaction.

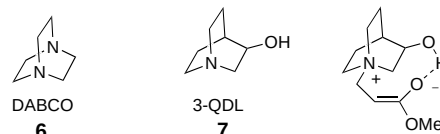
shift of a proton from the α -carbon to the oxygen atom of the alcoholate and extrusion of the catalyst.^[4] The densely functionalized Baylis–Hillman products can be stereoselectively transformed, for example, into azirines,^[5] epoxides,^[6] triols^[7] and *anti*-aldol products.^[8] In addition, α -methylene- β -hydroxycarbonyl compounds are versatile starting materials for the synthesis of a variety of natural and nonnatural target molecules.^[9, 10]

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Unfortunately, the applicability of the Baylis–Hillman reaction is very often limited by low rates and conversions and low, highly substrate-dependent yields. It is particularly important for the development of an efficient asymmetric version of the Baylis–Hillman reaction that these problems are adequately addressed. The use of high pressure or of the microwave technique resulted in a significant increase in the rate of the reaction; however, only for a few substrates.^[11] Increasing the reaction temperature above 20 °C resulted in polymerization of the sensitive acrylates. Recent work by Leahy and co-workers suggested that, counter-intentively, better yields and higher rates were observed at lower temperatures.^[12] These results can be explained by the different rates of the formation of the diastereomeric base–acrylate adducts (**2A** and **2B**).

The rate and the conversion of the Baylis–Hillman reaction was significantly improved when nucleophilic non-hindered bases, such as diaza[2.2.2]bicyclooctane (DABCO, **6**), rather than simple tertiary amines were used. Further

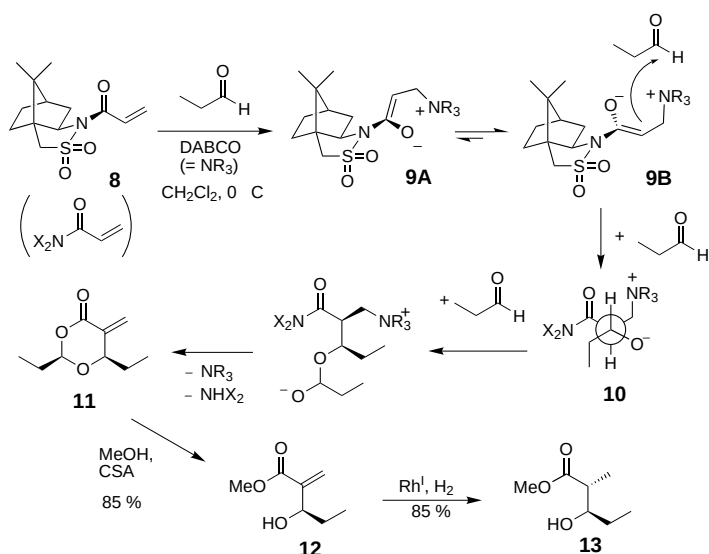


improvements were observed when 3-quinuclidinole (3-QDL, **7**) was employed, due to stabilization of the zwitterionic intermediate **2** by formation of intramolecular hydrogen bonds.^[13a–c] Similar effects were observed by the addition of methanol^[13d] or acetic acid^[13e] to the reaction mixture (formation of intermolecular hydrogen bonds) or by the presence of a hydroxy group in the acrylate.^[13f] The rate of the reaction was decreased by the presence of bulky substituents in the α -position of tertiary amines. This was explained by the decrease in the rate of the addition of the catalyst to the acrylate.^[14] Recent work by Aggarwal and co-workers has shown that very good rates and chemical yields could be obtained by using the non-nucleophilic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).^[15] However, the use of enolizable ketones led to formation of aldol products. The success of the use of DBU was explained by the assumption that the reaction of the zwitterionic intermediate with the acrylate rather than the attack of the catalyst on the acrylate

represents the rate-determining step of the reaction: although DBU represents a sterically hindered base, the zwitterionic intermediate is stabilized by delocalization of the positive charge. The concentration of the intermediate in the equilibrium is increased and, hence, the overall rate of the reaction is enhanced. However, this explanation is contradictory to the Curtin–Hammett principle. A stabilization of the zwitterionic intermediate and a significant enhancement of the rate of the reaction was also induced by the use of metal salts, such as $\text{La}(\text{OTf})_3$ and LiClO_4 (OTf = triflate = trifluoromethanesulfonate).^[16]

2. Asymmetric Baylis–Hillman Reactions of Chiral Michael Acceptors

During the course of the Baylis–Hillman reaction two stereocenters are formed, one of which remains in the Baylis–Hillman product. An obvious concept for the development of an asymmetric version of the reaction lies in the use of an enantiomerically pure acrylic acid derivative. The use of enantiomerically pure menthyl acrylates resulted, however only in certain cases, in respectable diastereomeric excesses.^[17] A significant improvement was reported in 1997 by Leahy and co-workers who used the Oppolzer sultame as a chiral auxiliary in DABCO-catalyzed Baylis–Hillman reactions (Scheme 2).^[18] In this reaction, the 1,3-dioxan-4-one **11** was obtained, which was transformed by methanolysis into



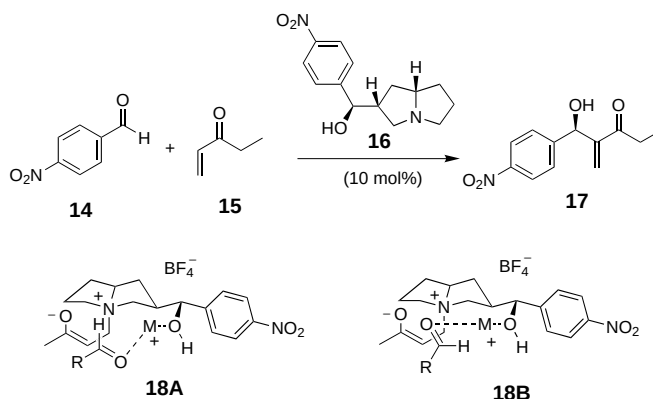
Scheme 2. DABCO-catalyzed Baylis–Hillman reaction according to Leahy et al. CSA = camphorsulfonic acid.

the α -methylene- β -hydroxyester **12** which was subsequently diastereoselectively hydrogenated to give the *anti*-aldol product **13**. The esters **12** were isolated in good yields. However, 15 equivalents of the aldehyde were required. The stereoselectivity can be explained by the following: Michael addition of the catalyst to the acrylate **8** results in formation of a *Z*-enolate which mainly resides in the *anti*-conformation **9B**, since in this case the dipole-repulsion between the sulfonyl and the carbonyl group is minimized. Owing to the steric interaction with the axial oxygen atom of the sulfonyl group,

the attack of the aldehyde proceeds diastereoselectively from the *re* side of the acrylate to give the adduct **10**, which subsequently reacts with a second aldehyde molecule to give a hemiacetal. Extrusion of the catalyst and cyclization with extrusion of the chiral auxiliary finally afforded the 1,3-dioxan-4-one **11**.

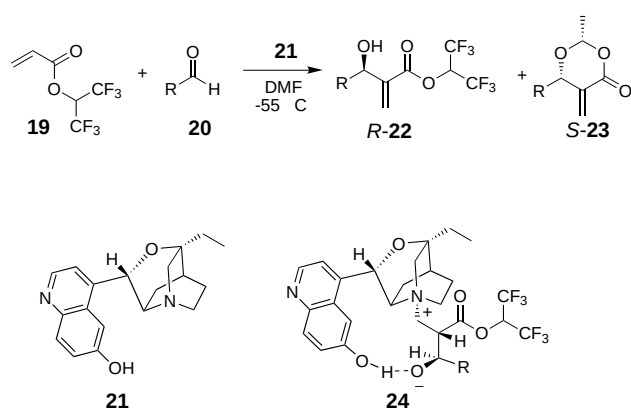
3. Asymmetric Baylis–Hillman Reactions of Achiral Michael Acceptors

Much work related to the development of a catalytic, enantioselective version of the Baylis–Hillman reaction by the use *chiral bases* has been reported. Only low enantiomeric excesses were obtained when brucin, *N*-methylprolinol, *N*-methylephedrine, and nicotine were employed. The use of cinchona alkaloids and of enantiopure 3-QDL **7** resulted in a significant increase of the rate of the reaction, but only in low enantiomeric excesses which decreased when the reactions were carried out under pressure.^[1b,17,19a] Enantiomeric excesses of 9–44% *ee* were obtained in the reaction of pyrimidine-5-carbaldehydes with acrylates using (*S*)-BINAP as the catalyst.^[19b] Enantioselectivities of 21–70% *ee* were observed in the reaction of ethyl- and methylvinyl ketone with aromatic aldehydes **14** using the chiral hydroxypyrrolizidine catalyst **16**, which was prepared in four steps starting from BOC-L-prolinol (Scheme 3).^[20] The enantioselectivity was explained by the predominant formation of intermediate **18A**, which is less sterically hindered than the isomeric intermediate **18B**. The employment of a reaction temperature of -40°C , the use of NaBF_4 as a cocatalyst, and the presence of a hydroxy group in the base (which allows the formation of intramolecular hydrogen bonds) resulted in good conversions and rates.



Scheme 3. Asymmetric Baylis–Hillman reaction using a chiral hydroxypyrrolizidine catalyst.

Very good enantioselectivities were recently reported by Hatakeyama and co-workers.^[21] The reaction of a variety of aldehydes **20** with the highly reactive 1,1,1,3,3,3-hexafluoroisopropylacrylate **19** using modified cinchona alkaloids as the catalyst resulted, at a temperature of -55°C , in the formation of the Baylis–Hillman products **22** in 31–58% yields with 91–99% *ee* (Scheme 4). The use of the tricyclic derivative **21**, which was prepared from quinidine in one step,^[22] proved crucial for obtaining high enantioselectivities. The success of catalyst **21** can be explained by the (compared with quinidine)

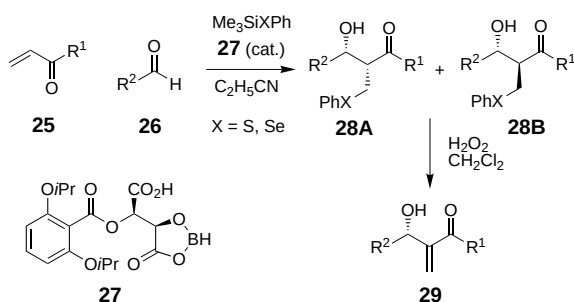


Scheme 4. Asymmetric Baylis–Hillman reaction using a chiral cinchona alkaloid catalyst.

increased nucleophilicity, by the presence of a free hydroxy group at the quinoline moiety, and by the *anti-open* conformation^[23] of the alkaloid which allow an optimal stabilization of the zwitterionic intermediate **24** by formation of intramolecular hydrogen bonds. Using the Oppolzer auxiliary (see above) only low enantioselectivities could be obtained for aldehydes branched at the α -position. In contrast, the Baylis–Hillman products derived from isobutyric aldehyde and cyclohexane carbaldehyde could be prepared in 31 and 36 % yields with 99 % *ee* when the catalyst **21** was used. A disadvantage of the method of Hatakeyama et al. results from the decrease of the yields due to formation of the dioxanones **23**, which were generated with opposite absolute configurations and with lower enantioselectivities compared with the products **22**. However, the formation of these undesired side products was helpful for the elucidation of the mechanism of the reaction.

4. Alternative Syntheses of Nonracemic Baylis–Hillman Products

An alternative approach for the synthesis of nonracemic Baylis–Hillman products was reported by Barrett and co-workers. In a two-step synthesis, which was preparatively equivalent to an asymmetric Baylis–Hillman reaction, α -methylene- β -hydroxyketones **29** were prepared with 34–94 % *ee* (Scheme 5).^[24] The overall yields of the sequence ranged between 18 (X = S, R¹ = Me, R² = Et) and 52 % (X = Se, R¹ = Me, R² = Me). In the first step, the enantioselective condensation of the α,β -unsaturated ketone **25** with the aldehyde **26** and trimethylsilylphenyl sulfide or selenide



Scheme 5. Baylis–Hillman analogous reaction according to Barrett et al.

afforded the diastereomeric β -hydroxyketones **28A** and **28B** with 63–97 % *ee*. This reaction was catalyzed by the chiral acyloxyborane **27**. The Baylis–Hillman products **29** were subsequently prepared by oxidative elimination using *m*-chloroperoxybenzoic acid or H₂O₂. The enantioselectivities of this step were in the range of 50 to 96 % *ee*.

Outlook

Many groups have contributed to the development of an efficient and enantioselective Baylis–Hillman reaction. All this work leads the way to the development of a useful and broadly applicable methodology in the future. In this context, the design of new catalysts based on the modification of cinchona alkaloids could play an important role. Besides the optimization of the enantioselectivities, the increase of the chemical yields, the conversions, and the reaction rates continue to represent important issues, particularly for “difficult” substrates.

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Nanoporous Structures by Design

Michael J. Zaworotko*

Whereas the development of reliable methods for crystal-structure prediction remains an elusive scientific challenge,^[1] its relevance to aspects of pharmaceutical and materials science has fueled rapid development of the concept of crystal engineering.^[2] Is crystal engineering synonymous with crystal-structure prediction? The simplest answer is, not yet. However, crystal engineering has emerged as a paradigm for the design and synthesis of new solid phases because its fundamental precept is that most crystal structures can be regarded as self-assembled entities,^[3] that is, they are “supermolecules par excellence”.^[4] In other words, crystal engineering can be regarded as being synonymous with supramolecular synthesis.

Crystal engineering has thus far focused upon chemical entities that are predisposed to form predictable, self-assembled networks that is, molecules or ions that are exofunctional and capable of forming 1D, 2D, or 3D networks. Crystal structure prediction is therefore simplified to an issue more related to the design of networks. That such a strategy can be successful is perhaps best exemplified by the recent observation of several new classes of open framework solids that exhibit the property of microporosity.

Zeolites represent the prototypal microporous solids, have channels and cavities of 4–10 Å,^[5] and have found widespread application in catalysis and separations.^[6] The first generation of synthetic “zeolite analogues” that were generated by crystal-engineering strategies consisted of rigid 3D networks based upon organic or metal–organic moieties. However, these zeolite analogues typically suffered from the phenomenon of interpenetration^[7] or from the irreversible collapse of the structure upon the removal of the guest molecules. Subsequent studies revealed a second generation of frameworks that are more closely analogous to zeolites in

that they are robust enough to survive the complete loss of the guest molecules and that they reversibly adsorb small volatile molecules. Interestingly, such structures can be purely organic and sustained by hydrogen bonds^[8] or they can be based upon coordination-polymer frameworks.^[9] However, these compounds do not compare to the inorganic (zeolite) structures in terms of either their surface area or their thermal stability.

Very recently, a third generation of synthetic frameworks that exhibit hitherto unseen levels of porosity, so called nanoporosity, and stability has been discovered. These structures are exemplified by the octahedral polymer $[\text{Zn}_4\text{O}(\text{bdc})_3]$ (bdc = benzene-1,4-dicarboxylate).^[10] $[\text{Zn}_4\text{O}(\text{bdc})_3]$ is a relatively simple and inexpensive material to prepare, is remarkably stable to loss or exchange of guests, and remains crystalline at temperatures above 300 °C. The feature that makes $[\text{Zn}_4\text{O}(\text{bdc})_3]$ special is that it exhibits a relative degree of porosity hitherto unprecedented in crystalline solids. Recently Kitagawa and co-workers reported the structure and porous nature of $[\text{Cu}(4,4'\text{-bipy})_2](\text{SiF}_6)$ (**1**).^[11] (bipy = bipyridine). Complex **1** is the latest addition to this new family of nanoporous materials and is important because of its implications for future design principles and because of its properties.

Design Principles

It would be natural to assume that 3D architectures present a higher level of complexity than 1D and 2D architectures. In many ways 3D architectures represent the ultimate challenge to crystal engineers since success leads directly to the control and prediction of crystal structures. It is therefore somewhat ironic that two of the simplest examples of predictable networks are exemplified by 3D networks, that is, those generated by the assembly of tetrahedral or octahedral nodes. Tetrahedral nodes are predisposed to generate diamondoid architectures, whereas octahedral nodes are expected to afford octahedral networks.

Diamondoid architectures using a tetrahedral metal as the node and cyanide ligands (CN^-) as the spacer represent

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prototypal examples of diamondoid coordination polymers. The compounds $\text{Zn}(\text{CN})_2$ and $\text{Cd}(\text{CN})_2$ form diamondoid networks with twofold interpenetration.^[12] However, $\text{Cd}(\text{CN})_2$ can also be obtained as a single network in which CCl_4 fills the cavity.^[12c] This result illustrates two principles that have broad implications: 1) interpenetration can be avoided in the presence of an appropriate template or guest molecule, and 2) such compounds might be regarded as catenated and noncatenated supramolecular isomers^[13] of each other.

Octahedral coordination polymers are exemplified by Prussian Blue, which is in a sense an octahedral version of $\text{Cd}(\text{CN})_2$. Prussian Blue avoids interpenetration because the cavities in the framework are filled by counterions. Synthetic octahedral networks were first reported in 1995. The complex $[\text{Ag}(\text{pyrazine})_3]\text{SbF}_6^{[14]}$ is sustained by octahedral Ag^I cations and pyrazine ligands that are relatively short compared to other bridging ligands; the framework is necessarily cationic. The zinc analogue of **1** $[\text{Zn}(4,4'\text{-bipy})_2](\text{SiF}_6)$ is a neutral framework and isostructural with **1**.^[15] Both these compounds represent a slightly different approach to building 3D structures, that is, the manipulation of existing 2D structures by cross-linking. Such a strategy is feasible if one selects an appropriate 2D structure that has functional groups aligned in the vertical direction and that resembles the “pillaring” effect in clays. Compound **1** can be regarded as having been generated from square grid coordination polymers^[16] that are cross-linked by $\mu\text{-SiF}_6$ anions. The SiF_6^{2-} counterions cross-link the square grids made by Zn centers and 4,4'-bipy ligands to form a rigid pseudooctahedral polymer with very predictable shape and dimensions (Scheme 1). Furthermore,



Scheme 1. From square grids to octahedral polymers. ● octahedral moiety (i.e. metal), — linear bifunctional ligand alternate linear ligand (e.g. anion).

the wall-like 4,4'-bipy ligands make it impossible for the structure to interpenetrate. Perhaps the most salient feature of **1** from a design perspective is that not only is the structure rational, it is entirely predictable. Indeed, **1** crystallizes in space group $P4/mmm$ with $Z=1$. In other words, the point group at the Zn and Cu centers (D_{4h}) is propagated into the space group symmetry. Furthermore, the cell parameters are effectively predetermined by the intermetallic separations.

Properties

The channels in **1** have an effective cross-section of approximately $8 \times 8 \text{ \AA}$ and represent almost 50% of the volume of the crystal. Solvent molecules are readily eliminated and, remarkably, the structure remains intact at 373 K

under vacuum. The pseudooctahedral framework has a large surface area that remains accessible to guest molecules (Figure 1). Indeed, the surface area of **1** is such that its

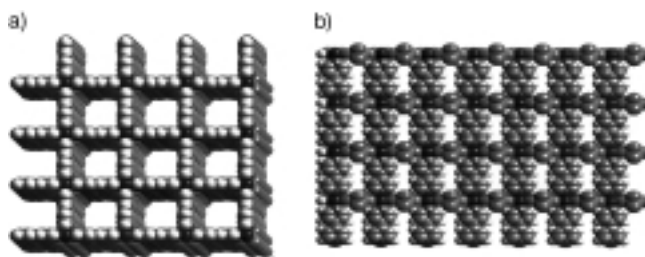


Figure 1. $[\text{Cu}(4,4'\text{-bipy})_2](\text{SiF}_6)_n$ —an octahedral coordination polymer with large channels. a) *ab* plane. b) *ac/bc* planes

capacity for methane gas adsorption is significantly greater, almost double, that of zeolite 5A, which had previously been the solid with the highest measured methane adsorption capacity.^[11] Indeed, **1** and $[\text{Zn}_4\text{O}(\text{bdc})_3]$ (approximately 60% of the structure is available and accessible in $[\text{Zn}_4\text{O}(\text{bdc})_3]$) offer significant increases over the typical accessibility of about 30% seen in zeolites.^[5]

The Future

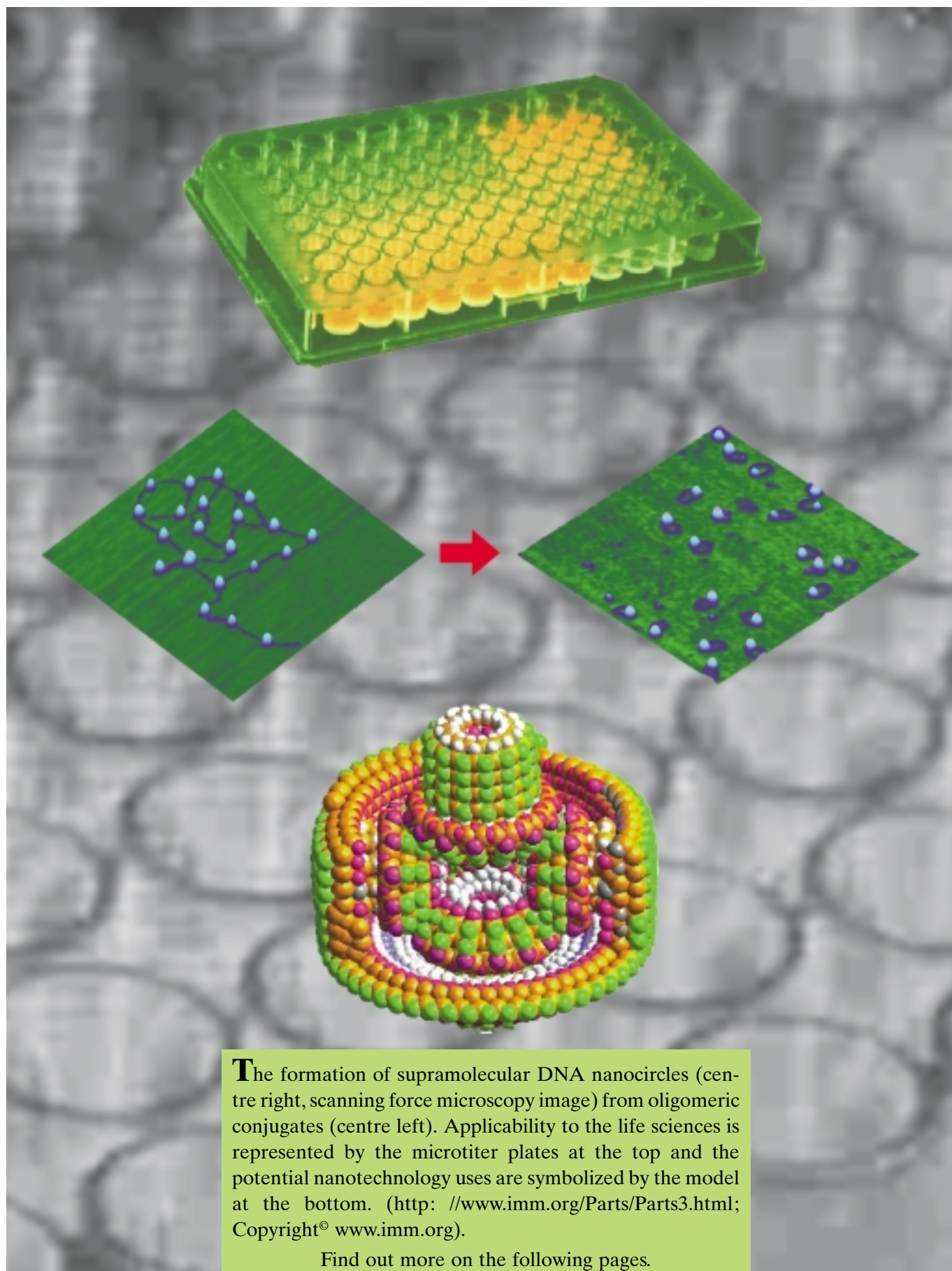
The ability to design networks from first principles affords a method to control and fine tune the shape and molecular recognition features of porous structures. Even for pseudooctahedral networks the diversity of components that are readily available and the means by which they can assemble spans the full range of chemistry. A work of M. C. Escher (Figure 2) offers a perspective that perfectly illustrates the situation—in principle, any octahedral moiety (for example a metal or a metal cluster) can serve as a node and the “spacer” can be a wide range of readily available ligands. It therefore seems



Figure 2. M. C. Escher's “Cubic Space Filling” reprinted with permission from Cordon Art B.V. copyright © Baarm, Holland 2000. All rights reserved.

plausible to assert that we have thus far only observed the prototypes of what should become a wide range of synthetic, designed coordination polymers. Furthermore, nanoporosity represents just one possible property of such solids. As the size of channels and cavities increases, the possibility of porous structures that can hold medium to large organic compounds and biomolecules becomes evident. We could therefore see applications in the areas of green chemistry (for example nanoscale reaction flasks for catalysis) and materials science (biocomposite materials for sensors, polar inclusion compounds). And what about crystal structure prediction? It remains a challenge of the highest order and has great relevance in its own right. However, it is not a prerequisite for the design of new structures. In the words of von Schnering, "The peasant who wants to harvest in his lifetime cannot wait for the ab initio theory of weather".^[17]

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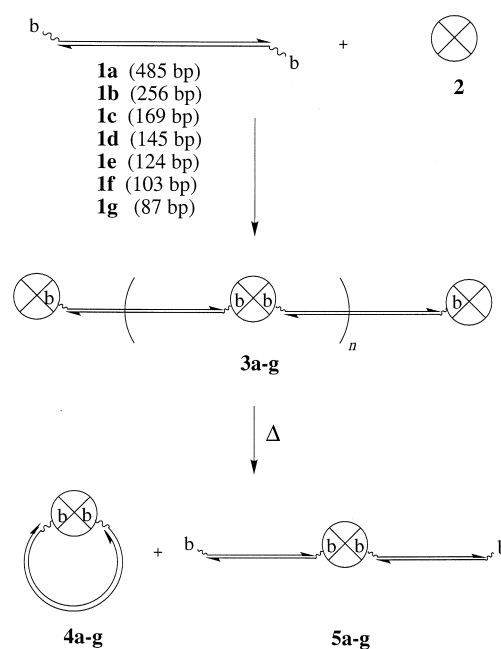
Supramolecular Nanocircles Consisting of Streptavidin and DNA**

Christof M. Niemeyer,* Michael Adler, Song Gao, and Lifeng Chi

The self-assembly of small molecular building blocks programmed to form larger nanometer-sized, structural and functional elements is an important goal of molecular nanotechnology.^[1] Due to its unique and simply predictable recognition capabilities, high physicochemical stability, mechanical rigidity, and high precision processing ability, DNA is a promising construction material for the fabrication of nanostructured systems.^[2, 3] As an example, the work of Seeman et al. indicates that DNA can be used to synthesize nanostructured scaffolding,^[2b,c] surface architecture,^[2d,e] and, even, nanomechanical devices.^[2f] Moreover, the highly specific hybridization of complementary nucleic acids can be used to selectively position macromolecules, such as proteins^[4] and metal nanoclusters.^[4b, 5]

We have recently reported on the synthesis of nanostructured oligomeric conjugates **3**, which can be obtained by the self-assembly of bisbiotinylated DNA **1** and the biotin-binding protein streptavidin (STV, **2**).^[6] Scanning force microscopy (SFM) analysis of the oligomers **3** revealed that the STV preferentially functions as a bi- or trivalent linker molecule between adjacent double-stranded DNA (dsDNA) fragments, despite its binding capacity for four molecules of biotin. Thus, linear and two-dimensionally branched supramolecular chains are formed (Scheme 1). Due to the remaining biotin-binding capacity, the DNA–STV oligomers **3** are powerful reagents for immuno-polymerase chain reactions (immuno-PCR),^[7] a highly sensitive method for the trace analysis of proteins and other antigens. We here report on the synthesis of novel supramolecular DNA nanocircles **4** which can conveniently be generated by thermal treatment of the oligomers **3**.

During the exploitation of the supramolecular DNA–STV oligomers **3** we intended to investigate their thermal denaturation response. For this purpose, oligomers were prepared by coupling of STV (**2**) with varying molar equivalents of a 256 base pair (bp) dsDNA fragment **1b**. In the case of a molar excess of STV the typical fingerprint-like band pattern was visible by gel-electrophoretic analysis representing the population of the supramolecular oligomers **3**. In contrast, an excess of dsDNA led to the formation of the typical blurry band of the high molecular weight oligomers in addition to



Scheme 1. Synthesis of supramolecular DNA–streptavidin nanocircles **4**. Oligomeric DNA–STV conjugates **3a–g** are prepared from 5',5'-bisbiotinylated double-stranded DNA (dsDNA) of various lengths **1a–g** and streptavidin (STV, **2**). Note that the schematic drawing of **3** is simplified, since branch points occur due to the presence of tri- and tetravalently conjugated STV (see Figure 2d). The oligomeric conjugates **3** are heated for two minutes at 95 °C and subsequently cooled down rapidly in an ice bath. As a consequence, supramolecular DNA nanocircles **4a–g** and linear DNA₂–STV conjugates **5a–g** are formed as the chief products. For simplification, complementary DNA strands are drawn as parallel lines. The 3'-ends are indicated by the arrow heads. It is unclear whether the two STV-bound dsDNA fragments are conjugated to adjacent or opposite subunits of the tetrameric STV. However, the model of Sinha and Chignell suggests that binding to opposite subunits should lead to more stable complexes.^[19]

distinct bands indicating the presence of free dsDNA and DNA–STV conjugates of varying stoichiometry, such as DNA₂–STV (Figure 1, lanes 1–4).^[6] The various preparations of **3b** were denatured by heating to 95 °C for two minutes, and subsequently, the samples were rapidly cooled in the ice bath. This treatment led to a significant simplification of the electrophoretic band patterns and the products were formed to a varying extent (Figure 1, lanes 5–8). The chief products are the dsDNA₂–STV conjugate **5b** (position d in Figure 1) and the nanocircle **4b** (position b).^[8] A comparison of lanes 4 and 8 indicates that free **1b** is reformed only when an excess of dsDNA was present initially (position a). Quantification of the bands by densitometry revealed that the formation of **4b** proceeded most efficiently (60 % yield) in the case of an initial 1:2 coupling ratio of DNA:STV.

Further studies showed a strong dependency of the product formation on the educt concentration. Increasing the concentration of **3** from 0.2 to 0.4 μM led to a decrease in the relative amount of **4** to about 45 %. An increase in the concentration of **3** to 1 μM further decreased the product yield to about 20 % of **4**, and, instead, high molecular weight oligomers were formed. The cooling rate subsequent to thermal denaturation of **3** also influenced the formation yields of the nanocircles. For example, the slower cooling rate of 0.5 °C min^{−1} led to the

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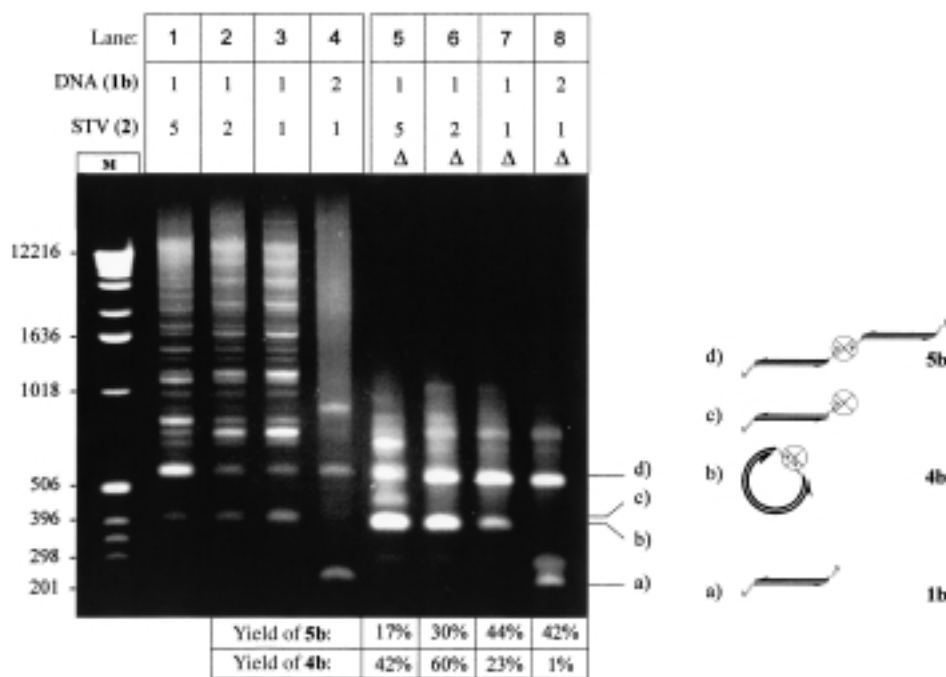


Figure 1. Electrophoretic analysis of the synthesis of nanocircle **4b**. A nondenaturing 1.5% agarose gel stained by ethidiumbromide is shown. Lanes 1–4 contain the oligomeric DNA–STV conjugates **3b**, prepared from varying coupling stoichiometries of STV and dsDNA **1b**. Thermal denaturation and rapid cooling in an ice bath leads to the transformation of the various preparations of **3b** to the low molecular weight products. The main products are the linear conjugate DNA₂–STV **5b** and the nanocircle **4b** (lanes 5–8). The dependency of the product formation on the initial coupling stoichiometry of DNA:STV is demonstrated by the percentage yields indicated below lanes 5–8. The numbers were obtained by densitometrical quantification of the band intensities. Lane M contains a DNA molecular weight marker; the lengths are given in base pairs.

predominant formation of highly oligomerized DNA–STV products, and the yield of **4** was decreased to about 35%. The quantitative oligomerization, indicated by the appearance of an immobile band in the gel electrophoretic analysis, was obtained by the slow cooling of a 1 μ M solution of **3**. The subsequent denaturation and rapid cooling of this oligomeric network, led to the reformation of products **4** and **5**, respectively. These results, which concern the dependency of the product formation on the temperature and concentration, were in agreement with earlier reports on the self-assembly of oligovalent building blocks.^[9, 10]

To further investigate the phenomenon of the circle formation, we modified the length of the dsDNA fragments in **3**, which led to the formation of nanocircles **4** of miscella-

neous size (**4a–g**). A comparison of the rates of product formation (Table 1) indicated that the yield of **4** decreased with a reduced length of the dsDNA. Instead, higher molecular weight products and free dsDNA were formed. This result should be due to the greater rigidity of short dsDNA fragments which counteracts intramolecular cyclization.^[11]

To characterize **4**, the cyclic structure was established by means of a Ferguson plot.^[12] In this analysis, the electrophoretic mobility of linear and cyclic DNA–STV species is plotted against the density of the gel matrix. The plot of the linear supramolecules revealed an identical slope of the species **5a–c**, while the slopes of the nanocircles **4a–c** grew with increasing length of the dsDNA. This observation allows one to estimate that the friction constant of nanocircles **4a** and **4b** is greater than the analogous linear conjugates **5a** and **5b**, while **4c** has a smaller friction coefficient than **5c**.^[13] Moreover, a comparison of the Ferguson plots of, for example, **5b** and a linear

500 bp dsDNA fragment indicates that the coupling of the STV molecule leads to a decreased friction constant. Probably, this is a consequence of the enhanced flexibility of the two DNA moieties linked by the central STV molecule in **5** compared to the rigid DNA double helix.

Samples of the nanocircles **4** were purified by electrophoresis and analyzed by SFM (Figure 2). The SFM visualization allowed clear verification of the supramolecular cyclic structure and the equimolar coupling ratio of the nanocircles for DNA sizes down to the 103 bp dsDNA of circle **4f**. Statistical analysis of the SFM images indicates that more than 90% of the supramolecules reveal the characteristic cyclic structure. The ring diameters measured by SFM correlate well with the theoretical values. However, the

Table 1. Yields and dimensions of the nanocircles **4**

dsDNA length [bp]	a	b	c	d	e	f	g
	485	256	169	145	124	103	87
amount of 4 prior to denaturation [%] ^[a]	30	10	10	4	2	<1	<1
amount of 4 after denaturation [%] ^[a]	77	60	62	49	50	52	28
amount of 5 after denaturation [%] ^[a]	20	30	25	12	13	11	17
diameter of 4 [nm] ^[b]	61	29	22	17	15	15	–
deviation [%] ^[b]	21	14	9	6	4	2	–
calculated diameter [nm] ^[c]	54.6	29.9	20.4	17.8	15.5	13.2	11.5

[a] Determined by densitometrical analysis of the gel electrophoretic band intensities; the average deviation is about $\pm 5\%$. [b] Determined from the scanning force microscopy images. The number of individual structures measured was more than 50. The data indicate the average arithmetical values and the percentage deviation of the average ring diameters. [c] Calculated from the length of the dsDNA fragments with assumption of a B-DNA conformation. The diameter of the STV was estimated to be 4.6 nm^[20] and the length of the two alkyl spacers between the DNA and the STV was estimated as 2 nm.

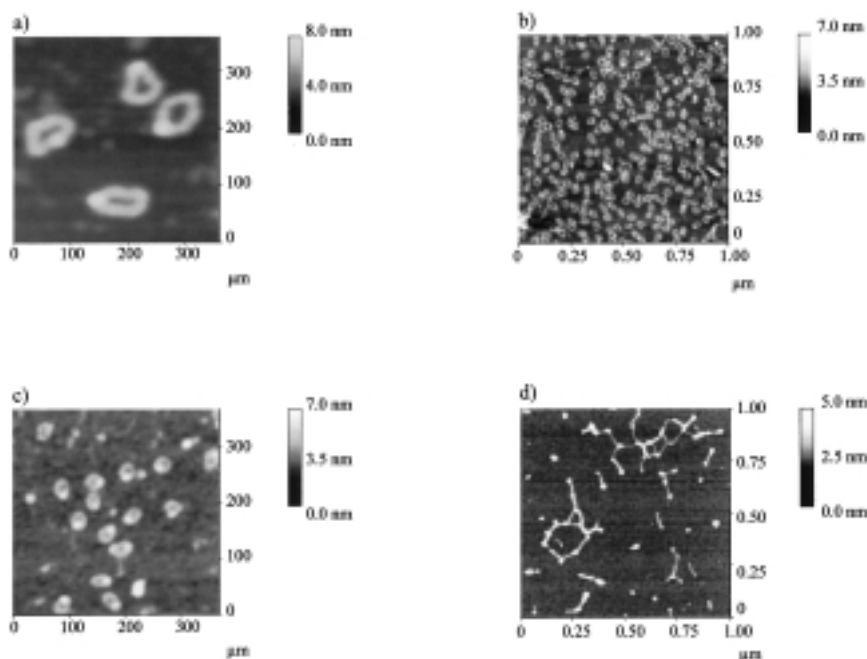


Figure 2. Scanning force microscopy (SFM) images of the DNA nanocircles **4a** (a), **4b** (b), **4c** (c), and the oligomeric DNA–STV conjugate **3b** (d).

average deviation of the various diameters which were determined decreases with reduced ring size (Table 1). This is most likely a consequence of the increasing ring tension within the small circles. As a consequence, the structure of **4c** is more similar to an ideal circle than that of **4a** (Figure 2).

The reasons for the dependency of product distribution on the initial coupling stoichiometry, as well as the mechanism of nanocircle formation, are as yet vague. However, it is known that the denaturation temperature of ligand-saturated STV is 112 °C.^[14] Thus, it seems probable that during thermal denaturation a quasi transition state species exists that consists of STV conjugated with two biotinylated single-stranded DNA molecules; these DNA molecules can intramolecularly hybridize, provided that they are complementary to each other. From the statistical point of view, however, only 50 % of the bivalently conjugated STV molecules in the quasi transition state carry two complementary single-stranded DNA moieties. Thus, the product yields of **4** that are up to 77 %, suggest that other mechanisms might contribute to the nanocircle formation. Denaturation of **3** in the presence of free D-biotin leads to the displacement of the dsDNA from the STV. This indicates that the interaction between the biotinylated dsDNA and STV becomes labile at elevated temperatures, which suggests that ligand exchange reactions might also contribute to the formation of **4**.^[15]

We have reported here on the simple and efficient synthesis of novel supramolecular DNA nanocircles **4**, obtainable by thermal denaturation and rapid cooling of the oligomeric DNA–STV networks **3**. The generation of DNA circles and their potential use as building blocks for the fabrication of nanostructured elements has previously been reported.^[10, 16, 17] This work extends the preceeding approaches since the endogenous STV molecule within the nanocircles can conveniently be used as an anchor group for further functional-

ization.^[4b, 18] Work regarding the modification and lateral organization of the nanocircles is under way.

Experimental Section

A solution of STV (24 μL, 1.0 μM) in Tris buffer (10 mM, pH 7.3) containing ethylenediaminetetraacetate (EDTA, 2 mM) was mixed with a solution of the bisbiotinylated dsDNA **1c** (6.8 μL, 1.75 μM) in the same buffer. The final concentration of dsDNA was 0.39 pmol μL⁻¹. The solution was incubated for 15 min at room temperature on a vertical shaker and, subsequently, diluted with the same Tris buffer to a final concentration of 0.2 μM of DNA. The oligomeric DNA–STV conjugate was denatured for 2 min at 95 °C and the solution was cooled to 0 °C in the ice bath. Analysis and purification of the products was achieved by nondenaturing gel electrophoresis on an agarose gel. The agarose concentration varied according to the length of the dsDNA fragment: 1 % (DNA > 400 bp), 1.5 % (400–150 bp), or 2.2 % (150–80 bp). The gel is stained with ethidiumbromide and the main product bands are cut out. The products are eluted from the gel slices by centrifugation for 10 min at 5000 rpm using a Micropure 0.22 μm filtration device (Millipore). Immobilization of **4** on mica and SFM analysis are carried out as previously described.^[6]

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Dynamic Observations of the Hydrolysis of a DPPC Monolayer at the Air/Water Interface Catalyzed by Phospholipase A₂**

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Many chemical reactions, especially those in the crystalline state, depend on the local environment of the reactants. Hence in order to study their mechanism one has to investigate these reactions as a function of local order, and in addition, the change of order should be measured as the reaction proceeds. For this purpose amphiphile monolayers are highly suited, because their order can be varied and characterized, and the chemical reaction can be followed by newly developed spectroscopic and microscopic techniques. In addition the reactants can be exchanged with the adjacent phase in a controlled way.

One biologically and technologically important system is the phospholipase A₂ (PLA₂) that catalyzes the hydrolysis of a phospholipid monolayer at an interface. It is known that this reaction depends upon the state of the monolayer. It is now possible to study the molecular arrangement within monolayers, even in individual domains, with microscopic resolution. For this investigation the main methods used are surface X-ray diffraction, FT-IR spectroscopy, and Brewster angle microscopy (BAM). These techniques are used here to follow the enzyme reaction in detail. The correlation of changes in the molecular arrangement with catalytic activity should provide an understanding of reaction mechanisms.

PLA₂ is a calcium-dependent enzyme abundant in living organisms. The enzymatic reaction with the membrane surface consists of a molecular recognition process and a cleavage reaction.^[1] PLA₂ stereoselectively hydrolyzes the *sn*-2 ester linkage of L-phospholipids to release fatty acids and lysophospholipids (Figure 1a). The activity of PLA₂ at the interface of aggregated substrates, such as phospholipid monolayers, is 10000-fold greater than with the corresponding monomeric substrate.^[2] PLA₂ has an α -helix-enriched conformation in aqueous solution (Figure 1b). During an enzymatic reaction a change in this conformation may occur as soon as the enzyme reaches the interface.

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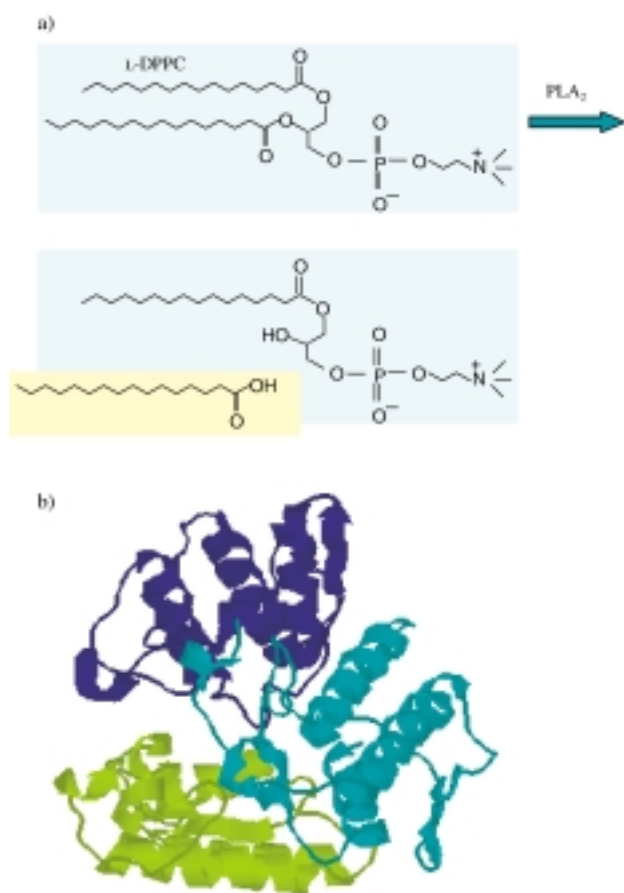


Figure 1. a) Schematic representation of the hydrolysis of an L-DPPC monolayer by phospholipase A₂ (PLA₂). b) Representation of the PLA₂ structure.

The BAM method opens a new possibility for the direct visualization of the texture of Langmuir monolayers.^[3] It allows the long-range orientational order of a phospholipid in a monolayer to be characterized from the optical anisotropy induced by the tilted aliphatic chains.^[4]

The results of the measurement of surface pressure as a function of time show that PLA₂ first causes an increase in surface pressure (Figure 2a). The pressure increase directly after the injection of the enzyme corresponds to the adsorption of PLA₂ onto the L-DPPC monolayer. The rate of the pressure increase ($d\pi/dt$) can be related to the adsorption rate which is proportional to the area change dA/dt according to [Eq. 1].

$$\frac{d\pi}{dt} = \frac{d\pi}{dA} \times \frac{dA}{dt} \quad (1)$$

An increase in the initial slope ($d\pi/dt$)₀ with pressure in the ratio 1.7:3.4:6.0 is observed whereas if $d\pi/dA$ is deduced from Figure 2b the ratio is 10:6:34. A comparison of the highest and lowest pressures at which the film is homogeneous shows that the pressure change is proportional to the change in $d\pi/dA$ and indicates a constant adsorption rate. For medium pressure, where the film is inhomogeneous, this proportionality does not hold because macroscopic and local compressibility deviate. In conclusion the adsorption rate is not determined by the free area of the surface of the film, but by

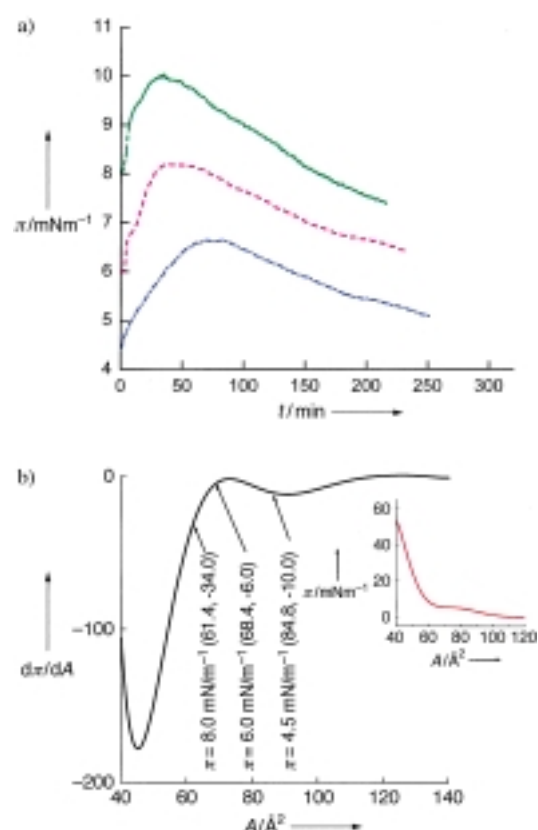


Figure 2. a) Variation of the surface pressure π as a function of time t . PLA₂ was injected into the subphase of the L-DPPC monolayer at different initial pressures: 4.5 (blue), 6.0 (red), 8.0 (green) mN m⁻¹. b) The slope $d\pi/dA$ of the DPPC isotherm (shown as an insert) as a function of molecular area A .

transport in the bulk phase to the interface. The cleavage reaction that follows adsorption explains the low maximum surface pressure value at lower starting pressure and why the increase in the surface pressure is limited. On the other hand it was found that the surface pressure could reach the same maximum value, of about 10 mN m⁻¹, for a D-DPPC monolayer after PLA₂ adsorption but without enzymatic cleavage.^[5] It was shown by FT-IR spectroscopy that of the reaction products the fatty acid remains at the interface and the lysophospholipid is partially dissolved into the solution.^[6] Hence, the area demand of the lipid at around 50 Å² per molecule is reduced to that of 20 Å² for the fatty acid, and the surface pressure should decrease.

Polarization-modulated infrared reflection absorption spectroscopy^[7] (PM-IRRAS) measurements of an L-DPPC monolayer revealed that PLA₂ has its maximum activity in the region where liquid-expanded (LE) and liquid-condensed (LC) phases coexist region.^[6] Micrographs of the morphology of L-DPPC monolayers attacked by PLA₂ in this phase-transition region show that the hydrolysis reaction starts at the interface between the liquid-expanded and condensed phases and progresses in the condensed phase.^[8] Defects in the packing form the preferential reaction sites. Depending on the sample's history^[9] the reaction starts either in the domain or at the edge. Domains damaged in the inner part, such as in the so called "pizza" type, were observed just 2 min after the

injection of the PLA_2 ($C_{\text{PLA}_2} = 0.975 \mu\text{g mL}^{-1}$, $\pi_0 = 8.0 \text{ mN m}^{-1}$; Figure 3). The lysolipid is one of the reaction products and leaves the interface, this therefore increases the free space available. As a consequence, the fraction of the ordered region becomes smaller, which is reflected in the change of domains (Figure 3).

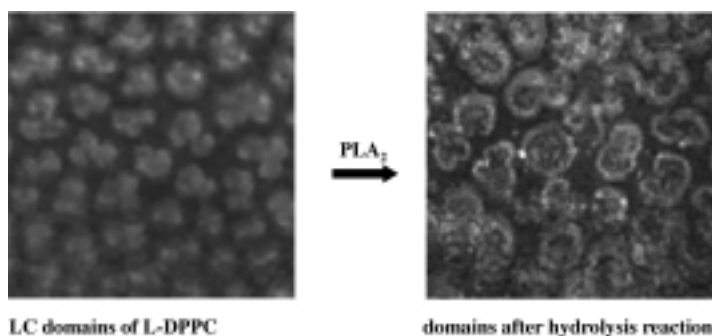


Figure 3. BAM images of a L-DPPC monolayer at the air/water interface before the injection of PLA_2 into the subphase and after the hydrolysis reaction.

The surface pressure decreases because of the loss of material at the interface, this again allows the penetration of the enzyme and a corresponding increase in pressure. The interplay of these factors results in the observed variation of surface pressure. It should be noted that in the absence of PLA_2 the size of the domains, not their number, changes with pressure. Enzyme addition in the absence of hydrolysis (D-enantiomer) does not affect the shape of the domain although the enzyme preferentially partitions at the domain boundaries.^[5]

Figures 4a–4d show the change of the shape of L-DPPC domains after the injection of the enzyme at an initial pressure of 7.5 mN m^{-1} . The shape of the L-DPPC domains (dark area corresponds to condensed phase) starts to change 300 s after the injection of the PLA_2 . The decrease in the area of the condensed-phase domains is attributed to the cleavage reaction. As shown in Figure 4b, there are two typical domain shapes: C- and O-form. The density of the C-form domains

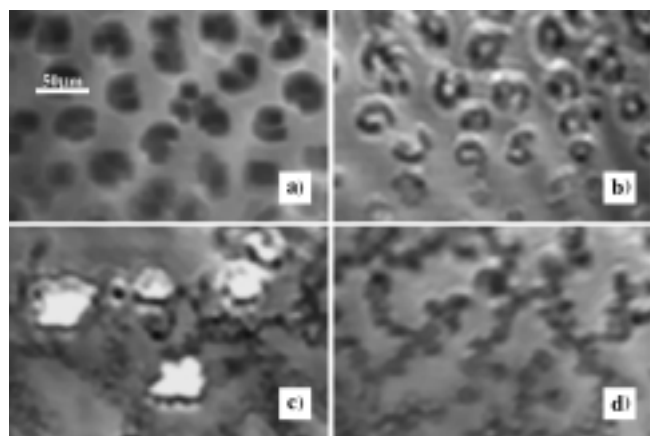


Figure 4. BAM images of an L-DPPC monolayer at the air/water interface during cleavage by PLA_2 with an initial surface pressure of $\pi_0 = 7.5 \text{ mN m}^{-1}$. For details see main text.

decreases drastically during the reaction, but their shape hardly changes. Therefore, we assume that the direction of hydrolysis in the C-form domains is along the curved domain boundaries. The long-range tilt orientation in the phospholipid monolayer has already been studied by BAM.^[4] From the morphology of the pure L-DPPC monolayer it can be deduced that the orientation of the molecules in the elongated domains changes continuously along the boundaries and consequently follows the curvature of each segment.^[10]

It can be deduced from the experiment, when the orientation of the molecules is considered, that the enzyme preferentially hydrolyzes the parts of the condensed domains with the same molecular chain orientation. If molecules with the same orientation (or reflectivity) are preferentially cleaved, C- or S-form domains are produced. The explanation is that the hydrolysis of molecules with the same orientation requires only a minimal change in the conformation of the enzyme during the cleavage. Figure 4b also displays O-shaped domains and indicates that the enzyme has hydrolyzed the central part of the domain, where, because of defects, the lipid molecules are less ordered. Thus, hollow O-form domains are produced. Finally, the domains can connect with each other to form a network.

A new kind of domain is observed about 80 min after the injection of the enzyme (Figure 4c). The shapes of these domains are very different from those of the DPPC monolayer and they have a relatively high reflectivity and are much brighter. The number of these domains increases with time and can be ascribed to the fact that the enzyme forms its own domains. This result is in good agreement with fluorescence microscopy studies on other proteins.^[11] After 120 min, the mixed film is expanded and then recompressed. The domains now have a star shape (Figure 4d). To understand the transformation of the domains, we have to note that the presence of the enzyme causes a change of the line tension at the domain boundary^[12] and that the monolayer is now a mixture of substrates and reaction products. One of these products remains at the interface as a fatty acid salt and probably forms phase-separated crystallites.

To show that the hydrolysis reaction first occurs in the condensed (LC) phase, the monolayer was compressed at a higher pressure. Figure 5 shows the BAM images taken during

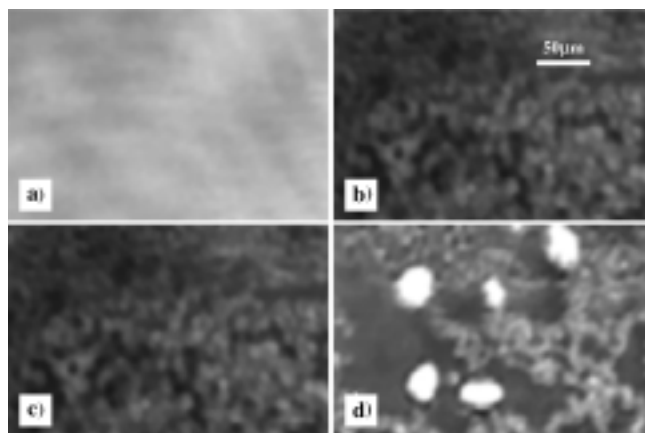


Figure 5. BAM images during the enzymatic reaction with an initial pressure of 15 mN m^{-1} . For details see main text.

a reaction at an initial pressure of 15.0 mNm^{-1} . The monolayer is a homogeneous LC phase at this pressure (Figure 5a). After the injection of the enzyme, morphology changes caused by the hydrolysis reaction are observed (Figure 5b). Unlike the reaction in the biphasic region, the cleavage reaction takes place randomly in the monolayer. A homogeneous film is converted into a network, the holes in the monolayer gradually enlarge, and the shape of the edges becomes irregular (Figure 5c). At this higher pressure, the enzyme began to form its own domains only an hour after injection (Figure 5d).

The first step of the hydrolysis reaction is the surface recognition process and includes the adsorption of the enzyme onto the lipid surface. X-ray studies have shown that the tilt angle of the phospholipid molecules changes upon adsorption of specific enzymes.^[5] This means that a change in the orientation of the lipid molecules is induced by the specific interaction between the lipid and the enzyme. As a result, the inner structure of the domains is different to that of the pure lipid. In addition to specific forces, long-range electrostatic forces can attract protein molecules to the domain boundary because the dipole moments normal to the surface of lipids and proteins are different.^[11] This electrostatic force can also affect the orientation of the lipid molecules. In the interfacial recognition process at the start of the hydrolysis reaction both the enzyme as well as the phospholipid molecules could change their conformation.

In conclusion we have shown that enzyme penetration, the hydrolysis reaction, and the dissolution of the reaction products into the bulk phase determine the enzyme-catalyzed hydrolysis of phospholipids. These three processes can be separated by studying well-defined monolayers using BAM and film balance techniques. We anticipate that these results observed for the reaction of PLA₂ with L-DPPC can be generalized for a whole class of interfacial bioreactions. This is possible because the bilayer membrane is generally regarded to be a fluid phase. However, strong variations in the density and order of the membrane can lead locally to domain boundaries as discussed here. The amphiphile monolayers were used here as models to study stereospecific reactions in general, because they enable structural control and the microscopic follow up of reactions at interfaces. Although these monolayers are in an ordered state they allow access to the reactants through exchange with the adjacent bulk phase.

Experimental Section

A high-resolution Brewster angle microscope (Optrel, Germany) was mounted onto a computer-interfaced Langmuir trough (R&K, Germany) with a film balance. The reflected light was detected by an analyzer and a CCD camera. The output signal was recorded on a video recorder. The images were captured afterwards through a frame grabber and processed by using special software to adjust the contrast and to correct the image distortion caused by observation at the Brewster angle. The surface tension was measured within 0.1 mNm^{-1} by the Wilhelmy method. The enzyme solution was injected into the subphase with a small syringe.

L-DPPC, D-DPPC, and PLA₂, from *Crotalus atrox* Venom, were purchased from Sigma and used without further purification. Chloroform (99%) was purchased from ACROS. In all experiments the subphase was an aqueous buffer solution at pH 8.9, which contained 15 mM NaCl, 5 mM CaCl₂, and 10 mM Tris. Experiments were performed at a temperature of $20.0 \pm 0.1^\circ\text{C}$.

The concentration of the PLA₂ in the subphase was 0.714 mgmL^{-1} . All the water used in the experiments was purified by a Milli-Q system.

In the hydrolysis experiment, a 1 mM solution of L-DPPC in chloroform was spread on the surface of the buffer. The monolayer was compressed to the desired initial pressure and then the enzyme solution was injected into the subphase. The film pressure, and morphology of the formed domains were recorded simultaneously, the isotherm was recorded after 120 min.

In the experiment with D-DPPC monolayers, the monolayer was spread by the same method and slowly compressed to the coexistence region. Then the enzyme was injected into the subphase. After 60 minutes, the monolayer was expanded until the LC phase disappeared. The BAM images were recorded during the subsequent recompression. In all experiments the compression rate was $2.5 \times 10^{-2} \text{ nm}^2$ per molecule per minute.

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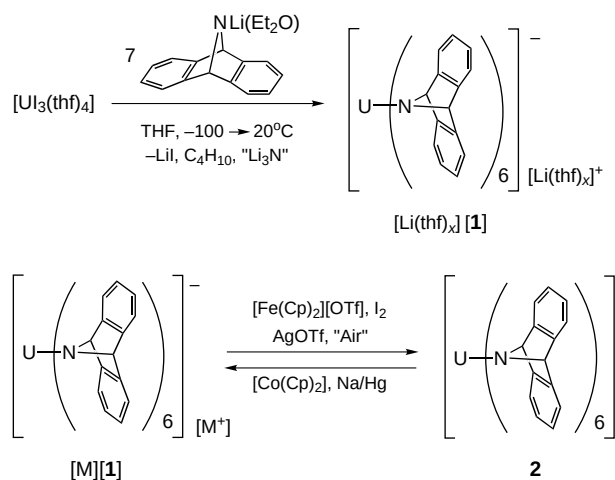
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Uranium Hexakisamido Complexes**

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Here we report on the synthesis and characterization of the unique hexakisamidouranium(v) anion $[U(dbabh)_6]^-$ (**1**, Hdbabh = 2,3:5,6-dibenzo-7-azabicyclo[2.2.1]hepta-2,5-diene^[1]) and its one-electron oxidation product, namely the neutral homoleptic uranium(vi) derivative $[U(dbabh)_6]$ (**2**). Employed previously in the synthesis of terminal nitrido derivatives of chromium(vi), the dbabh ligand is redox-active such that elimination of anthracene from a complex containing it effects a two-electron oxidation.^[2] The latter feature is critical to the present synthesis of anion **1**, a synthesis beginning with uranium(III) and employing anthracene elimination as the means for oxidation to uranium(v).

Accordingly, treatment of a thawing solution of $[UI_3(thf)_4]^{[3]}$ in THF with a cold ethereal suspension of $[Li(dbabh)(OEt_2)]$ (7 equiv) provided an orange crystalline precipitate of the lithium salt $[Li(thf)_x][1]$ in 85% yield. Anthracene (ca. one equivalent per mol uranium) was shown by ¹H NMR spectroscopy to be a by-product of the reaction. Additionally, the yield of anion **1** was found to be maximized when seven equivalents of $[Li(dbabh)(OEt_2)]$ were employed, supporting the stoichiometry for the reaction as proposed in Scheme 1. Recrystallization of $[Li(thf)_x][1]$ from a saturated



Scheme 1. Synthesis of the homoleptic hexakisamidouranium(v) and (vi) complexes. Cp = C₅H₅, Tf = SO₂CF₃.

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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.

solution of $[PPh_4][Br]$ in dichloromethane/hexane at $-40^\circ C$ afforded cube-shaped, exceedingly air-sensitive but thermally stable crystals of the salt $[PPh_4][1]$.

The hexakisamidouranium(v) complex $[Li(thf)_x][1]$ was found to be EPR-silent at room temperature, but its X-band EPR spectrum in a dilute frozen acetonitrile/toluene solution at 20 or 100 K evinced a single broad isotropic signal centered at $|g| = 1.12$ (Figure 1).^[4] The isotropic g value observed for **1** is unexceptional given that other pseudo-octahedral uranium(v) complexes exhibit a similar parameter, some exam-

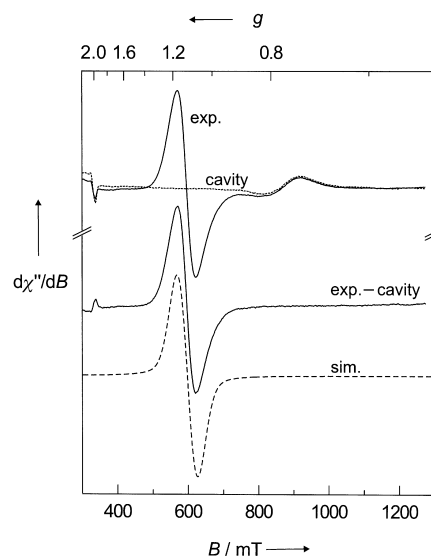


Figure 1. X-band EPR spectrum of $[Li(thf)_x][1]$ recorded in CH₃CN/toluene at 20 K. Shown are the measured spectrum (exp.), that of the sample chamber (cavity), the difference between the two spectra (exp. – cavity), and the simulated spectrum (sim.).

ples being $[UCl_5(SOCl_2)]$ ($g_{calcd} = -1.18$, $|g_{exp}| = 1.1(15)$) and $[U(OEt)_5]_2$ ($g_{calcd} = -1.16$).^[5] SQUID magnetometry revealed the magnetic moment for microcrystalline $[nBu_4N][1]$ to be $1.16 \mu_B$ in a temperature range between 5 and 35 K.^[6] The low g value together with the magnetic susceptibility data are characteristic of an ion with a 5f¹ electron configuration in a cubic crystal field, perturbed additionally by spin-orbit coupling.^[7] In contrast, it was shown recently that low-symmetry organometallic derivatives of uranium(v) supported by η^5 -C₅H₅ or η^8 -C₈H₈ ligation exhibit highly anisotropic g values ranging from 3.3–0.7.^[8]

While salts of the paramagnetic anion **1** are extremely air-sensitive, the corresponding neutral uranium(vi) derivative $[U(dbabh)_6]$ (**2**) is remarkably robust and is the only detectable product of air-oxidation of anion **1**. Salts of anion **1** were seen to exhibit a one-electron oxidation wave at -1.01 V versus the ferrocene/ferrocenium couple (see Supporting Information). Chemical oxidation of **1** thus was conveniently accomplished with silver triflate, a regimen providing **2** in 95% yield from $[Li(thf)_x][1]$. Recrystallization from toluene at $-40^\circ C$ over a few days delivered **2** as dark purple prisms.

Single-crystal X-ray diffraction studies were carried out for salt $[PPh_4][1]$ ^[9] and for neutral **2** (Figure 2).^[10] In both cases, two crystallographically independent but chemically equiv-

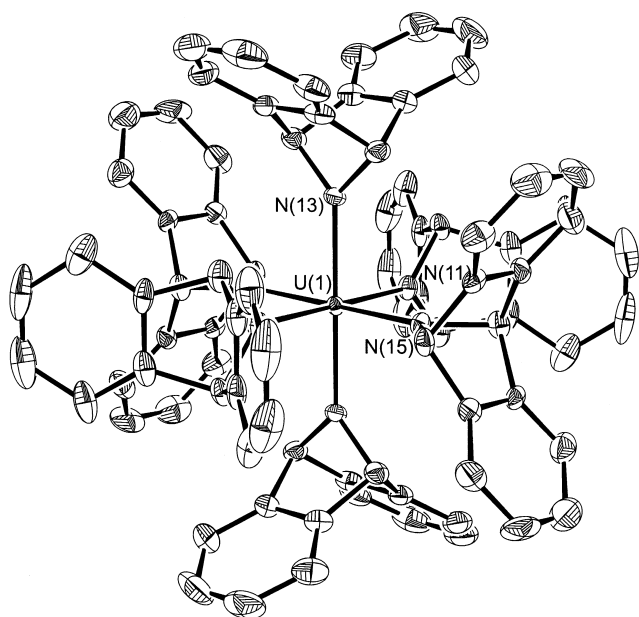


Figure 2. Molecular structure of $[U(dbabh)_6]$ (**2**) (see text for details).

alent molecules were found to be present in the asymmetric unit. Both uranium atoms in **1** and **2** reside at crystallographic inversion centers, such that there are three crystallographic U–N distances per uranium center. The six amido nitrogen atoms in the uranium(v) and (vi) species define a near-perfect octahedron, the U–N distances ranging from 2.230(11) to 2.267(13) Å in anion **1** and from 2.178(6) to 2.208(5) Å in neutral **2**. The observation of slightly shorter bond lengths in **2** than in **1** is explicable in terms of the higher formal oxidation state for the uranium center in the former. N–U–N angles for **1** and **2** are 90° to within experimental error, in accord with the point group symmetry T_h , which is approximated by the $[U(dbabh)_6]^{0/1-}$ molecules. A point of interest with respect to ligand conformation is that the angles about each of the nitrogen atoms sum to 360(11)° in **1** and 360(5)° in **2**, such that the dbabh ligands are disposed favorably for π donation to the respective uranium centers.

A single ligand environment is evidenced in the solution NMR spectra of **2**, consistent with the molecule's T_h symmetry.^[11] The line widths in the ^1H NMR spectra of **2** were determined to be less than 1.5 Hz and the observed shifts are in a range typical for organic derivatives of the ligand, such as H(dbabh) or Br(dbabh).^[1] On the other hand, it is found that the ^1H NMR spectra of mixtures of diamagnetic **2** and salts of paramagnetic anion **1** represent an average of the two independent spectra. This phenomenon, being indicative of self-exchange electron transfer in the **1/2** couple, warrants further investigation.

Two distinct absorption bands are observed in the electronic absorption spectrum of **2**, one at 353 nm ($\epsilon = 2200 \text{ M}^{-1} \text{ cm}^{-1}$), the other at 501 nm ($\epsilon = 1200 \text{ M}^{-1} \text{ cm}^{-1}$) (see Supporting Information).^[12]

Group theoretical considerations and density functional theory (DFT) calculations indicate that in anion **1** the ground state involves population of a nonbonding $5f_{xyz}$ orbital by the unpaired electron, while in neutral **2** the HOMO is triply

degenerate and consists of amido to uranium π -bonding molecular orbitals.^[13] In the latter orbitals, the character is largely nitrogen 2p, but contributions from uranium 5f and 6d functions also are indicated (Figure 3).

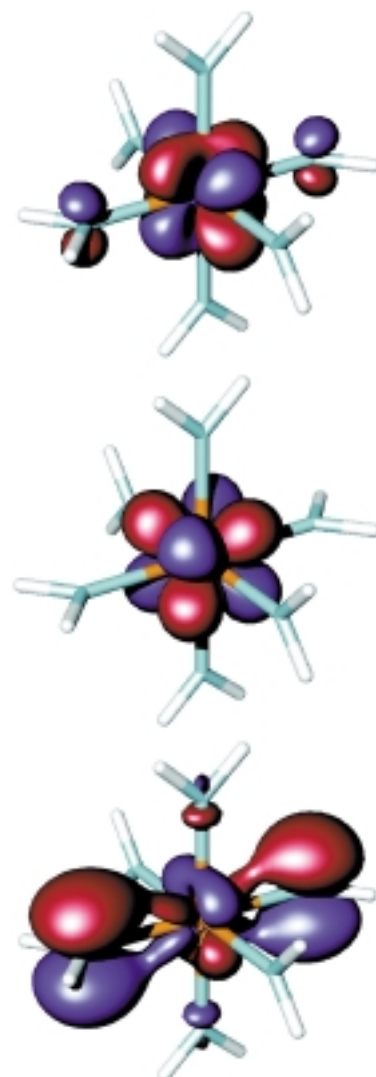


Figure 3. Antibonding LUMO (top), SOMO (middle), and HOMO (bottom) of the model $[U(NH_2)_6]^-$ ion (LUMO and HOMO are threefold degenerate).

Anion **1** and its redox partner, neutral **2** are unique in uranium chemistry for being isolable hexakisamido complexes, and also for providing a robust redox couple in which the addition or removal of an electron is accompanied by minimal structural change. Other examples of hexakisamido-uranium derivatives include $[U(NMe_2)_6]$, but the latter evidently has not been isolated, being characterized only by its proton NMR spectrum.^[14] Hexakisamido complexes are more common in transition metal chemistry, being exemplified by $[M(NMe_2)_6]$ ($M = Mo, W$);^[15, 16] however, the latter neutral complexes are not known to have well-defined anionic counterparts. The stability of $[U(dbabh)_6]$ in two states of charge is reminiscent of the behavior of other robust couples that retain their structural integrity, such as the archetypal ferrocene/ferrocenium couple.

Experimental Section

[Li(thf)₃][1], [nBu₄N][1], [Ph₄P][1]: A cold suspension of [Li(dbabh)]·Et₂O (350 mg, 1.28 mmol) in diethyl ether (7 mL) was added to a cold solution (−90 °C) of [U₂(thf)₄] (172 mg, 0.18 mmol) in THF (7 mL) and stirred. After 2 h at −50 °C the resulting greenish-brown solution was allowed to reach room temperature and was filtered. Within 36 h, orange crystals of [Li(thf)₃][U(dbabh)₆] were filtered off, washed with diethyl ether, and dried in vacuum (225 mg, *x* = 0, 0.16 mmol, 89 %). Recrystallization from a saturated solution of [nBu₄N][ClO₄] in THF yielded orange needle-shaped crystals of [nBu₄N][U(dbabh)₆]. Block-shaped single crystals of [Ph₄P][U(dbabh)₆] suitable for an X-ray diffraction analysis were obtained from a CH₂Cl₂/hexane solution containing [PPh₄][Br]. ¹H NMR (300 MHz, CD₃CN, 23 °C): δ = 4.42 (s, 2H, Δ*ν*_{1/2} = 110 Hz), 6.73 (s, 4H, Δ*ν*_{1/2} = 13 Hz), 6.86 (s, 4H, Δ*ν*_{1/2} = 10 Hz); elemental analysis (%) calcd for [nBu₄N][1]: C 73.47, H 5.92, N 6.03; found: C 73.28, H 6.08, N 6.03.

2: Reaction of a solution of [Li(thf)₃][1] (350 mg, 0.25 mmol) in acetonitrile (20 mL) with a solution of silver trifluoromethanesulfonate (AgOSO₂CF₃ (AgOTf); 72 mg, 0.28 mmol) or with stoichiometric amounts of [(C₅H₅)₂Fe][X] (X = SO₃CF₃ (OTf), B[3,5-(CF₃)₂C₆H₃]₄) in acetonitrile resulted in the spontaneous formation of colloidal silver metal and precipitation of **2**. After filtering off the residues and washing the solids with tetrahydrofuran, the filtrate was evaporated to dryness yielding **2** (330 mg; 0.24 mmol, 95 %). Recrystallization from toluene at −40 °C yielded dark purple prism-shaped crystals of **2** suitable for X-ray diffraction analysis. ¹H NMR (500 MHz, C₆D₆, 25 °C): δ = 5.72 (s, 2H, Δ*ν*_{1/2} = 1.3 Hz), 6.99 (q, 4H, Δ*ν*_{1/2} = 1.5 Hz), 7.14 (q, 4H, Δ*ν*_{1/2} = 1.5 Hz); ¹³C NMR (500 MHz, C₆D₆, 25 °C): δ = 71.5 (s), 122.3 (s), 126.6 (s), 159.9 (s); elemental analysis (%) calcd for **2**: C 72.51, H 4.35, N 6.04; found: C 73.11, H 4.49, N 5.85.

The X-band EPR spectra of [Li(thf)₃][1] were performed on a Bruker EMX spectrometer equipped with a helium flow cryostat (Oxford Instruments ESP 310) in the temperature range 20–300 K. The magnetization of crystalline powdered samples of [nBu₄N][1] was recorded between 5–300 K at 0.1, 0.3, 0.5, and 0.7 T with a SQUID magnetometer (Quantum Design). Values of the magnetic susceptibility were corrected for the underlying diamagnetic increment (*χ*_{dia} = −922.1 × 10^{−6} cm³mol^{−1}) by using tabulated Pascal constants and the effect of the blank sample holder (gelatine capsule/straw).

Cyclic voltammetry was performed under N₂ in dry THF containing predried and recrystallized tetra-*n*-butylammonium perchlorate (TBA-ClO₄; Aldrich, ca. 0.9 M). A platinum disk electrode (1.6 mm diameter, Bioanalytical Systems), a platinum wire, and a silver wire were employed as the working electrode. A one-cell compartment was used in the cyclic voltammetry measurements. The electrochemical response was collected with the assistance of an Eco-Chemie Autolab potentiostat (pgstat20) and the GPES 4.3 software package. The ferrocenium/ferrocene couple was used as internal standard. Scan rates varied between 25 and 160 mV s^{−1}.

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- [9] Crystallographic details for [Ph₄P][1]: Orange block-shaped crystals grown from a concentrated solution of [Li(thf)₃][U(dbabh)₆] and excess of [PPh₄][Br] in CH₂Cl₂/hexane at −40 °C were coated with Paratone N oil (Exxon) on a microscope slide. A crystal of approximate dimensions 0.1 × 0.1 × 0.1 mm was selected and mounted with wax on a glass fiber. A total of 27648 reflections (−10 ≤ *h* ≤ 15, −30 ≤ *k* ≤ 30, −28 ≤ *l* ≤ 27) were collected at *T* = 173(2) K in the *θ* range of 1.51 to 20.00° of which 9083 were unique (*R*_{int} = 0.0774); MoK_α radiation (λ = 0.71073 Å). The structure was solved by Patterson methods (SHELXTL V5.0, G. M. Sheldrick and Siemens Industrial Automation, Inc., 1985) in conjunction with standard difference Fourier techniques. The symmetry transformation used to generate equivalent atoms were 1) −*x*, −*y* + 1, −*z* + 1; 2) −*x*, −*y* + 1, −*z* + 2. All non-hydrogen atoms were refined anisotropically and hydrogen atoms were placed in calculated (*d*_{C–H} = 0.96 Å) positions. The residual peak and hole electron density was 0.522 and −0.516 e Å^{−3}. The absorption coefficient was 1.783 mm^{−1}. The least-squares refinement converged normally with residuals of *R*₁ = 0.1273 (all data), *wR*₂ = 0.1754, and GOF = 1.189 (*I* > 2σ(*I*)). The extinction coefficient was negligible (0.00054(5)). C₁₀₉H₈₂Cl₂N₆PU, space group *P*2₁/*c*, monoclinic *a* = 13.74580(10), *b* = 27.7384(4), *c* = 26.1124(4) Å, β = 101.5730(10)°, *V* = 9753.9 Å³, *Z* = 4, ρ_{calcd} = 1.236 g cm^{−3}, *F*(000) = 3676, *R*(*F*) = 0.0946, *wR*(*F*²) = 0.1540.^[18]
- [10] Crystallographic details for **2**: Purple prism-shaped crystals grown from a concentrated solution of toluene at −40 °C were coated with Paratone N oil (Exxon) on a microscope slide. A crystal of approximate dimensions 0.60 × 0.35 × 0.33 mm was selected and mounted with wax on a glass fiber. A total of 18355 reflections (−30 ≤ *h* ≤ 30, −22 ≤ *k* ≤ 18, −18 ≤ *l* ≤ 17) were collected at *T* = 183(2) K in the *θ* range of 1.27 to 23.26° of which 6586 were unique (*R*_{int} = 0.0443); MoK_α radiation (λ = 0.71073 Å). The structure was solved by Patterson methods (SHELXTL V5.0, G. M. Sheldrick and Siemens Industrial Automation, Inc., 1985) in conjunction with standard difference Fourier techniques. The symmetry transformation used to generate equivalent atoms were 1) −*x*, −*y* + 1, −*z* + 1; 2) −*x*, −*y*, −*z*; 3) −*x*, *y*, −*z* + 1; 4) *x*, −*y* + 1, *z*; 5) −*x*, *y*, −*z*; 6) *x*, −*y*, *z*. All non-hydrogen atoms were refined anisotropically and hydrogen atoms were placed in calculated (*d*_{C–H} = 0.96 Å) positions. Excess electron density was observed but no chemical assignment could be made except the possibility of solvent. The residual peak and hole electron density was 0.938 and −0.620 e Å^{−3} after the data was treated with the SQUEEZE algorithm to reduce the effects of excess electron density.^[17] The absorption coefficient was 1.860 mm^{−1}. The least-squares refinement converged normally with residuals of *R*₁ = 0.0637 (all data), *wR*₂ = 0.0805, and GOF = 1.086 (*I* > 2σ(*I*)). The extinction coefficient was negligible (0.00000(2)). C₈₄H₆₀N₆U, space group *C*2/*m*, monoclinic *a* = 27.7468(3), *b* = 20.0934(2), *c* = 16.8916(2) Å, β = 108.9560(10)°, *V* = 8906.8(2) Å³, *Z* = 2, ρ_{calcd} = 1.038 g cm^{−3}, *F*(000) = 2792, *R*(*F*) = 0.0321, *wR*(*F*²) = 0.716.^[18]
- [11] The ¹H NMR spectrum of **2** recorded in C₆D₆ evinces a characteristic singlet at δ = 5.72 assigned to the bridgehead protons of the coordinated dbabh ligands. The aromatic protons, eight per dbabh unit, give rise to two well-resolved quadruplets centered at δ = 6.99 and 7.14. A 2D ¹³C{¹H} correlated NMR spectrum reveals that the bridgehead carbon resonance is located at δ = 71.5, while the proton resonance at δ = 7.14 correlates with the ¹³C peak at δ = 122.3. The proton resonance centered at δ = 6.99 shows a crosspeak at δ = 126.6. The ¹³C resonance at δ = 159.9 therefore is assigned to the bridgehead-adjacent carbon atoms.
- [12] Additional features include a shoulder located on the bathochromic edge of the 501 nm band, along with tailing to the near IR region. These two additional features were simulated with low-intensity Gaussian lines at 660 nm (ε = 400 M^{−1}cm^{−1}) and 900 nm (ε = 70 M^{−1}cm^{−1}).
- [13] The geometry of the model compound [U(NH₂)₆] was optimized by using the Vosko, Wilk, and Nusair (VWN) local density approximation. We also used Becke's (1988) exchange correction and Perdew (1986) correlation for the gradient corrections. Calculations also include scalar relativistic effects (Pauli Formalism). Since the Amsterdam-Density-Functional-Package is not able to calculate *T*_h symmetry, the calculations were performed using *D*_{2h} symmetry. The H–N–H angle was constrained to 95° as obtained by calculations on the

anionic amide dbabh. U: triple- ζ basis set with polarization functions frozen core (5d); N: triple- ζ basis set with polarization functions frozen core (1s); H: double- ζ basis set with polarization functions including H polarization.

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- [18] 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-142254 ([PPh₄] **1**) and CCDC-142255 (**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).

Base-Induced Disproportionation of Elemental Gold**

Anja-Verena Mudring and Martin Jansen*

Elemental gold is the paragon of precious, not only in a chemical sense. This evaluation is based on its general chemical inactivity which seems to weaken, however, with respect to extremely electron-rich reaction partners. Thus, in spite of its high melting point, gold reacts under mild conditions with molten alkali metals. Particularly the formation of Cs₃AuO from CsAu and Cs₂O by interdiffusion in the solid state indicates that in such compounds gold is able to exist as an anion with an excess, localized electron.^[1] Here, a decisive role is played by the high-electron affinity of gold, which comes close to that of iodine. This obvious relationship to the chemistry of halogens is well supported by crystal-chemical arguments. Thus, there are a number of isotypic alkali auride oxides and alkali halogenide oxides in which Au[−] takes over the same crystal-chemical function as a halogenide.^[2]

We have now observed another amazing parallel to the chemistry of halogens: like these, gold seems to disproportionate under the influence of bases. In reactions of gold with the alkali metals cesium, rubidium, or potassium, and the corresponding alkali metal oxides in ratios of alkali metal to gold of three or higher, anti-perovskites of formula type M₃AuO develop.^[3] If less alkali metal is used, the disproportionation of gold into Au⁺ and Au[−] is observed and the novel alkali metal auride aurates(i) Rb₇Au₅O₂ ([RbAu]₄[Rb₃AuO₂]) and Cs₇Au₅O₂ ([CsAu]<₄[Cs₃AuO₂]) are formed. The structural features clearly indicate the presence of monovalent gold in the form

of characteristic AuO₂^{3−} dumbbells along with Au[−] with its typical distances between alkali metal and Au[−]. Independent of this, the presence of gold in the two mentioned valence states was confirmed by Mössbauer spectroscopy; both the isomeric shift and the quadrupolar splitting confirm the presence of Au⁺ and Au[−] next to each other.^[4]

At first glance it seems paradox that gold, in a positive oxidation state, can form during the reducing influence of the alkali metals. The effect of some oxidizing agents can be excluded since—even when handling the extremely oxidation-sensitive reactants in inadequate working conditions—traces of oxygen are immediately absorbed by the alkali metal. Thus, the positively charged gold must have formed through disproportionation, whereas in strong analogy to the disproportionation of halogens, as induced by bases, the stabilization of the positive oxidation state by complexation is the most important contribution to the driving force of this reaction. But, also the high electron affinity of gold, as well as the stabilization of Au[−] by coulombic interactions with the alkali ions are significant components for the stabilization.

According to the results of single-crystal structure analyses,^[5, 6] which have been confirmed by powder X-ray diffraction, Rb₇Au₅O₂ and Cs₇Au₅O₂ crystallize as representatives of a completely new structure type (Pearson Code oI28), which unites both the characteristic structural features of oxoaurates(i) and ionic aurides. Remarkably, both corresponding structure parts are spatially separated in such a way that the complete structure can be regarded as an intergrowth of layers of composition Cs₃AuO₂ and (CsAu)₄ (Figure 1).

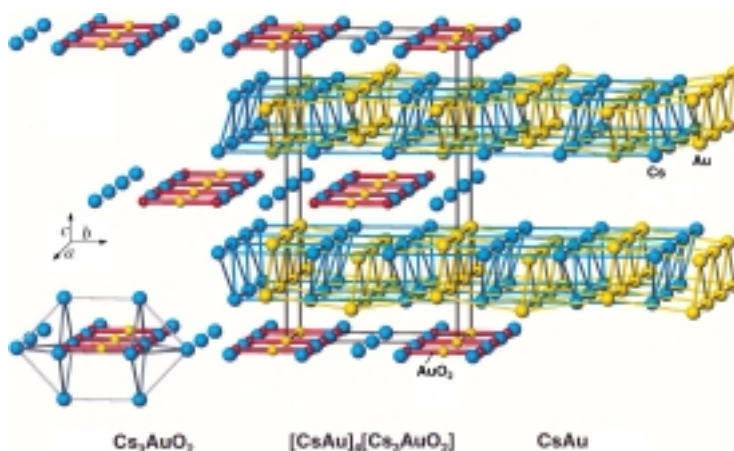


Figure 1. Perspective representation of the crystal structure of Cs₇Au₅O₂, (left: Cs₃AuO₂ partial structure, right: CsAu partial structure, center: [CsAu]₄[Cs₃AuO₂] with unit cell). The connecting lines between Cs (blue) and Au (yellow) highlight the layered-type of structure and emphasize the connection to the tungsten carbide type. Mean bond lengths in Cs₇Au₅O₂: $d(\text{Au}^+-\text{O}) = 200$, $d(\text{Au}^--\text{Cs}) = 375$ pm. For the isotypic compound Rb₇Au₅O₂: $d(\text{Au}^+-\text{O}) = 200$, $d(\text{Au}^--\text{Rb}) = 372$ pm.

According to the site symmetry (*mmm*), the AuO₂^{3−} ions, which show the expected bond lengths, are exactly linear^[7] ($d(\text{Au}-\text{O}) = 200$ pm), and are surrounded by a bicapped cube of ten alkali metal ions. An analogue of this building unit is found in pure Cs₃AuO₂.^[8] In the auride partial structure, the alkali metals and gold form approximately the same nets of trigonal prisms, interpenetrating each other. Thus, this part of the structure corresponds to a distorted section of the

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tungsten carbide type. In an alternative approach, the Cs^+/Au^- partial structure can be derived from the binary component $\text{CsAu}^{[9]}$ (CsCl-type), by cutting out a disk which is oriented along (101), and is of a half a face diagonal of the unit cell of CsAu in thickness. The latter description seems in this respect more suitable because gold is located rather on the faces of the trigonal-prismatic cesium polyhedra than in their centers. In addition, these prisms are completed to give cubes by cesium atoms from the Cs_3AuO_2 layer.

The two auride aurates introduced here complement the group of compounds carrying the same element in a positive, as well as a negative oxidation state by representatives being remarkably stable. In spite of the proximity in space of Au^- and Au^+ (shortest distance $d(\text{Au}^+-\text{Au}^-) = 428$ pm), no redox lability is found.

Experimental Section

$\text{Rb}_7\text{Au}_5\text{O}_2$ and $\text{Cs}_7\text{Au}_5\text{O}_2$ were obtained as single-phase solids by reaction of the required amounts of alkali metal aurides MAu ($\text{M} = \text{Rb}, \text{Cs}$) with gold, and the corresponding alkali oxides under argon at $T = 425^\circ\text{C}$ for one day. Samples of higher crystallinity were obtained by starting from alkali metal, gold, and alkali metal oxide, and by using an excess of alkali metal which is distilled off in dynamic vacuum on completion of the reaction. The alkali metals themselves were prepared by reducing the corresponding chlorides with calcium, and were distilled before usage.^[10] The alkali metal monoxides were made by oxidizing the respective alkali metal with the appropriate amount of oxygen.^[11] Elemental gold was precipitated by reducing tetrachloric gold acid with sodium oxalate.^[12]

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- [4] A. Kyeck, F. E. Wagner, A.-V. Mudring, M. Jansen, unpublished results. $\text{Cs}_7\text{Au}_5\text{O}_2$ ($\text{Cs}_3\text{AuO}_2 \cdot 4\text{CsAu}$): $\text{Au}(+1)$: IS 3.21(1) mms^{-1} , QS 7.07(1) mms^{-1} , $\text{Au}(-1)$: IS 5.76(1) mms^{-1} , QS 3.48(1) mms^{-1} . Compare with CsAuO : IS 2.54(1), QS 6.39(1) mms^{-1} ; CsAu : IS 7.00(1) mms^{-1} .
- [5] Crystallographic data of $\text{Rb}_7\text{Au}_5\text{O}_2$: orthorhombic, space group *Immm* (no. 71), $a = 567.1(1)$, $b = 930.1(2)$, $c = 1659.3(3)$ pm, $V = 875.3(3) \times 10^6$ pm³, $\rho_{\text{calc}} = 6.128$ g cm³, $Z = 2$, $\mu_{\text{MoK}\alpha} = 61.071$ mm⁻¹, $F(000) = 1340$, $\lambda = 71.073$ pm, Enraf-Nonius CAD4 diffractometer, graphite monochromator, $T = 293$ K, $\omega/2\theta$ scan, 1014 measured reflections, 265 symmetry-independent reflections, 30 refined parameters. Empirical absorption correction (psi-scans). Structure solution (heavy atoms) with direct methods.^[13] Subsequent difference Fourier analysis provided the positions of oxygen atoms. All positions can be refined anisotropically giving $R_1 = 0.043$ and $wR_2 = 0.1110$.^[14]
- [6] Crystallographic data of $\text{Cs}_7\text{Au}_5\text{O}_2$: orthorhombic, space group *Immm* (no. 71), $a = 599.4(1)$, $b = 960.6(3)$, $c = 1721(1)$ pm, $V = 990.8(8) \times 10^6$ pm³, $\rho_{\text{calc}} = 6.572$ g cm³, $Z = 2$, $\mu_{\text{MoK}\alpha} = 49.54$ mm⁻¹, $F(000) = 1592$, $\lambda = 71.073$ pm, Enraf-Nonius CAD4 diffractometer, graphite monochromator, $T = 293$ K, $\omega/2\theta$ scan, 3395 measured reflections, 476 symmetry-independent reflections, 30 refined parameters. Empirical absorption correction (psi-scans). Structure solution (heavy atoms) with direct methods.^[13] Subsequent difference Fourier analysis provided the positions of oxygen atoms. All positions can be refined anisotropically giving $R_1 = 0.0431$ and $wR_2 = 0.0756$. Further details on the crystal structure investigation(s) may be obtained from the Fachinformationszentrum Karlsruhe, 76344 Eggenstein-Leopoldshafen, Germany (fax: (+49)7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository numbers CSD-411333 ($\text{Rb}_7\text{Au}_5\text{O}_2$) and CSD-411334 ($\text{Cs}_7\text{Au}_5\text{O}_2$).

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Unusual C–H...Se=C Interactions in Aldols of Chiral *N*-Acyl Selones Detected by Gradient-Selected ¹H–⁷⁷Se HMQC NMR Spectroscopy and X-ray Crystallography**

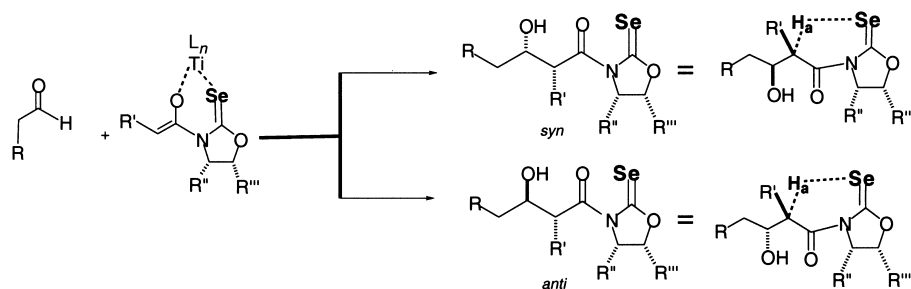
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During the past decade we have been exploring the uses of the selenocarbonyl group both as an analytical tool to interrogate chiral centers^[1] (using ⁷⁷Se NMR spectroscopy) and as a platform for the development of new asymmetric chemical methods associated with selone-based chiral derivatizing agents (CDAs). In the course of these studies, we have uncovered a new type of aldol reaction using chiral *N*-acyl selone reagents (Scheme 1), in which the selenocarbonyl group plays a pivotal role in the stereoselectivity of these reactions.^[2] An unexpected observation in the aldol products was proton couplings to the selenium atom of the CDA.

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[+] Molecular mechanics and ab initio calculations

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Scheme 1. TiCl_4 -mediated stereoselective aldol reactions of chiral *N*-acyl selones. R = alkyl, alkenyl, aryl, NHFmoc (Fmoc = 9-fluorenylmethoxycarbonyl), OBn; R' = Me, OBn; R'' = Me, *i*Pr, Bn; R''' = H, Ph.

Intrigued by this observation, we systematically examined these aldol structures by gradient-selected ^1H – ^{77}Se HMQC NMR spectroscopy, X-ray crystallography, and modeling calculations. While these structural investigations were initially undertaken to gain insight into the orientation and the high selectivities shown for these selone aldol reactions, they ultimately led to the observation of $\text{C}\text{--}\text{H}\cdots\text{Se}=\text{C}$ hydrogen bonds in all of the aldol products.

Though controversial, $\text{C}\text{--}\text{H}\cdots\text{O},\text{N}$ hydrogen bonds are generally classified as weak interactions.^[3] The observation of the corresponding $\text{C}\text{--}\text{H}\cdots\text{S},\text{Se}$ interactions^[4] is very rare, and the $\text{C}\text{--}\text{H}\cdots\text{Se}=\text{C}$ interaction, to the best of our knowledge, is reported here for the first time. Single-crystal X-ray analysis of three of the aldols (**1**–**3**) indicated $\text{C}\text{--}\text{H}\cdots\text{Se}$ distances of

2.63, 2.71, and 2.72 Å (Figure 1); these values are smaller than the sum of the van der Waals radii. The chemical shifts of the aldol H_a protons of $\delta = 5.2$ – 6.9 indicate significant deshielding of this proton. To date, we have observed $J_{\text{H}\text{--}^{77}\text{Se}}$ couplings for all of the aldols we have investigated. For the *syn*-aldols, we observed an apparent unique doublet in each 1D proton-coupled (H_a) selenium spectrum ($J_{\text{H}\text{--}^{77}\text{Se}} = 5$ – 6 Hz) indicating that the β -hydroxy group is not significantly hydrogen bonding to the selenium center as we had initially expected. The proton resonance signals were unambiguously assigned and the J_{H} values were determined from ^1H – ^1H DQF-COSY spectra for both *anti*-**3** and *syn*-**4**. The gradient-selected ^1H – ^{77}Se HMQC spectra of *syn*-**4** and *anti*-**3** are illustrated in Figure 2.^[5] The *syn*-**4** selenium interacts with three proton spin systems. The strongest interaction arises from the H_a . Minor interactions are observed for the oxazolidine ring methyl and α -methyl groups. For the *anti*-aldols, the 1D proton-coupled ^{77}Se spectra exhibited more than one spin system interacting with the sele-

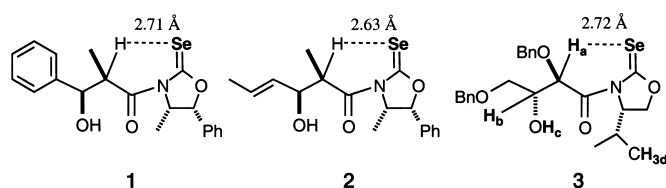


Figure 1. Structures of **1**–**3** (ORTEP representation).^[11]

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To analyze the energetics of the observed $\text{C}\text{--}\text{H}\cdots\text{Se}=\text{C}$ interactions we have performed molecular mechanics and ab initio calculations on model system **5**.^[6] The structural data of

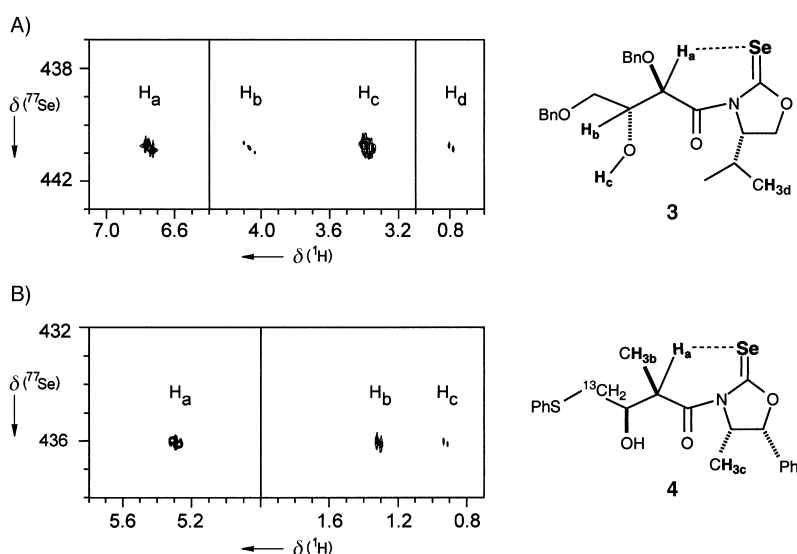


Figure 2. Gradient-selected ^1H – ^{77}Se HMQC spectra for **3** (A; right panel is fifty times greater) and **4** (B; right panel is 40 times greater) in CDCl_3 .

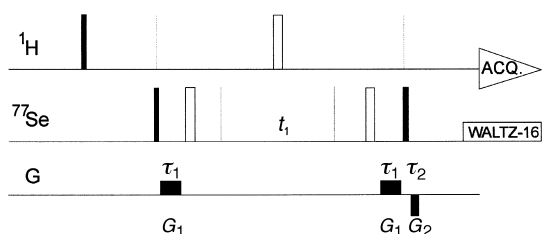
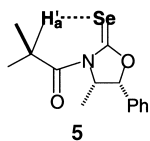


Figure 3. Pulse sequence used for ^1H - ^{77}Se HMQC experiment. Black, open, and shaded rectangles represent 90° , 180° . Rectangles on line G designate gradient pulses. ACQ = acquisition; WALTZ-16 ^1H - ^{77}Se decoupling.

the molecule **2** were used as a starting point for the calculations. The large side chain ($-\text{CH}(\text{OH})-\text{CH}=\text{CH}-\text{CH}_3$) was replaced with a methyl group to give structure **5** which significantly reduced the calculation time. MM+ structural optimization was carried out exclusively for the relaxation of the three protons of the newly introduced methyl group. All other single-point calculations were carried out on the obtained preoptimized coordinates at the



RHF or ROHF level with a SBKJC basis set using the GAMESS program package.^[7] These calculations indicate that the $\text{Se}\cdots\text{H}_a'$ interaction has an energy equivalent to $-1.488\text{ kcal mol}^{-1}$. This additional stabilization could potentially originate from the interactions with the carbonyl oxygen atom. However, the distance from H_a' to the carbonyl oxygen atom is more than $4\text{ \AA}$, and we found no electron overlap from the α -carbon radical to the carbonyl oxygen atom; whereas the distance from H_a' to the Se atom is only $2.63\text{ \AA}$. In addition, the Se and H_a' have an overlap of 0.0212 electrons; this strongly suggests that stabilization is a result of the $\text{C}-\text{H}_a'\cdots\text{Se}=\text{C}$ hydrogen bond.

Numerous reports of $\text{C}-\text{H}\cdots\text{O}$ interactions have not only been found and characterized in organometallic and organic structural chemistry, but also in biological systems such as oligomeric nucleic acids and proteins.^[8] The importance of these weak interactions has become increasingly evident in providing structural elements to macromolecular systems and in playing a dominant role in the stereoselectivity of chemical reactions. However, there are few reports of $\text{C}-\text{H}\cdots\text{S}$ interactions,^[4a] and only three reports of $\text{C}-\text{H}\cdots\text{Se}$ interactions. Tomoda and co-workers^[4b] reported the shortest $\text{C}-\text{H}\cdots\text{Se}$ distance of $2.94\text{ \AA}$, while Vij and co-workers^[4b] have reported distances of 2.98 and $3.26\text{ \AA}$ for their saphyrin molecules.

Our results indicate that we have uncovered a fundamental new type of non-opportunistic hydrogen bond in the aldols investigated.^[9] In addition, we have for some time been puzzling over the reasons our chiral selones have the ability to interrogate remotely disposed chiral centers by ^{77}Se NMR spectroscopy, especially when there are no intervening aromatic or vinyl groups to facilitate the communication. An explanation for the remarkable ability of the selenium atom to report on the status of a chiral center up to eight bonds removed from the observing selenium nucleus^[10] is provided by the $\text{C}-\text{H}\cdots\text{Se}$ interaction. This interaction allows

both the necessary conformational amide rigidity and the critical truncation in the communication pathway between the chiral center and the "observing" selenium atom.

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$$\frac{2 G_1 \tau_1}{G_2 \tau_2} = \frac{\gamma_{\text{H}}}{\gamma_{\text{Se}}} = 5.245 \quad (1)$$

–81.035 kcal mol^{–1} and the average energy necessary to break a C–H bond is 80.841 kcal mol^{–1}. The extent of covalent versus electrostatic contributions to hydrogen bonding have been the subject of numerous discussions in the chemical literature (see, for example, J. J. Dannenberg, L. Haskamp, A. Masunov, *J. Phys. Chem. A* **1999**, *103*, 7083, and references therein) and depend on the particular system studied. Calculated Mulliken partial charges are +0.077 for H_a' and –0.13 for Se indicating an attractive interaction. The difference between an average bond order of C–H bonds (0.904; this value is less than the expected due to the destabilization of C–H bond by the oxazoline ring system) and of the C–H_a' bond (0.888) directly gives the destabilization by 0.016 bond orders and therefore the C–H_a' bond is destabilized by 1.293 kcal mol^{–1} (C–H = 80.841 kcal mol^{–1}).^[6] The Se...H_a' interaction is equal to the total stabilization of the system (–0.194 kcal mol^{–1}) minus the destabilization of the C...H_a' bond (1.293 kcal mol^{–1}), that is, 1.487 kcal mol^{–1}.

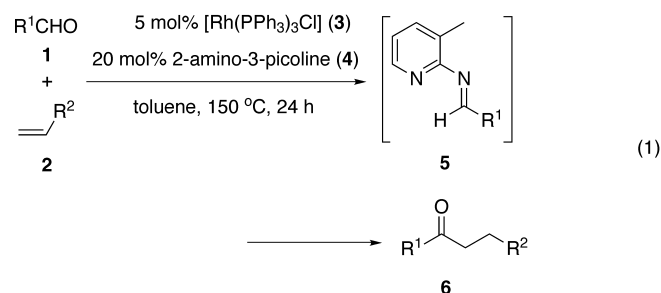
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A Highly Active Catalyst System for Intermolecular Hydroacylation**

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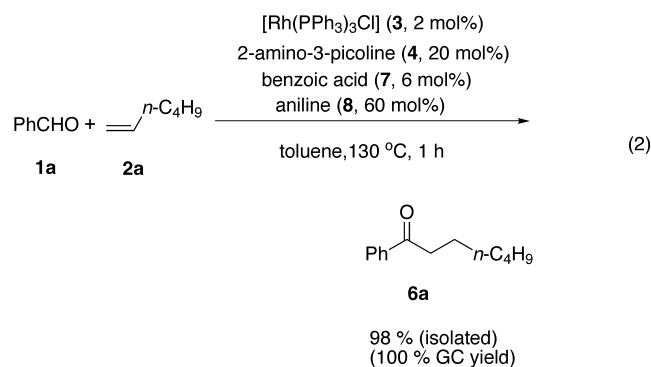
Hydroacylation^[1–3] is a useful synthetic method for obtaining ketones from aldehydes and olefins by using C–H bond activation by transition metal complexes. Although the intramolecular hydroacylation of 4-pentenals has been extensively studied,^[1] only a few successful intermolecular reactions have been reported.^[2a–f] To effect intermolecular hydroacylation, ethylene,^[2a,b] carbon monoxide,^[2c] or vinylsilanes with a Co^I catalyst^[2d,e] have been used to suppress the decarbonylation that results in catalytically inactive metal carbonyl species. We have developed a general intermolecular hydroacylation of 1-alkenes by using an Rh^I complex and 2-amino-3-picoline as cocatalyst, whereby aldimine **5** is assumed to be a key intermediate that suppresses decarbonylation and permits

C–H bond activation [Eq. (1)].^[3] Herein we report an efficient intermolecular hydroacylation for which a catalyst system was designed to ensure facile formation of the intermediate aldimine.



Recently, we found that the reactivity in the hydroacylation depicted in Equation (1) improved when benzaldehyde (**1a**) contaminated by benzoic acid (**7**)^[4] was used as the substrate.^[5] Benzoic acid was assumed to catalyze the condensation of aldehydes **1** with **4** to generate **5**; this may be the rate-determining step of the reaction (see below). This observation prompted us to search for a way to facilitate the formation of **5**, and we found a remarkable enhancement of the reactivity occurred when aniline (**8**), as well as **3**, **4**, and **7**, was used as an additive.

In our experiment **1a** was treated with 1-hexene (**2a**) at 130 °C for 1 h in the presence of 2 mol % of [Rh(PPh₃)₃Cl] (**3**), 20 mol % of **4**, 6 mol % of **7**, and 60 mol % of **8** as cocatalysts to give heptanophenone (**6a**) in 98 % yield after chromatographic separation [Eq. (2)]. A significant decrease in reactivity was observed when the reaction was performed under



the same conditions but without **7** and **8** (Figure 1). For example, while the reaction was complete (100 % GC yield) after 1 h when both **7** and **8** were added, only a 9 % yield of **6a** was obtained when the reaction was performed without additives. The yield increased to 28 % with the addition of **7**.

The postulated mechanism is depicted in Scheme 1. Cycle A represents the mechanism for the catalyst system consisting of **3** and **4**. The first step is believed to be the formation of aldimine **9** from **1a** and **4**. Aldimine **9** reacts with **2a** to yield ketimine **10** by hydroiminoacylation.^[6] The resulting ketimine **10** is hydrolyzed by H₂O, generated from

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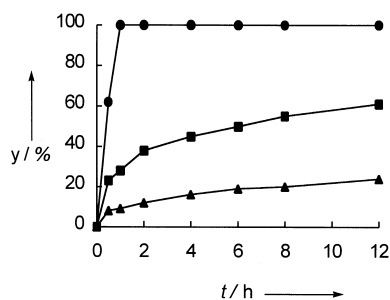
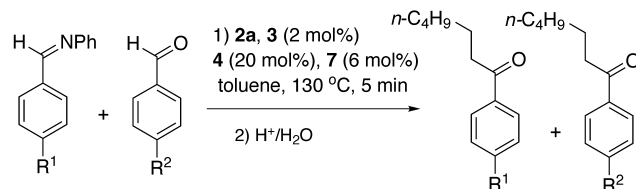


Figure 1. Conversion (yield of **6a**) versus time plot for the hydroacylation of **2a** with **1a** in the presence of 2 mol % of **3**, and 20 mol % of **4** in toluene at 130 °C. Results for the reaction with **7** and **8** (●), the reaction with **7** (■), and the reaction without any additives (▲) are shown (y = yield of **6a** determined by GC).

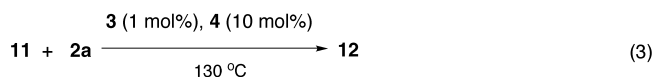


Reactants		Products (yield)	
11 ($R^1 = H$),	1b ($R^2 = OMe$)	6a ($R^1 = H$, 21 %)	13 ($R^2 = OMe$, 5 %)
14 ($R^1 = OMe$),	1a ($R^2 = H$)	13 ($R^1 = OMe$, 26 %)	6a ($R^2 = H$, 5 %)

Scheme 2. The reactions of **2a** with aldimines **11** and **14** and aldehydes **1a** and **1b** in the presence of **3**, **4**, and **7**.

mechanism of cycle A in Scheme 1.^[12] These results imply that the transimination of **11** with **4** is more facile than the direct condensation of **1a** and **4** as a route to **9**.

To examine the effect of carboxylic acid on transimination an alkylation of **11** with **2a** was carried out in the presence of a cocatalyst system comprising **3** and **4** [Eq. (3)]; the results are



shown in Figure 2. With the addition of **7**, the reactivity increased and the reaction was complete in 3 h, while it took more than 18 h to obtain a 90 % yield of **12** in the absence of **7**. This result strongly suggests that transimination is catalyzed by carboxylic acid.

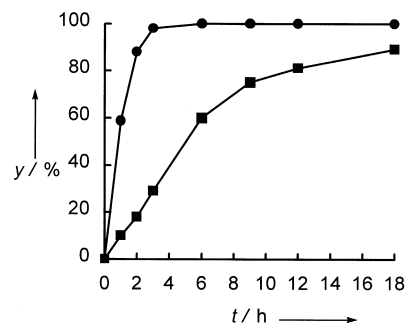


Figure 2. The effect of **7** on the alkylation of imine **11** with **2a** in the presence of 2 mol % of **3**, and 20 mol % of **4** [Eq. (3)]. Results for the reaction with **7** (●) and the reaction without **7** (■) are shown (y = yield of **12** determined by GC).

the reaction of **1a** and **4**, to produce **6a** with the regeneration of **4**. The role of benzoic acid might be to catalyze the condensation of **4** and **1a** to generate intermediate **9**, which resulted in an increased reactivity.^[7]

Cycle B depicts the reaction mechanism with a cocatalyst system consisting of **8**, **3**, **4**, and **7**, in which imine **11** may also be involved.^[8] The initial formation of **11** from **1a** and **8** is followed by transimination^[9] of **11** with **4** to give **9**. A similar type of semicarbazone formation from carbonyl compounds catalyzed by aniline in an acidic medium was previously reported.^[10a] This process, known as nucleophilic catalysis,^[10] consists of the initial formation of the imine compound and consecutive transimination with semicarbazide to form semicarbazone. We also demonstrated the conversion of **11** into ketimine **12** by transition metal catalyzed alkylation through transimination with **4**.^[11] To confirm this mechanism, the following experiments were carried out.

When **2a** was treated with aldimine **11** and anisaldehyde **1b** at 130 °C for 5 min in the presence of a catalyst system consisting of **3**, **4**, and **7**, a mixture of **6a** and **13** was isolated in 21 and 5 % yield, respectively, after hydrolysis (Scheme 2). In contrast, the reaction of aldimine **14** and benzaldehyde **1a** with **2a** produced a mixture of **6a** and **13** in 5 and 26 % yield, respectively. Regardless of the substituents, aldimines that undergo hydroacylation by transimination (cycle B of Scheme 1) are more reactive than aldehydes that follow the

The reactions of various aldehydes and olefins are summarized in Table 1. All terminal olefins were hydroacylated in fairly good yields within 1 h. However, trimethylvinylsilane (**2e**) was so reactive that the reaction went to completion with only 1.1 equivalents of **2e** (entry 5). The reactions of pentafluorostyrene (**2f**) and allyl phenyl ether (**2g**) were complete after 40 min (entries 6 and 7). However, internal olefins such as cyclohexene and 2-pentene failed to undergo hydroacylation. The reaction with aldehydes bearing various substituents gave moderate yields of the corresponding ketones (entries 8–11). Even aliphatic aldehyde underwent hydroacylation to give ketones in moderate yield (entry 12).

Table 1. The reaction of various aldehydes and olefins.

$$\text{R}^1\text{-CHO} + \text{CH}_2=\text{CH-R}^2 \xrightarrow[\text{toluene, 130 }^\circ\text{C, 1 h}]{\text{3 (2 mol\%), 8 (60 mol\%), 4 (20 mol\%), 7 (6 mol\%)}} \text{R}^1\text{-C(=O)-CH}_2\text{-CH}_2\text{-R}^2$$

Entry	R ¹ (1)	R ² (2) ^[a]	Product	Yield [%] ^[b]
1	Ph (1a)	<i>n</i> -C ₄ H ₉ (2a)	6a	98 (100)
2	Ph (1a)	<i>n</i> -C ₃ H ₇ (2b)	6b	83 (86)
3	Ph (1a)	<i>n</i> -C ₆ H ₁₃ (2c)	6c	99 (100)
4	Ph (1a)	<i>t</i> Bu (2d)	6d	84 (87)
5	Ph (1a)	Me ₃ Si (2e)	6e	95 (100) ^[c]
6	Ph (1a)	C ₆ F ₅ (2f)	6f	98 (100) ^[d]
7	Ph (1a)	PhOCH ₂ (2g)	6g	95 (100) ^[d]
8	<i>p</i> MeOC ₆ H ₄ (1b)	<i>n</i> -C ₄ H ₉ (2a)	13	79 (80)
9	<i>p</i> CF ₃ C ₆ H ₄ (1c)	<i>n</i> -C ₄ H ₉ (2a)	6h	71 (86)
10	<i>p</i> Me ₂ NC ₆ H ₄ (1d)	<i>n</i> -C ₄ H ₉ (2a)	6i	60 (64)
11	PhC ₆ H ₄ (1e)	<i>n</i> -C ₄ H ₉ (2a)	6j	95 (98)
12	PhCH ₂ CH ₂ (1f)	<i>n</i> -C ₄ H ₉ (2a)	6k	71 ^[c]

[a] Five equivalents based on aldehyde were used. [b] Yield of product after isolation; GC yields are given in parenthesis. [c] 1.1 equivalents of 2e was used. [d] Reaction time was 40 min. [e] 10% of the aldol condensation product of 1f was obtained.

In summary, we have presented an efficient catalytic system for intermolecular hydroacylation. Further work is now directed toward understanding the mechanistic details of this reaction.

Experimental Section

Typical procedure for preparation of ketone 6a (Table 1, entry 5): A screw-capped pressure vial (1 mL) was charged with freshly purified benzaldehyde (1a, 0.5 mmol), 2-amino-3-picoline (4, 0.1 mmol), benzoic acid (7, 0.03 mmol), aniline (8, 0.3 mmol), 1-hexene (2a, 2.5 mmol), and toluene (80 mg). After the mixture had been stirred at room temperature for several minutes, [Rh(PPh₃)₃Cl] (3, 0.01 mmol) was added, and then it was stirred at 130 °C for 1 h. After cooling the reaction mixture to room temperature, it was purified by column chromatography (SiO₂, *n*-hexane/ethyl acetate 4/1) to yield pure 6a (0.49 mmol, 98% yield).

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- [7] The reactivity of 2a with 9 towards hydroiminoacylation was not enhanced by the addition of 7. This implies that carboxylic acid does not affect the hydroiminoacylation step. Therefore, we assume that the rate-determining step is the formation of 9.
- [8] It is also possible that 1a condenses with 4 to form 9. However, the condensation of 1a with 8 is more facile than with 4. When an equimolar mixture of 1a, 4, and 8 was heated at 130 °C for 10 min 1a was completely consumed and the ratio of 9:11 was determined as 10:90 by GC.
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Asymmetric Synthesis of a Chiral Secondary Grignard Reagent**

Reinhard W. Hoffmann,* Bettina Hölzer, Oliver Knopff, and Klaus Harms

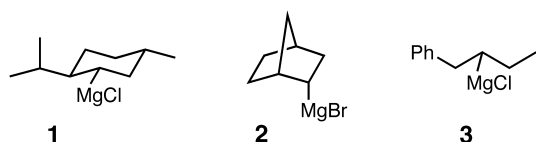
Chiral organometallic reagents are of interest in stereoselective synthesis. This holds in particular for chiral α -heterosubstituted organolithium and Grignard reagents.^[1] However, their reactions with electrophiles do not always take a stereochemically homogenous pathway. It is not clear

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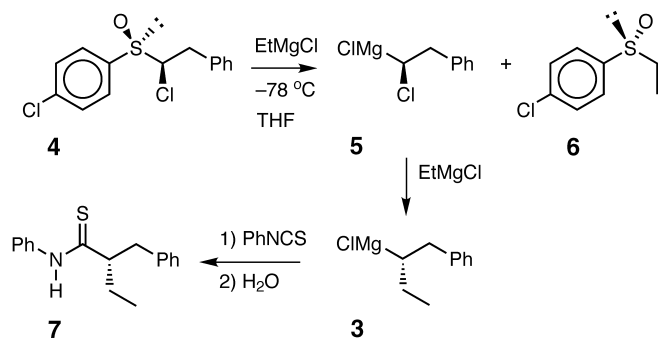
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whether the result (inversion, retention, or partial racemization) is predominantly due to the nature of the electrophile or to which extent the presence of the heteroatom in the α -position is involved in the stereochemical outcome. Clearer information on the intrinsic stereochemical course of reactions of organolithium and Grignard reagents with electrophiles could be obtained if “unsubstituted” chiral Grignard reagents were available. In this context reagents such as **1**^[2] or **2**^[3] should be suitable. Since **1** and **2** have, however, more than



one stereogenic center, it remains open, to which extent the stereochemical course of the reaction of Grignard reagents **1** or **2** depends on the chirality of the molecular backbone. Unambiguous results could therefore be obtained if simple chiral secondary Grignard reagents such as **3** could be used for stereochemical studies. We report here on the “synthesis” of the Grignard reagent **3** (> 90% *ee*) and the stereochemistry of its oxidation to the secondary alcohol **8** (see Table 1).

Enantiomerically pure Grignard reagents such as **3** are not accessible from enantiomerically pure secondary alkyl halides by reaction with magnesium metal,^[4] since electron transfer processes and the intervention of radicals^[5] annihilates the stereochemical information. Likewise, neither halogen/magnesium nor sulfoxide/magnesium exchange reactions can be used to generate **3**, because the latter as a simple secondary Grignard reagent is too rich in energy to allow its generation in a thermodynamically driven Grignard exchange reaction. A reaction which is suitable for the generation of **3** is the carbenoid homologation reaction^[6] using α -haloalkyl Grignard reagents **5** as a starting point. The route to generate enantiomerically pure secondary Grignard reagents **3** was open, once we succeeded in generating the enantiomerically pure α -chloroalkyl Grignard reagent **5** by a sulfoxide/magnesium exchange reaction on diastereomerically pure α -chloroalkyl sulfoxide **4**.^[7] Subsequent reaction of **5** with an excess



of ethylmagnesium chloride between -50 and -30 °C furnished the desired Grignard reagent **3**. Ethylmagnesium chloride was chosen for two reasons: First, racemization of the intermediate **3** is slowest if chloride, as an anion of low

nucleophilicity, is present.^[8] Second, the carbenoid homologation reaction of **5** to give **3** is least complicated by formation of a “rearranged” Grignard product,^[9] if ethylmagnesium halide is used in THF.

The solution of **3** generated by reaction of the sulfoxide **4** with an excess of ethylmagnesium chloride (5–10 equiv)^[10] was quenched with phenylisothiocyanate at -78 °C and subsequently allowed to warm to room temperature resulting in the formation of 56% of the thioamide **7**. HPLC showed **7** to be of 93% *ee*. From the solution of **7** a small amount of crystalline material was obtained, identified by HPLC as **7** of 78% *ee*. X-ray structure analysis^[11] of one of these crystals and HPLC analysis of this particular crystal allowed the assignment of the absolute configuration of the major enantiomer of **7** as shown. This demonstrates that the carbenoid homologation reaction of **5** to give **3** proceeded with inversion of configuration.^[12] We attribute the small loss in enantiomeric purity to a competing racemization at the stage of the intermediate **5**. The secondary Grignard reagent **3** appears to be configurationally stable at -78 °C. Warming of the solution of **3** to -10 °C, however, leads to slow racemization in a first order process with $k = (3.46 \pm 0.05) \times 10^{-5} \text{ s}^{-1}$ corresponding to a half life of about 5 h. This holds for the Grignard reagent **3** in the given solvent and reaction mixture. For comparison, the epimerization of **2** to *exo*-norbornylmagnesium bromide occurs at room temperature in diethyl ether with a half life of about 5 h.^[3, 13]

Access to the Grignard reagent **3** with known absolute configuration^[12] and an enantioselectivity of about 90% *ee* allows the study of the stereochemical course of the reaction of Grignard reagents and provides more detailed mechanistic insights. We illustrate this with reference to the oxidation of **3** to give the secondary alcohol **8** (Table 1). The absolute configuration of the laevorotatory alcohol is known.^[14] Oxidation of organolithium and Grignard reagents may occur by the transfer of an oxygen atom, but may also be initiated by electron transfer to the oxidizing agent. The stereochemical

Table 1. Stereochemical course of the oxidation of the Grignard reagent **3** to the alcohol **8**.

Oxidant	Yield (8) [%]	<i>ee</i> (8) [%]
MoO ₅ · Py · DMPU ^[a]	9 84	92
PhSO ₂ -N ⁺ (Ph)-O ⁻	10 80	91
	11 80	88
Me ₃ Si-O-SiMe ₃	12 20	82
Ti(O <i>i</i> Pr) ₄ /tBuOOH	13 82	71
Li-O-	75	32
O=O	89	15

[a] Py = Pyridine, DMPU = *N,N'*-dimethylpropylene urea.

course of the oxidation of **3** to the alcohol **8** reveals (cf. Table 1) that depending on the nature of the oxidizing agent the one or the other mechanism may predominate. The molybdenum peroxide **9**,^[15] the Davis oxaziridine **10**,^[16] and the peroxyborate **11**^[17] oxidize **3** to **8** under retention of configuration and essentially complete retention of the enantiomeric purity (>90%). On oxidation with bis(trimethylsilyl)peroxide **12**^[18] racemization occurs to a noticeable extent. Extensive racemization was observed on oxidation of **3** with the peroxotitanium reagent **13**,^[19] with lithium *tert*-butylhydroperoxide,^[20] or with dioxygen.

We have described here a route to an enantiomerically enriched chiral secondary Grignard reagent **3** which may serve as a probe to give insights into the mechanisms of Grignard reactions, as demonstrated by the stereochemistry of its oxidation to the alcohol **8**.

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Unusually Stable Vinyl Cations**

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Dedicated to Professor Paul von Ragué Schleyer on the occasion of his 70th birthday

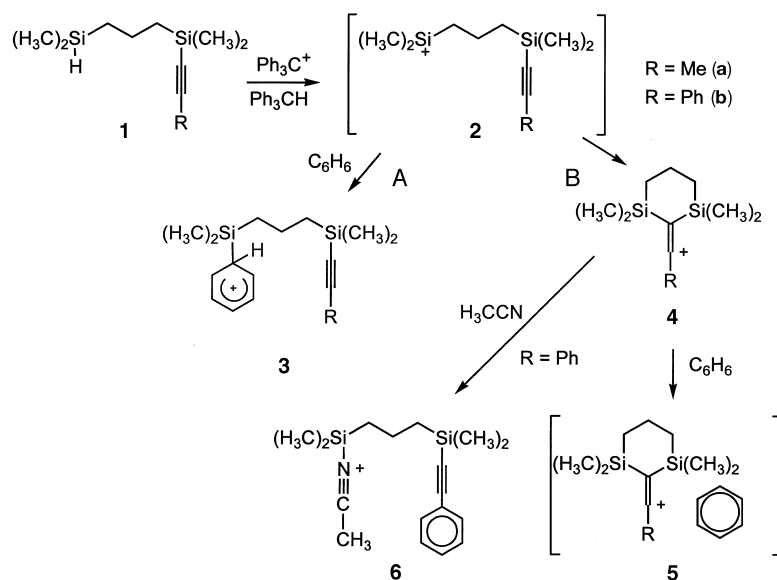
Recent progress in silylium ion chemistry^[1] has opened a novel route for the synthesis of stable carbocations in arene solvents at room temperature. The addition of an arene complex of triethylsilylium to the C=C bond in 1,1-diphenyl ethene has been used to generate a room-temperature stable β -silyl-substituted carbenium ion.^[2,3] Similarly, we have used the intramolecular addition of a silylium ion to a C=C bond to generate the 2-silanorbornyl cation.^[4] Vinyl cations,^[5] dicoordinated carbocations in which the positive charge is located at a sp-hybridized carbon of a double bond, have been established as reaction intermediates in numerous reactions, such as the solvolysis of activated haloalkenes^[6] and alkenes bearing super leaving groups like triflate and nonaflate^[7] and protonation reactions of alkynes and allenes.^[8] Some persistent vinyl cations have been generated by protonation of alkynes^[9] and allenes^[10] in superacidic media at temperatures below –100 °C. These cations have been characterized by NMR spectroscopy supported by quantum-mechanical calculations. Herein we report the synthesis of unusually stable vinyl cations by intramolecular addition of transient silylium ions to C≡C bonds.

Hydride transfer^[11] between 1-alkyl- and 1-aryl-substituted 3,7-disila-3,3,7-dimethyl-octyne-1 (**1**) and trityl cation in benzene is expected to give silylium ion **2** as the first intermediate. The silylium ion **2** may react intermolecularly

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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.

(path A, Scheme 1) with the aromatic solvent to give the silylated arenium ion **3**,^[12] or could add intramolecularly (path B, Scheme 1) to the C≡C bond yielding the β -disilacyclohexenyliene-substituted vinyl cation **4**. The results of density functional calculations^[13] at the hybrid B3LYP/6-31G(d)^[14] + ZPVE level of theory reveal that the reactions of the intermediate silylium ions **2a** and **2b** and benzene to form the arenium ions **3a** and **3b**, respectively, are exothermic (by 20.9 and 20.6 kcal mol⁻¹, respectively). The intramolecular cyclization reactions of **2a,b** to give the vinyl cations **4a,b**, however, are exothermic by 33.0 and 41.7 kcal mol⁻¹, respectively, and thus are more favorable. The association energies A_E between vinyl cations **4a,b** and benzene as calculated for the complexes **5a,b** are relatively small ($A_E = 3.8$ and 3.1 kcal mol⁻¹, respectively). Therefore, it is unlikely that these complexes are stable at ambient conditions in benzene and the vinyl cations **4a,b** are best described as free cations with no direct coordination to the solvent. In conclusion, the analysis of the computational data suggests that vinyl cations **4** should be assessable by the reaction sequence B (Scheme 1) and that they should be stable compounds at ambient conditions in the absence of strong nucleophiles.

Addition of **1b** to a vigorously stirred solution of trityl tetrakis(pentafluorophenyl)borate (TPFPB) in benzene at room temperature instantaneously gives a deeply colored solution which separates into two layers. ¹H and ¹³C NMR spectra show that the slightly yellow upper layer contains only triphenylmethane. The ¹H, ¹³C, ²⁹Si, and ¹⁹F NMR spectra obtained from the red-brown lower layer of the solution are consistent with the formation of the vinyl cation **4b** (Table 1, Figure 1).^[15] The ²⁹Si NMR spectrum shows only one signal at $\delta = 22.8$, indicating the formation of a symmetrical species. This is also supported by the number of signals in the ¹³C NMR spectrum.^[15] The ¹³C chemical shifts of $\delta = 185.8$ (C^α) and 84.1 (C^β) are characteristic for the $C^\beta=C^{\alpha+}$ moiety of vinyl cations. The relatively high-field resonance of the aromatic *ipso*-carbon atom C^β ($\delta = 113.7$) is typical for the *ipso*-C(sp²)-C $^\alpha$ (sp) linkage in aryl-substituted vinyl cations.^[16] The strongly

deshielded *ortho*- and *para*-carbon atoms of the phenyl substituent indicate considerable charge transfer into the phenyl ring. The NMR chemical shifts of **4b** do not change significantly when the cation is generated in toluene instead of benzene. However, noteworthy changes occur when dry acetonitrile is added to a solution of **4b** in benzene. Two new ²⁹Si NMR signals at $\delta = +35$ and -16 indicate the formation of the linear nitrilium ion **6**.^[17] ¹³C and ¹H NMR data give further support for the formation of **6** (see Table 1 and Supporting Information).

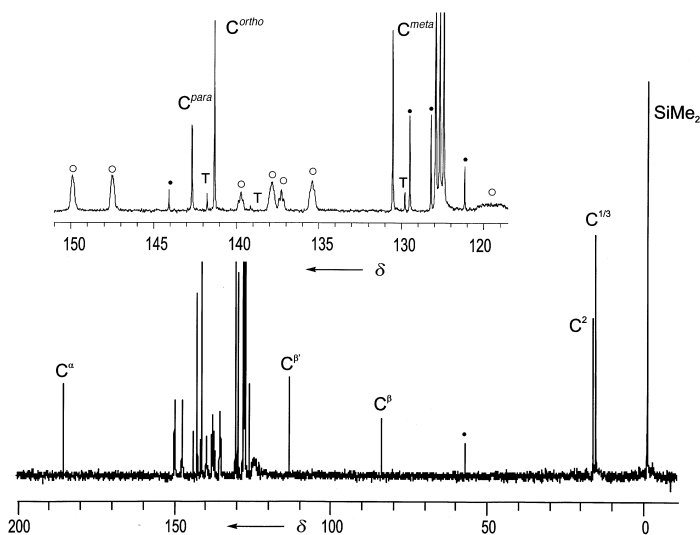
The α -methyl-substituted vinyl cation **4a** is formed analogously to **4b** in a clean reaction by addition of **1a** to a benzene solution of TPFPB. The structure of **4a** is fully supported by the measured ¹H, ¹⁹F, ¹³C, and ²⁹Si NMR data.^[18]

The NMR spectra obtained from a solution of **4b** in benzene remained unchanged for weeks in

 Table 1. NMR Chemical shifts of cations **4** and **6**.

	¹³ C NMR						²⁹ Si NMR
	$\delta(C^\alpha)$	$\delta(C^\beta)$	$\delta(C^\beta)$	$\delta(C^{ortho})$	$\delta(C^{meta})$	$\delta(C^{para})$	$\delta(Si)$
4a ^[a]	184.8	77.7	9.3	—	—	—	24.1
4b ^[a]	185.8	84.1	113.7	141.6	130.9	143.0	22.8
4b ^[b]	185.3	84.1	113.7	141.7	130.9	143.0	22.8
6 ^[c]	106.7	93.0	123.4	128.7	132.1	129.1	33.2, -16.4

[a] At 300 K, in [D₆] benzene. [b] At 300 K, in [D₈] toluene. [c] At 300 K, in [D₆] benzene/CH₃CN.


 Figure 1. 100 MHz ¹³C NMR spectra of **4b** in C₆D₆ at 300 K. (○: [B(C₆F₅)₄]⁻, ●: Ph₃CH, T: Ph₃C⁺)

the absence of nucleophiles; **4a**, however, decomposes during a period of several days into unidentified products. Both compounds, **4a** and **4b**, are strongly stabilized by the well-documented hyperconjugative effect of two β -silyl substituents.^[3, 19] Cation **4b** is further stabilized by the electron-donating ability of the α -phenyl group. Both effects become

apparent in the calculated geometry and in the natural bond orbital (NBO) charge distribution^[20] of **4b** (Figure 2). The high thermostability of the β -disilyl- α -aryl vinyl cation **4b** is in contrast to previous results for β -monosilyl-substituted α -aryl vinyl cations, as for example the α -mesityl-substituted cation **7**, which could be observed in superacidic media only at temperatures below -130°C .^[9a, 16]

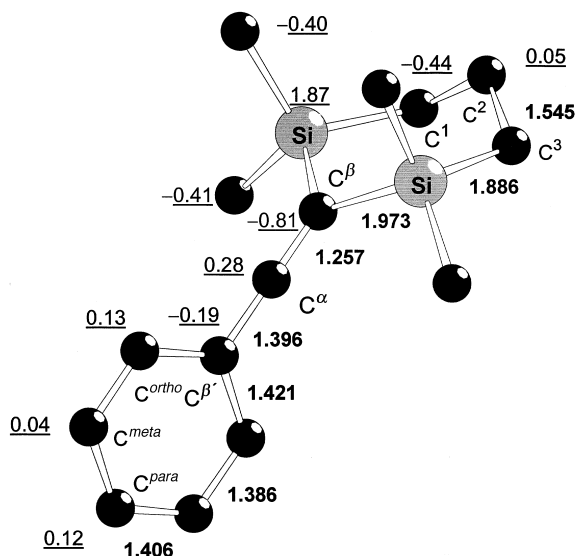
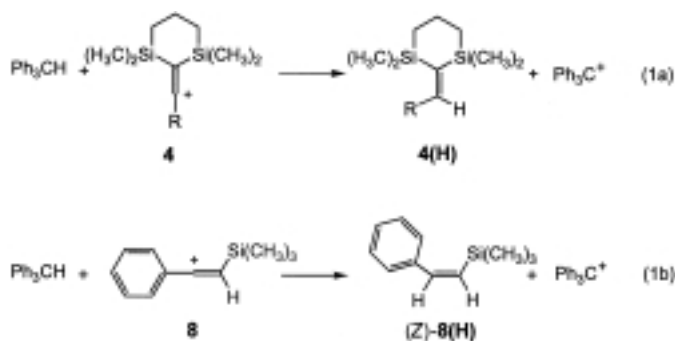
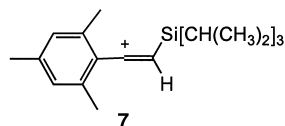


Figure 2. Calculated structure and NBO charge distribution of **4b** (at B3LYP/6-31G(d), H atoms are omitted for clarity). Bond lengths in Å (bold), NBO charges summed in heavy atoms in au (underlined).

According to the isodesmic reactions (1a) and (1b), **4b** is only 1.7 kcal mol^{-1} less stable than the trityl cation, and



$19.6\text{ kcal mol}^{-1}$ lower in energy than the α -phenyl- β -trimethylsilyl-substituted vinyl cation **8**,^[21] a close model for the experimentally investigated cation **7** (at B3LYP/6-31G(d)).^[9a]



The facile generation and stability of **4b** in solution at ambient temperature as compared to β -monosilyl-substituted vinyl cations such as **7** can be attributed to the electronic and steric

effects of the second β -silyl substituent which enhances the thermodynamic and kinetic stability. The α -methyl-substituted vinyl cation **4a** is thermodynamically significantly less stabilized than **4b** (i.e. it is $12.5\text{ kcal mol}^{-1}$ higher in energy than Ph_3C^+ , [Eq. (1a)]) due to the weaker electron-donating effect of the α -methyl group, however, it is still lower in energy than **8** by 8.8 kcal mol^{-1} .

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- $\delta(\text{CF}_2\text{Cl}_2) = 0$, external): $\delta = -136.9$ (d, 2F, $J(\text{F},\text{F}) = 8.4$ Hz; F^{meta}), -167.7 (tr, 1F, $J(\text{F},\text{F}) = 20.7$ Hz; F^{para}), -171.5 (tr, 2F, $J(\text{F},\text{F}) = 16.8$ Hz; F^{ortho}); $^{13}\text{C}\{^1\text{H}\}$ NMR (100.63 MHz, $[\text{D}_6]\text{benzene}$, 300 K, $\delta(\text{C}_6\text{D}_6) = 128.0$): $\delta = 185.8$ (s; C^{α}), 149.1 (d, $^1J(\text{C},\text{F}) = 241.5$ Hz; $\text{C}^{\text{meta}} [\text{B}(\text{C}_6\text{F}_5)_4]$), 143.0 (s; C^{para}), 141.6 (s; C^{ortho}), 139.2 (d, $^1J(\text{C},\text{F}) = 251.6$ Hz; $\text{C}^{\text{para}} [\text{B}(\text{C}_6\text{F}_5)_4]$), 137.0 (d, $^1J(\text{C},\text{F}) = 251.6$ Hz; $\text{C}^{\text{ortho}} [\text{B}(\text{C}_6\text{F}_5)_4]$), 130.9 (s; C^{meta}), 125.2 (br; $\text{C}^{\text{ipso}} [\text{B}(\text{C}_6\text{F}_5)_4]$), 113.7 (s; C^{β}), 84.1 (s; C^{β}), 16.3 (s; C^2), 15.0 (s; $\text{C}^{1/3}$), -0.7 (s; CH_3); $^{29}\text{Si}\{^1\text{H}\}$ NMR (79.50 MHz, $[\text{D}_6]\text{benzene}$, 300 K, $\delta((\text{H}_3\text{C})_2\text{SiHCl}) = 11.7$, external): $\delta = 22.8$.
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[(Cp*RuCl)₂(μ-Cl)₂]: Bond-Stretch or Spin-State Isomerism?*

John E. McGrady*

The phenomenon of bond-stretch isomerism,^[1] the ability of a single molecule to exist in two distinct forms differing only in the length of one or more bonds, has been the source of intense debate ever since the first example, *cis-mer*-[Mo(O)Cl₂(PMe₂Ph)₃], was reported by Chatt and co-workers.^[2] Related examples such as [W(O)Cl₂(Me₃tacn)]⁺^[3] (Me₃tacn = *N,N',N''*-trimethyl-1,4,7-triazocyclononane) and [Nb(O)Cl₃(PMe₃)]^[4] subsequently emerged, and appeared to

establish bond-stretch isomerism as a general feature of the chemistry of metal–ligand multiple bonds. This new form of isomerism naturally aroused much interest among theoretical chemists, notably Hoffmann and co-workers, who used extended Hückel theory to study strained cyclic hydrocarbon species,^[1] and, much later, the metal–oxo species noted above.^[5] As a result of these calculations, they postulated that a redistribution of electrons within the π framework of the molecule, involving either a crossing of occupied and unoccupied orbitals or a second-order Jahn–Teller effect, could account for the isomerism in [W(O)Cl₂(Me₃tacn)]⁺ and *cis-mer*-[Mo(O)Cl₂(PMe₂Ph)₃], respectively. In 1991, however, Hall and Song used more sophisticated ab initio methods to reinvestigate these claims, and reported that they were unable to locate a double minimum on the potential energy surface in either case.^[6] At around the same time, a reexamination of the structure of *cis-mer*-[Mo(O)Cl₂(PMe₂Ph)₃] showed that the apparently different Mo=O bond lengths were in fact an artifact of compositional disorder, caused by traces of *mer*-[MoCl₃(PMe₂Ph)₃].^[7] This report, in conjunction with the available theoretical data, prompted Parkin to observe in his 1993 review that there was “presently no definitive evidence of bond-stretch isomerism”.^[8]

Three years previously, Kölle and co-workers^[9] had reported that two isomers were present in the unit cell of [(Cp*RuCl)₂(μ-Cl)₂] (**1**) (Cp* = C₅Me₅; Figure 1) with very different Ru–Ru separations, 2.930(1) Å (**1a**) and 3.752(1) Å (**1b**). This system has not, however, been widely accepted as

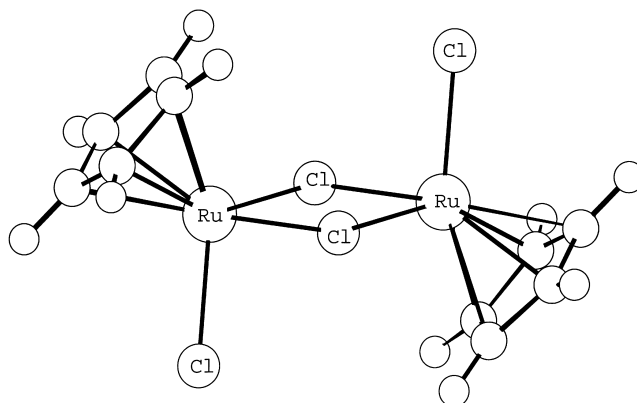


Figure 1. Molecular structure of [(Cp*RuCl)₂(μ-Cl)₂] (**1**).

an example of bond-stretch isomerism because the nature of the coupling between the metal ions differs in the two isomers: antiferromagnetic in **1a**, ferromagnetic in **1b**. Hoffmann and Parkin have gone to some lengths^[10] to distinguish “bond-stretch” isomerism, where both isomers lie on the same potential energy surface, from “spin-state” isomerism, where a change in multiplicity occurs.^[11] Within the limits of Hoffmann’s definition, the Kölle complex is therefore properly classified as an example of “spin-state” isomerism. Herein broken-symmetry density functional theory is used to establish the fundamental electronic reasons for the co-existence of two isomers in **1**. The validity of the distinction between “bond-stretch” and “spin-state” isomerism is then reexamined in the context of dimetallic clusters.

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Optimization of the structure of the broken-symmetry state of $[(\text{CpRuCl})_2(\mu\text{-Cl})_2]$ ($\text{Cp} = \text{C}_5\text{H}_5$) does indeed reveal two minima, denoted S_1 and S_2 , depending on the starting geometry. The calculated Ru–Ru separations of 2.881 Å and 3.753 Å (Table 1) for S_1 and S_2 , respectively, are remarkably similar to those in **1a** and **1b**, as are the other bond lengths and angles. Whilst both S_1 and S_2 correspond to spin singlet

Table 1. Optimized structural parameters, spin densities, and relative energies of S_1 – S_3 and T_1 . Structural parameters of **1a** and **1b** are shown for comparison.

	Ru–Ru [Å]	Ru–Cl ^[a] [Å]	Ru–Cl _{br} ^[a] [Å]	Ru–C [Å]	Cl _i –Ru–Ru ^[a] [°]	Spin density	E_{rel} [kJ mol ^{−1}]
calculated values							
S_1	2.881	2.429	2.422	2.259	88.2	0.0	0.0
S_2	3.753	2.370	2.480	2.263	93.4	± 0.71	+ 4.0
S_3	3.658	2.409	2.463	2.252	89.8	± 0.61	+ 9.0
T_1	3.782	2.377	2.464	2.259	94.7	+ 0.68	+ 1.0
crystallographic values							
1a	2.930(1)	2.418(2)	2.366(1)	2.191	89.7		
1b	3.752(1)	2.365(2)	2.445(1)	2.173	95.8		

[a] t = terminal Cl atom, br = bridging Cl atom.

configurations, the distribution of the ten valence electrons within the twelve spin orbitals of the t_{2g} manifold differs: $(a')^6(a'')^4$ in S_1 , and $(a')^8(a'')^2$ in S_2 . In S_1 , a net spin density of zero at each metal center indicates that all orbitals are completely delocalized. The Ru–Ru σ^* character in the LUMO ($4a'$, Figure 2a) confirms that a single σ bond is present, as postulated previously by Burdett et al.^[9b] In S_2 , in contrast, the spin- α and spin- β orbitals are localized on opposite sides of the molecule (Figure 2b), as a result of which, nonzero spin densities (± 0.71 electrons) emerge at each ruthenium center. Full potential energy curves for the two configurations, $(a')^6(a'')^4$ and $(a')^8(a'')^2$, are shown in Figure 3 (full and dashed lines, respectively). For the former,

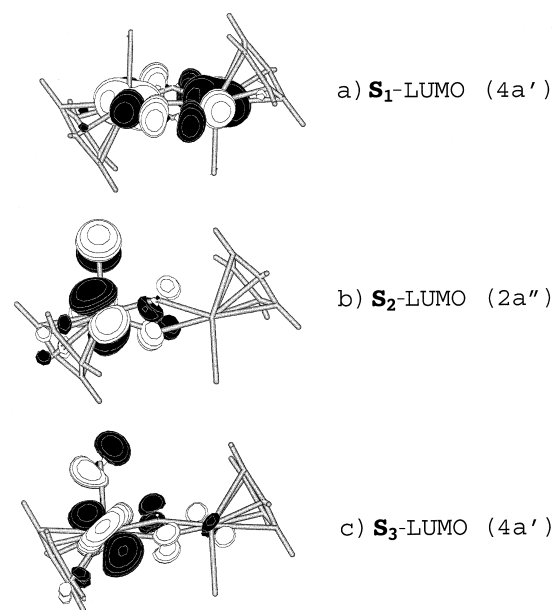


Figure 2. Lowest unoccupied spin orbitals of S_1 – S_3 . The orbitals shown have α spin; the corresponding spin- β orbitals are mirror images of those shown.

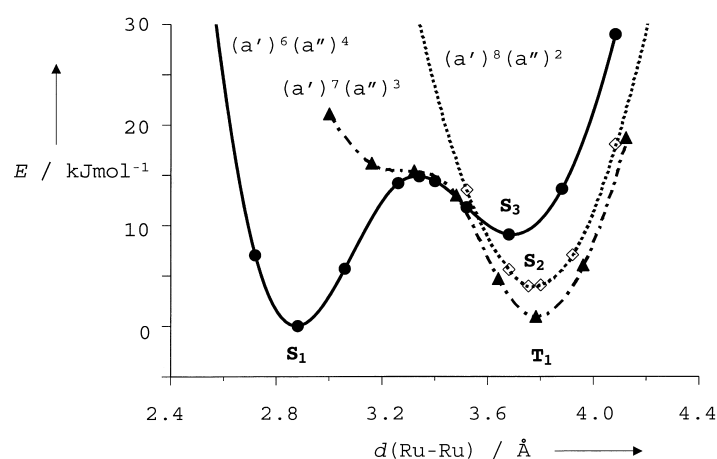


Figure 3. Potential energy curves for singlet and triplet configurations of $[(\text{CpRuCl})_2(\mu\text{-Cl})_2]$.

a local minimum (S_3) is found at Ru–Ru = 3.658 Å, in addition to the one at 2.881 Å (S_1). The double minimum arises because both Ru–Ru σ and π orbitals transform as a' in C_s symmetry, and at S_3 , π character, rather than σ character, dominates in the LUMO (Figure 2c). Although configuration interaction cannot be explicitly accommodated within the confines of density functional theory, its influence on the potential energy curves in Figure 3 can be deduced readily. Both configurations give rise to states of $^1A'$ symmetry and so configuration interaction will mix the two, most strongly in the region where the curves cross. This mixing causes the singlet ground state to pass smoothly from the covalently bonded limit (S_1) to the weakly antiferromagnetically coupled state (S_2).

The location of two widely separated minima on the potential energy surface clearly indicates the existence of two distinct electronic mechanisms for conferring stability on the molecule. At short separations the primary stabilizing force is the σ bond between the metal atoms. At longer separations the loss of this σ -bond is offset by an increase in the exchange energy associated with the nonzero spin density on each individual metal center.^[12a, 13] Where the covalent bonding and exchange stabilization are of similar magnitude, as they are in this case, two distinct minima can emerge on the potential energy surface. In the absence of ligands bridging the two metal centers, the weakly coupled limit simply corresponds to the dissociation limit of the bond. Thus a bridged bimetallic core is the minimum structural motif required for the occurrence of two distinct minima.

Whilst the calculated structural parameters of S_2 are very similar to those of isomer **1b**, the singlet spin state is inconsistent with the experimentally observed ferromagnetism.^[9] At long Ru–Ru separations, however, three triplet configurations are also present in the valence region. These configurations correspond to single occupation of a) two orbitals of a' symmetry, b) two orbitals of a'' symmetry, and c) one orbital of each symmetry. Of these, only the third configuration $(a')^7(a'')^3$, in which the two singly occupied orbitals are orthogonal, has a ground state (T_1) that is more stable than S_2 . The Ru–Ru separations of S_2 and T_1 are very similar, and so, based on both structural and energetic

evidence the prime candidates for the ground states of the isomers **1a** and **1b** appear to be **S₁** and **T₁**, respectively. The relative energies of **S₁** and **T₁** are consistent with the greater observed stability of **1a**, although the calculated separation of only 1 kJ mol⁻¹ is substantially lower than the experimental value of 15 kJ mol⁻¹. It has been noted previously, however, that the gradient-corrected density functionals used in this work tend to underestimate the strength of metal–metal bonds.^[12b] If the calculations are repeated without gradient corrections the general features of the curves remain unchanged but the **S₁**–**T₁** energy separation rises to 50 kJ mol⁻¹, substantially higher than the experimental estimate. The purpose of this paper is not to assess the merits of different functionals and this comparison is provided simply to illustrate that the calculation of relatively small energy differences remains a considerable challenge to computational chemistry. In the present context, the critical point to emphasize is that density functional theory predicts the presence of two minima on the potential energy surface with an energetic separation that is consistent with the coexistence of two isomers in a single crystal. The calculated barrier that separates the two minima (15 kJ mol⁻¹) is also consistent with experiment, being low enough to allow rapid equilibration in solution, but high enough to prevent interconversion in the solid state.

Having established the physical basis for the coexistence of two distinct isomers we can now address the question raised in the title—should the Kölle complex be classified as an example of “bond-stretch” or “spin-state” isomerism? In the strict Hoffmann definition the answer is clearly the latter because a change of spin state does occur. However, the nature of the spin-state transition in dimetallic species is distinctly different from that in monomeric spin–crossover complexes. In the latter, the low-spin/high-spin transition is intrinsically linked to the change in structure, because metal–ligand antibonding orbitals must be populated in the high-spin state. In contrast, in dimetallic systems the spin-state transition involves only a reorientation of spins on the metal centers and is associated with very minor structural changes (compare the structures of **S₂** and **T₁**). Moreover, the underlying stabilizing mechanism, the exchange energy, is the same in both **S₂** and **T₁**. There is therefore no *causal* link between the change in structure and the change in spin state. The isomerism observed in complex **1** is, in fact, best described as an equilibrium between a covalently bonded species and a diradical. When viewed in this context the system is very similar to the strained cyclic hydrocarbon species that formed the basis of Hoffmann’s original work on bond-stretch isomerism.^[1] In summary, despite the change in spin state associated with the isomerism in **1** this system fulfills all the criteria required of “bond-stretch” isomerism, and should be recognized as a genuine example thereof.

Computational Methods

All calculations were performed by using the Amsterdam Density Functional (ADF99) program.^[14] Double- ζ + polarization and triple- ζ basis sets were used to describe the main group and ruthenium atoms, respectively. The local density approximation was employed in all cases^[15] along with the local exchange–correlation potential of Vosko, Wilk, and Nusair^[16] and

gradient corrections to exchange (Becke)^[17] and correlation (Perdew).^[18] Unsubstituted Cp rings, constrained to local *D*_{5h} symmetry, were used to model Cp* ligands. The broken-symmetry methodology,^[19] which involves the removal of all symmetry elements connecting the ruthenium centers (corresponding to a reduction in symmetry from *C*_{2h} to *C*_s), was used for all singlet configurations.

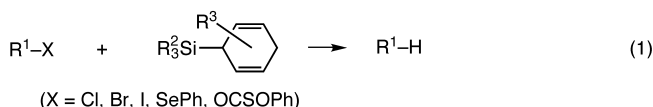
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Silylated Cyclohexadienes: New Alternatives to Tributyltin Hydride in Free Radical Chemistry**

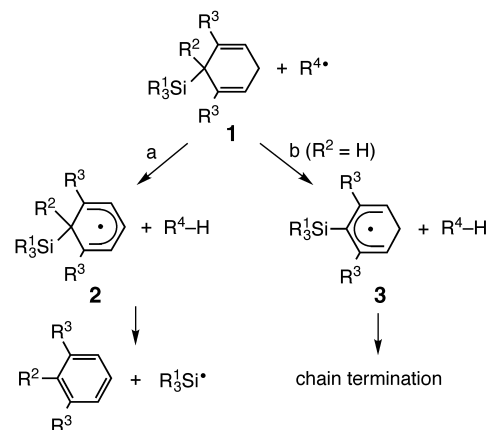
Armido Studer* and Stephan Amrein

Although tin hydrides (Bu_3SnH , Ph_3SnH , Me_3SnH) have successfully been used in free radical chemistry over the last 40 years,^[1] there are drawbacks associated with such tin-based radical reducing agents. The toxicity of organostannanes necessitates special handling in their disposal, and problems with product purification are often observed. Many approaches have therefore been presented to circumvent these problems.^[2] Protocols in which tin hydrides are used in catalytic amounts have been described.^[3] In addition polymer-bound,^[4] fluorous,^[5] water-soluble^[6] and acid-soluble^[7] tin hydrides have been prepared. Special workup procedures for tin-mediated reactions have been introduced;^[8] but, in all these systems, trace quantities of tin can remain after standard laboratory purification. Recently, disilaanthracenes^[9] and tetraaryldisilanes^[10] were used in radical reactions instead of tin hydrides; however, these silanes are difficult and labor-intensive to prepare. Substituted germanes^[11] are expensive, and phosphorus-based reducing agents^[12] are not well-developed. Polarity reversal catalysis using silanes together with thiols has only found limited application in radical chemistry.^[13] The best alternative to the tin hydrides documented thus far is Chatgililoglu's tris(trimethylsilyl)silane ($(\text{TMS})_3\text{SiH}$),^[14] which is easily oxidized by air. We now introduce silylated 1,4-cyclohexadienes as new, readily prepared radical reducing agents. We demonstrate that they can replace the toxic tin hydrides in various radical reactions such as dehalogenations, deselanations, deoxygenations, cyclizations, and intermolecular radical addition reactions [Eq. (1)].



It is well known that cyclohexadienes can serve as hydrogen donors in alkyl radical reductions.^[15, 16] However, 1,4-cyclohexadiene is not a tin hydride substitute in alkyl halide reductions since the cyclohexadienyl radical formed after

initial hydrogen transfer cannot propagate a radical chain by halogen abstraction. With appropriate substituents the 1,4-cyclohexadienes should participate as reducing agents in radical chain reactions: We speculated that silylated cyclohexadienes **1** would function as a substitute for tin hydrides in radical dehalogenations (Scheme 1). Reduction of a radical $\cdot\text{R}^4$ by **1** should afford cyclohexadienyl radical **2** (path a).

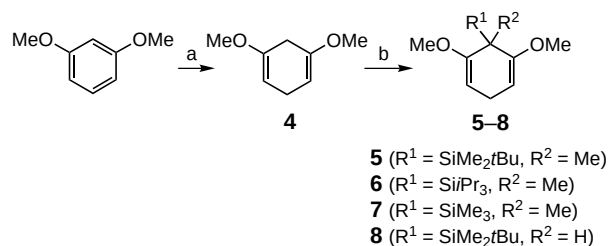


Scheme 1. 1,4-Cyclohexadienes **1** as radical reducing agents.

Fragmentation (rearomatization) of **2** will then give the corresponding silyl radical which is able to propagate the chain by halogen abstraction. If $\text{R}^2 = \text{H}$, pathway b can occur leading to chain termination. In order to drive the fragmentation of **2** towards the desired formation of the silyl radical, substituent R^2 should neither be a hydrogen atom^[17] nor a substituent which would afford a stabilized radical upon fragmentation.

We considered the ready multigram synthesis of any new hydrogen donor to replace tin hydride an important criteria. Based on this practical and the mechanistical considerations discussed above, **5–7** were prepared starting from commercially available resorcin dimethyl ether by Birch reduction ($\rightarrow$ **4**) and subsequent one-pot silylation–methylation (Scheme 2). For example on a 30g-preparative scale, **5** was obtained in 80% overall yield as a crystalline solid that can easily be stored.^[18] Moreover, the sequence we have developed allows ready access to new derivatives of this type.

To test whether these new reagents serve as effective replacements for tin hydrides, we first studied the dehalogenation of 1-bromoadamantane with **5** (1.3 equiv, 0.3 equiv α,α' -azobisisobutyronitrile (AIBN)) in various solvents (0.2 M).



Scheme 2. a) NH_3 , Na, EtOH, Et_2O ; 95%. b) 1. $t\text{BuLi}$, HMPA, THF; 2. $\text{R}_2\text{R}''\text{SiCl}$; 3. $n\text{BuLi}$; 4. $(\text{MeO})_2\text{SO}_2$.

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In refluxing hexane, reduction occurred quantitatively (Table 1, entry 1). Slightly lower yields were obtained when the reaction was performed in benzene (78 %),^[19] *t*BuOH (80 %), or THF (60 %). Subsequent experiments were therefore conducted in hexane (Table 1). Reduction of 1-bromo-

Table 1. Radical reductions of various substrates using **5**–**8** (1.3 equiv) and AIBN (0.3 equiv) in refluxing hexane (0.2 M).

Entry	RX	Si Reagent	<i>t</i> [h]	Yield (RH) ^[a] [%]
1		5	5.5	99
2		6	4.5	99
3		7	7	35
4		8	8	27
5		5	3.5	99
6		6	4	99
7		5	5	98
8		6	3.5	99
9		5	18	99 ^[b]
10		6	15	92 ^[b]
11		6	7.5	76 ^[b]
12		6	2	99
13 ^[c]		5	15	58
14 ^[d]		6	16	93
15		5	15	74 ^[b]
16		6	15	92 ^[b]
17		5	18	91 ^[b]
18		5	5	63 ^[b]

[a] Yield determined by GC analysis using C₁₄H₃₀ as internal standard. [b] Yield of isolated product. [c] With two equivalents of **5** and 0.6 equivalents of AIBN. [d] With three equivalents of **6** and one equivalent of AIBN.

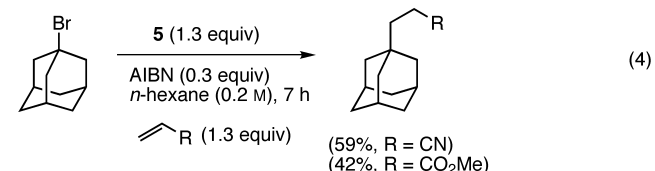
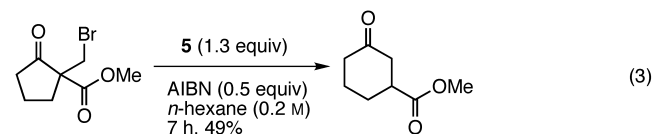
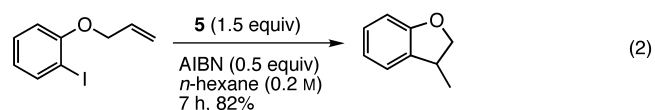
adamantane with **6** worked equally well (entry 2), whereas adamantane was obtained in only 35 % in the reduction with the trimethylsilyl derivative **7** (entry 3); but **7** was completely consumed. We further found that **7** slowly decomposes even at 4 °C. Consequently, owing to its instability, **7** is not a suitable tin hydride substitute. Si-reagent **8** lacking the methyl substituent (R²=H) afforded adamantane in only 27 % yield (entry 4).^[20] Thus, **5** and **6** became the reagents of choice for our subsequent studies.

In addition to AIBN, other radical initiators can be used for the reduction of bromoadamantane with **5** such as Et₃B (0.2 equiv)/O₂^[21] (99 %). The same reaction at room temper-

ature afforded only traces of adamantane. Either the reduction step or the rearomatization or even both processes are too slow at room temperature. Pleasingly, the reduction can be conducted perfectly well under an atmosphere of air (≈22 % O₂) without using an initiator to give adamantane in quantitative yield (99 %).^[22]

To test the scope and the limitations of **5** and **6**, a series of reductions using various radical precursors that are typically employed was studied (Table 1). Primary, secondary, and aromatic halogenides were efficiently reduced (entries 5–14, Table 1). Barton–McCombie deoxygenation^[23] of secondary alcohols using phenyl thionocarbonate esters^[24] worked equally well (entries 15–17).^[25] As expected, phenylselenides are readily reduced with **5** (entry 18).

Another common set of synthetic reactions that utilize trialkyltin hydrides are radical cyclizations and intermolecular addition reactions. Cyclization of *o*-iodophenyl allyl ether [Eq. (2)] and the Beckwith–Dowd ring enlargement reaction^[26] could be conducted with our new reagent [Eq. (3)]. Intermolecular addition reactions worked rather well [Eq. (4)]. Importantly, the syringe pump technique is not necessary to conduct these intermolecular addition reactions. Furthermore, the olefin is used in only a small excess (1.3 equiv).^[27]



Finally, we also estimated the rate constant of the hydrogen transfer from reagent **5** to a primary radical. We have chosen the 5-*exo*-cyclization of the 5-hexenyl radical as the free radical clock.^[28] A rate constant of $1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ was estimated for the reduction of the intermediate primary alkyl radical at 70 °C. Thus, hydrogen transfer from **5** is about 10 times slower than the corresponding reaction with (TMS)₃SiH,^[29] about 55 times slower than that with Bu₃SnH,^[30] and almost as fast as that with Bu₃GeH.^[31]

With the presented readily accessible, radical reducing agents, reagents are available that can replace the toxic tin hydrides commonly applied.

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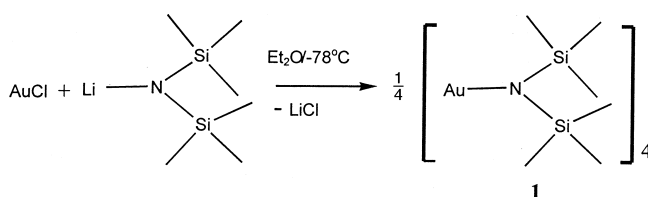
[[Au(μ -N(SiMe₃)₂)]₄]: The First Base-Free Gold Amide**

Scott D. Bunge, Oliver Just, and William S. Rees, Jr.*

*Dedicated to Professor Herbert Schumann
on the occasion of his 65th birthday*

Throughout the Periodic Table, the use of the bis(trimethylsilyl)amido ligand N(SiMe₃)₂[−] has played a central role in the synthesis and characterization of metal and metalloid complexes with low coordination numbers.^[1] Despite the extensive use, only a few examples of noble- or heavier coinage-metal derivatives have been reported. This deficiency may be attributed to the instability of these compounds, which arises from the combination of a soft metal with a hard amide ligand.^[2] Herein, the first synthesis and X-ray structural characterization of a base-free homoleptic gold(I) di(silyl)-amide, [[Au(μ -N(SiMe₃)₂)]₄] (**1**), is reported.

The gold(I) amide **1** was prepared in 19% yield by the metathesis reaction of lithium bis(trimethylsilyl)amide with gold(I) chloride in diethyl ether (Scheme 1). ¹H NMR and ¹³C NMR spectroscopic analyses of **1** in CDCl₃ revealed the presence of singlets at δ = 0.34 and 6.707, respectively. The EI and CI mass spectra revealed a tetranuclear parent ion, with the expected fragment ions.



Scheme 1. Synthetic route leading to the formation of **1**.

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Several other gold tetramers have been reported previously; $[\{\text{Au}[\text{SSi}(\text{OrBu})_3\}]_4\}$,^[3a] $[\{\text{Au}[\text{TeC}(\text{SiMe}_3)_3\}]_4\}$,^[3b] $[\{\text{Au}[\text{SC}(\text{SiMe}_3)_3\}]_4\}$,^[3b] $[\{\text{Au}(\text{PhNNPh})\}]_4$,^[4a] and $[\{\text{Au}(\text{PhNCHNPh})\}]_4$.^[4b]

The development of gold(I) chemistry is dominated by the viewpoint that gold is a prototypical soft Lewis acid, which forms its most stable complexes with soft Lewis bases. Accordingly, the synthesis of gold(I) complexes with a hard Lewis base, such as nitrogen, has been limited to a select number of complexes.^[5] Schmidbaur et al., have reported the following base-stabilized gold–nitrogen derivatives; $[\text{AuN}(\text{SiMe}_3)_2(\text{PMe}_3)]$ and $[\text{Au}_2\text{N}(\text{SiMe}_3)_2(\text{PET}_3)_2]\text{BF}_4$ (**2**).^[6a,b] It is notable that these complexes employ phosphane donors to stabilize the valency configuration of gold.

Compound **1** was shown by single-crystal X-ray diffraction to be tetrameric and solvent-free in the solid state (Figure 1).

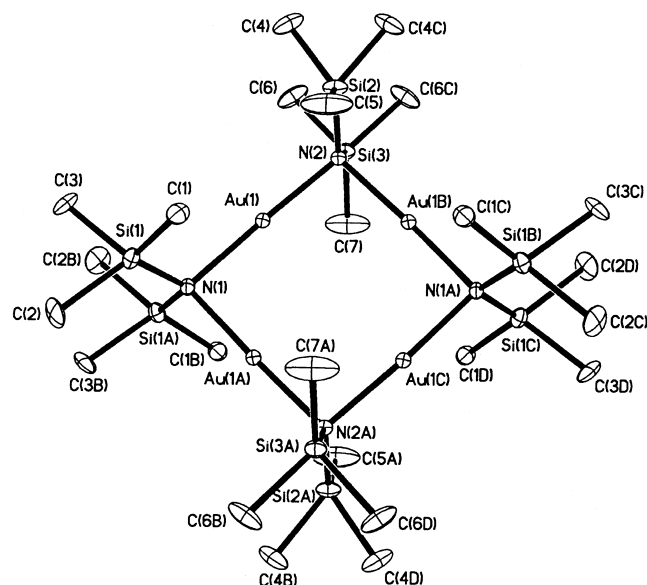
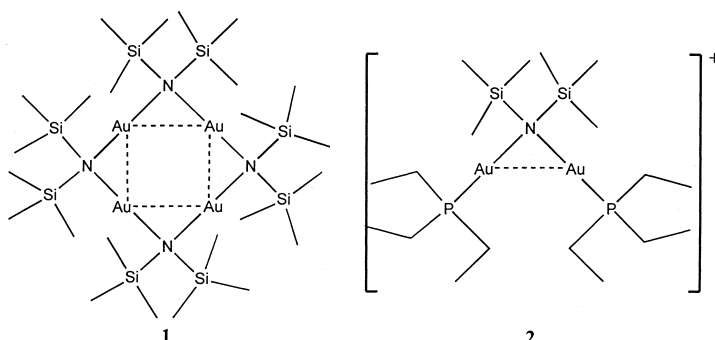


Figure 1. An ORTEP plot representation (50% probability) for **1**. Hydrogen atoms have been omitted for clarity. Selected interatomic distances [Å] and angles [°]: Au(1)–N(1) 2.082(3), Au(1)–N(2) 2.086(3), Au(1)–Au(1A) 3.0100(3), Au(1)–Au(1B) 3.0355(3), Si(1)–N(1) 1.773(3); Au(1)–N(1)–Au(1A) 92.38(19), Au(1)–N(2)–Au(1B) 93.60(19), Si(3)–N(2)–Si(2) 120.9(3).

The molecular structure is tetranuclear, based on a square of two-coordinate gold atoms. Each Au atom is linked to its neighbor by a singly bridging amido ligand, the nitrogen donor sites thus being four-coordinate and the N–Au–N arrangements being essentially linear. The Au–N interatomic distances observed for compound **1** (2.082, 2.086 Å) are similar to previously reported values. The Au–N–Au angles in **1** are 92.38° and 93.60°, which correspond to Au–Au distances of 3.0100 Å and 3.0355 Å, respectively.

Schmidbaur defines aurophilicity as “the unprecedented affinity between gold atoms even with ‘closed-shell’ electronic configurations and equivalent electrical charges.”^[7] The interatomic distance usually observed for these aurophilic interactions is on the order of 3.00 ± 0.25 Å, and the energy associated with these interactions is similar to the energetics of hydrogen bonds. A comparison of Au–Au and Au–N

interatomic distances for **1** and **2** (Scheme 2) reveals that the Au–Au interatomic distances in the base-free, air-stable compound **1** are significantly shorter than those in **2**. Therefore, it can be presumed that aurophilic interactions are, in part, stabilizing the tetramer.



Scheme 2. Au–Au and Au–N interatomic distances [Å] in **1** and **2**. Compound **1** (this work): Au–Au 3.0100(3), 3.0355(3); Au–N 2.082(3); compound **2** (reference [6a]): Au–Au 3.071(1); Au–N 2.115(9).

Planar, tetranuclear coinage metal(I) clusters singly bridged by monoanionic amido ligands previously have been reported for copper and silver.^[8]

In summary, the first gold(I) amide $[\{\text{Au}[\mu\text{-N}(\text{SiMe}_3)_2\}]_4\}$ has been synthesized without the stabilization of a Lewis base. This result may lead to new directions in the chemistry of gold(I) complexes.

Experimental Section

1: *n*BuLi (1.1 mL of a 2.68 M solution in hexane) was added dropwise to hexamethyldisilazane $\text{HN}(\text{SiMe}_3)_2$ (0.48 g, 2.97 mmol) in diethyl ether (50 mL) at -78°C under an argon atmosphere. Subsequently, the reaction mixture was allowed to warm to ambient temperature on its own and was left to stir for 2 h, completing the generation of the lithium amide in situ. The solution was recooled to -78°C and AuCl (0.69 g, 2.97 mmol) was added through a solid addition tube. After the mixture had been stirred overnight at -78°C under light exclusion, the solvent was removed and the product was extracted with hexane (10 mL). Following filtration, colorless crystals of **1** were grown from hexane over five days at -40°C . Yield: 0.20 g (18.9%); Colorless crystals turned purple at 110°C ; ^1H NMR (300 MHz, CDCl_3): δ = 0.34 (s, 72 H, 24 CH_3); ^{13}C NMR (300 MHz, CDCl_3): δ = 6.707 (s, 24 CH_3); MS (EI, 70 eV, 400°C): m/z : 1429 [$M^+ + \text{H}^+$], 1413, 554, 699, 342, 130, 73; elemental analysis calcd (%) for $\text{Au}_4\text{N}_4\text{Si}_8\text{C}_{24}\text{H}_{72}$: C 20.17, H 5.08, N 3.92; found: C 19.85, H 5.00, N 3.96.

Crystal data: $\text{Au}_4\text{N}_4\text{Si}_8\text{C}_{24}\text{H}_{72}$, M_r = 1429.44 g cm^{-3} , crystal dimensions $0.256 \times 0.160 \times 0.128$ mm, monoclinic, space group $C2/m$, a = 20.1768(8), b = 13.6852(6), c = 9.2556(4) Å, β = 116.1870(10)°; V = 2293.37(17) Å³, Z = 2, ρ_{calcd} = 2.070 g cm^{-3} , Siemens SMART CCD diffractometer, $1.87 < \theta < 28.28^\circ$, $\text{MoK}\alpha$ radiation (λ = 0.71073 Å), ω scans, T = 173(2) K; of 7267 measured reflections, 2827 were independent and 2572 observed with $I > 2\sigma(I)$, $-25 < h < 26$, $-18 < k < 18$, $-9 < l < 12$; R_1 = 0.0233, wR_2 = 0.0610, GOF = 1.037 for 149 parameters, $\Delta\rho_{\text{max}}$ = 1.509 e Å^{-3} . The structure was solved by direct methods (SHELXS-97) and refined by full-matrix least-squares procedures (SHELXL-97), Lorentzian and polarization corrections and absorption correction SADABS were applied, μ = 12.983 mm^{-1} , min./max. transmission 0.1358/0.2873. For C5 and C7, hydrogen atoms were refined as HFIX from SHELXTL using an appropriate riding model with varied thermal parameters. All other carbon atoms were disordered. 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-142594. Copies of the data can be obtained free of charge on application to CCDC,

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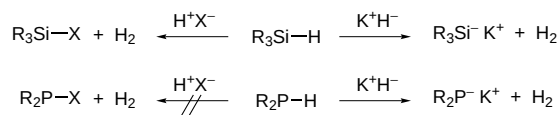
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Umpolung of P–H Bonds**

Dietrich Gudat,* Asadollah Haghighverdi, and Martin Nieger

Dedicated to Professor Gerd Becker
on the occasion of his 60th birthday

Bonds between p-block elements E and hydrogen are employed as versatile synthons in numerous synthetic transformations.^[1] Their reactivity follows a systematic course that is characterized by a change from hydridic (E–H bonds involving elements of Group 13) to protonic (E–H bonds involving elements of Groups 15–17) character of the hydrogen atom. Hydrogen compounds of Group 14 elements are a borderline case: whereas the protonic character of the hydrogen atom dominates for C–H bonds, Si–H bonds may react both as the source of a hydride or of a proton (Scheme 1).^[1, 2]



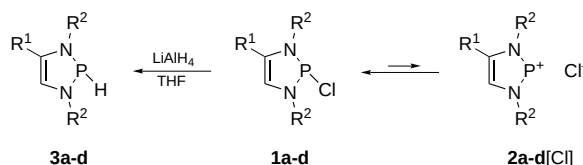
Scheme 1. X = halogen, OR.

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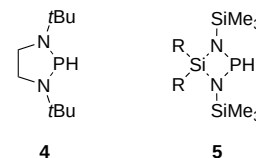
Supporting information for this article is available on the WWW under
http://www.wiley-vch.de/home/angewandte/ or from the author.

The reactivity of P–H bonds in phosphane derivatives is generally determined by the protonic character of the hydrogen atom (Scheme 1), even though in view of the similar electronegativities ($\chi^{\text{AR}}(\text{H}) = 2.2$, $\chi^{\text{AR}}(\text{P}) = 2.06$) and resulting low bond polarities it appears conceivable to achieve an umpolung of the reactivity by means of suitable substituent effects.^[3] Taking into account our observation that π -delocalization effects which can be described in terms of σ^* aromaticity^[4] render the P–Cl bonds in *P*-chloro-1,3,2-diazaphospholenes **1** more polar and favor dissociation under formation of the cations **2**,^[5] it appeared of interest to establish if the same effects can also be applied to generate hydridic reactivity of the P–H bonds in *P*-hydrido-1,3,2-diazaphospholenes **3** (Scheme 2).



Scheme 2. R¹ = H, R² = *t*Bu (**1a–3a**), 2,4,6-Me₃C₆H₂ (Mes) (**1c–3c**); R¹ = Cl, R² = *t*Bu (**1b–3b**), Mes (**1d–3d**).

The target compounds **3a–d** are formed in clean reactions upon treatment of **1a–d** with stoichiometric amounts of LiBEt₃H or LiAlH₄ in THF and were isolated as light yellow, air- and moisture-sensitive oils or solids after distillative work-up (**3a, b**) or crystallization (**3d**). The constitution of all products can be deduced unequivocally from their spectroscopic data. The ³¹P NMR signals ($\delta^{31}\text{P} = 57.1$ (**3a**), 71.6 (**3b**), 64.0 (**3c**), 75.8 (**3d**)) appear at slightly lower field than in the 1,3,2-diazaphospholidine **4** ($\delta^{31}\text{P} = 57.9$ ^[6]). The P,H coupling constants display a remarkable substitution dependence: whereas the couplings in the *N*-*tert*-butyl derivatives **3a, b** ($^1J(\text{P,H}) = 181$ (**3a**), 219 (**3b**) Hz) are clearly larger than in **4** ($^1J(\text{P,H}) = 156$ Hz^[6]), those in the *N*-mesityl compounds **3c, d** ($^1J(\text{P,H}) = 139$ (**3c**), 147 (**3d**) Hz) come close to the extremely low values of the four-membered heterocycles **5** (R = Me, Ph; $^1J(\text{P,H}) = 125$ –127 Hz^[7]). Following common concepts, the decrease of $^1J(\text{P,H})$ can be related with decreasing p-character and lengthening of the P–H bond and should thus indicate a bond-weakening effect. This hypothesis is corroborated by the red shift of the P–H stretching vibration frequencies in **3a–d** ($\tilde{\nu}(\text{PH}) = 2120$ –2202 cm⁻¹) as compared to those of known cyclic and acyclic diaminophosphanes ($\tilde{\nu}(\text{PH}) = 2220$ –2340 cm⁻¹^[6, 8]), and by the result of a single-crystal X-ray diffraction study of **3d**.^[9]



The structure of crystalline **3d** is composed of isolated molecules that display no significant intermolecular interactions (Figure 1).^[11] The five-membered ring features an “envelope” conformation with the phosphorus atom sticking out of the plane formed by the remaining ring atoms, and the attached hydrogen atom adopting a “flagpole” position. The

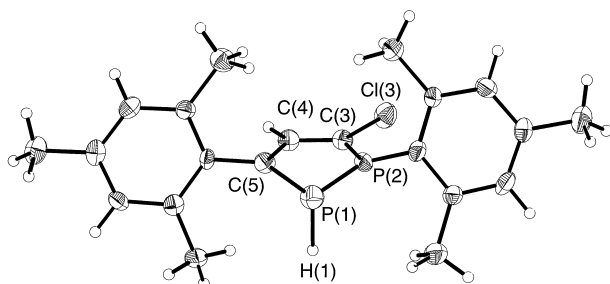


Figure 1. Molecular structure of **3d** in the crystal (50% probability thermal ellipsoids); important distances [Å] and angles [°]: P(1)-H(1) 1.51(4), P(1)-N(5) 1.709(3), P(1)-N(2) 1.722(3), N(2)-C(3) 1.407(5), C(3)-C(4) 1.327(5), C(3)-Cl(3) 1.720(4), C(4)-N(5) 1.410(5), N(5)-P(1)-N(2) 89.92(16), N(5)-P(1)-H(1) 97.8(14), N(2)-P(1)-H(1) 99.3(14).

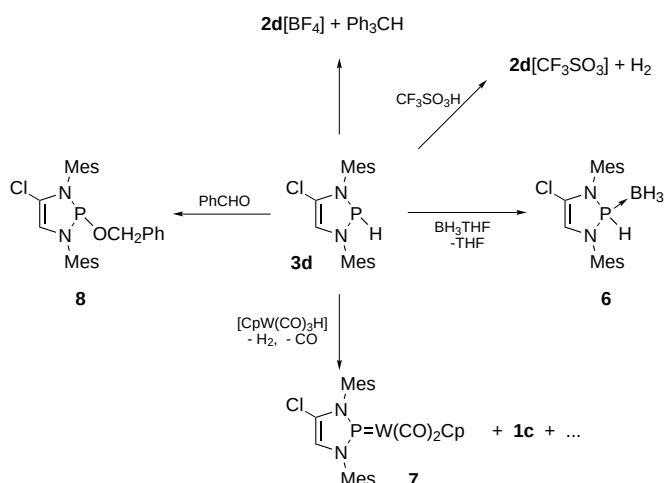
P-H distance (1.51(4) Å) is notably longer than known P-H bond lengths in phosphanes (1.288 ± 0.09 Å^[12]). The endocyclic bond lengths (P-N 1.709(3), 1.722(3), C-N 1.407(5), 1.410(5), C=C 1.327(5) Å) range as in **1a**, **c** and **2a**^[5, 13] between those of genuine single and double bonds, even if the extent of bond length equalization decreases from **2a** over **1a**, **c** to **3d**.

In accord with model calculations^[14] these findings may be interpreted by assuming that hyperconjugative interaction between the six π electrons in the C_2N_2 unit and the $\sigma^*(P-H)$ orbital results (as in the case of **1**^[5]) in partial delocalization of π electrons in the ring. The lower extent of this “ σ^* aromaticity”^[4] in **3d** as compared to that in **1a**, **c** is consistent with the lower acceptor ability of the $\sigma^*(P-H)$ as compared to that of a $\sigma^*(P-Cl)$ orbital. Direct consequences of this π delocalization are a weakening of the P-H bond and a concomitant increase of charge density at the hydrogen atom (i.e. increasing hydridic character), which become immediately evident from comparison of computed P-H distances, $\nu(PH)$ vibrational frequencies, and atomic charges of the model compounds **A–C** (Table 1).

Table 1. Computed (at the MP2/6-31 + g(d,p(P-H))-level)^[14] P-H distances, $\nu(P-H)$ vibrational frequencies, and atomic charges $q(H)$ (from natural bond order (NBO) population analyses) in the model compounds **A–C**.

	A	B	C
$r(P-H)$ [Å]	1.447	1.426	1.406
$\nu(P-H)$ [cm ⁻¹]	2217	2361	2509
$q(H)$	-0.14	-0.11	-0.08

Chemical studies confirm that the enhancement of the hydridic character of the P-H bond, which has been inferred from spectroscopic and structural criteria, leads in fact to an umpolung of the reactivity. Thus, **3d** reacts with $[Ph_3C]^+[BF_4]^-$ under hydride transfer to give a quantitative yield of triphenylmethane and the phosphonium ion **2d** (Scheme 3).^[15] The same cation is also formed upon treatment of **3d** with trifluoromethanesulfonic acid. The reaction proceeds with gas evolution (H_2) and was found by ³¹P NMR spectroscopic



Scheme 3. Reactions of **3d**.

studies to occur spontaneously and without detectable intermediates even at -78°C in CH_2Cl_2 . Evidence for a quaternization, which is typical for secondary or tertiary phosphanes, was obtained in no case, even if the formation of the stable BH_3 adduct **6** from **3d** and $BH_3 \cdot THF$ suggests a sufficient basicity for the phosphorus atom.

Reaction of **3d** with the transition metal hydride $[CpW(CO)_3H]$ affords as main product (ca. 50%) the phosphonium complex **7**,^[16] which was spectroscopically identified, together with **1c** (ca. 25%) and further as yet not characterized by-products. Whereas the formation of **7** can be explained by condensation of the hydridic P-H and acidic W-H functionalities of **3d** and $[CpW(CO)_3H]$, respectively, which proceeds with cleavage of H_2 and subsequent elimination of CO, **1c** arises presumably from C-Cl/P-H meta-thesis between two molecules of **3d**.^[18]

Beside the observation of H_2 elimination in reactions with compounds containing acidic protons, the umpolung of the P-H bonds in **3** becomes further manifest in an inverse regioselectivity during the addition to carbonyl compounds. Thus, reaction of **3d** with benzaldehyde proceeds not as expected^[19] with formation of a α -hydroxybenzyl phosphane, but yields rather as the only product the benzyloxy derivative **8**, which was isolated as a yellow oil and characterized by spectroscopic techniques.^[16] The regioselectivity of the addition can be deduced unequivocally from the appearance of the signal of a benzylic CH_2 moiety in the $^{13}C\{^1H\}$ DEPT spectrum, and the absence of a characteristic $\nu(OH)$ band in the IR spectrum.

Experimental Section

3a–d: A solution of **1a–d** (10 mmol) in THF (50 mL) was added dropwise at 4°C to a suspension of $LiAlH_4$ (2.5 mmol) in THF (10 mL). Stirring was continued for 1 h, the solvent removed in vacuo, and the oily residue dissolved in hexane (50 mL). The formed precipitate was filtered off over Celite and the solvent removed in vacuo. The crude product was characterized by NMR spectroscopy (**3c**), or purified by means of vacuum distillation (**3a**, **b**) or recrystallization from pentane at -20°C (**3d**). **3a**: b.p. 50°C (1 mbar), yield 65%; ³¹P NMR (121.5 MHz, C_6D_6): $\delta = 57.1$ (d, $^1J(P,H) = 181$ Hz); ¹H NMR (300 MHz, C_6D_6): $\delta = 1.14$ (d, $^4J(P,H) = 0.9$ Hz, 18H), 5.97 (d, $^3J(P,H) = 4.0$ Hz, 2H), 6.07 (d, $^1J(P,H) = 181$ Hz, 1H); MS (16 eV): m/z (%): 200 (10) [M^+], 199 (78) [$M^+ - H$], 143 (28)

$[M^+ - C_4H_8]$, 87 (100) $[M^+ - 2C_4H_8]$; IR (gas): $\tilde{\nu} = 2176\text{ cm}^{-1}$ (P–H). **3b**: b.p. 55 °C (1 mbar), yield 78%; ^{31}P NMR (121.5 MHz, C_6D_6): $\delta = 71.6$ (d, $^1J(\text{P,H}) = 219\text{ Hz}$); ^1H NMR (300 MHz, C_6D_6): $\delta = 0.99$ (d, $^4J(\text{P,H}) = 0.7\text{ Hz}$, 9H), 1.33 (d, $^4J(\text{P,H}) = 1.7\text{ Hz}$, 9H), 6.16 (d, $^3J(\text{P,H}) = 3.3\text{ Hz}$, 1H), 6.23 (d, $^1J(\text{P,H}) = 219\text{ Hz}$, 1H); IR (Nujol): $\tilde{\nu} = 2202\text{ cm}^{-1}$ (P–H). **3c**: ^{31}P NMR (121.5 MHz, C_6D_6): $\delta = 64.0$ (d, $^1J(\text{P,H}) = 139\text{ Hz}$); ^1H NMR (300 MHz, C_6D_6): $\delta = 2.44$ (s, 6H), 2.48 (s, 6H), 2.56 (s, 3H), 2.59 (s, 3H), 6.16 (d, $^3J(\text{P,H}) = 1.8\text{ Hz}$, 2H), 7.04 (br, 4H), 7.13 (d, $^1J(\text{P,H}) = 139\text{ Hz}$, 1H); **3d**: m.p. 87–89 °C, yield 72%; ^{31}P NMR (121.5 MHz, C_6D_6): $\delta = 75.8$ (d, $^1J(\text{P,H}) = 147\text{ Hz}$); ^1H NMR (300 MHz, C_6D_6): $\delta = 2.09$ (s, 6H), 2.23 (s, 6H), 2.23 (s, 3H), 2.37 (s, 3H), 5.87 (d, $^3J(\text{P,H}) = 0.9\text{ Hz}$, 1H), 6.67 (s, 2H), 6.70 (s, 1H), 6.72 (s, 1H), 7.17 (d, $^1J(\text{P,H}) = 147\text{ Hz}$, 1H); MS (16 eV): m/z (%): 358(43) $[M^+]$, 357(100) $[M^+ - H]$; IR (gas): $\tilde{\nu} = 2120\text{ cm}^{-1}$ (P–H).

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Stereoselective Synthesis and Palladium-Catalyzed Transformations of 2-Alkylidene-5-vinyltetrahydrofurans**

Peter Langer* and Edith Holtz

Domino and sequential reactions are of interest in modern organic chemistry since they enable the rapid assembly of complex products.^[1] In the course of our studies on the development of domino reactions of dianions and dianion equivalents,^[2] we have recently reported the first cyclizations of dilithiated 1,3-dicarbonyl compounds with oxalic acid dielectrophiles.^[3] These reactions allow an efficient, regio- and stereoselective synthesis of the pharmacologically relevant substance class of γ -alkylidenebutenolides. Although a variety of simple condensation reactions of dianions with monofunctional alkyl halides are known, only a few domino dialkylation reactions of dianions with difunctional alkyl

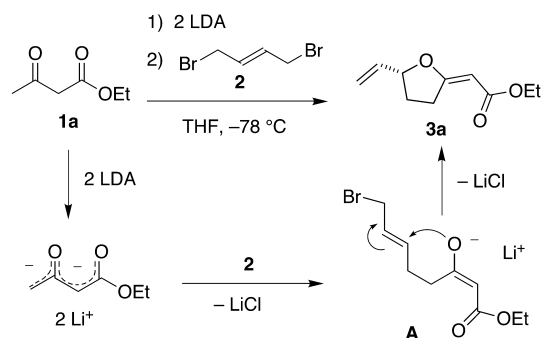
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halides have been reported so far.^[4] This can be explained by the high reactivity of the dianions and by the lability of the dielectrophiles which can give rise to polymerization, formation of open-chain 2:1 products, or single-electron transfer (SET) processes rather than cyclization: for example, reaction of the dianion of methyl acetoacetate with 1,*n*-dibromoalkanes was reported to give mixtures of open-chain monoalkylated products and of 2:1 condensation products.^[5] The reaction of dianions of 1,3-dicarbonyl compounds with 1,4-dichloro-2-butene was equally disappointing and resulted in formation of mixtures of open-chain products in low yields.^[5]

Herein, we report that reaction of dianions of 1,3-dicarbonyl compounds **1** with 1,4-dibromo-2-butene (**2**) results in regioselective formation of a wide range of 2-alkylidene-5-vinyltetrahydrofurans **3** and selective generation of up to three stereocenters (see Scheme 1). From a methodology viewpoint, these reactions represent the, to the best of our knowledge, first C,O-cyclodialkylations of 1,3-dicarbonyl dianions with **2**.^[6] 2-Alkylidene-tetrahydrofurans are not only of pharmacological relevance but also represent interesting building blocks for the synthesis of terpenes^[7a,b] and medium-sized lactones.^[7c] To demonstrate the usefulness of our cyclization products containing a unique functionality, we have developed a new palladium(0)-catalyzed rearrangement reaction which allows the direct transformation of bicyclic 2-alkylidene-5-vinyltetrahydrofurans into functionalized bicyclo[3.2.1]octan-8-ones. This rearrangement has, to the best of our knowledge, not been previously noted. It is noteworthy that the bicyclo[3.2.1]octane skeleton is present in a large number of pharmacologically important natural products including aphidicolan,^[8] kaurane,^[9] and stemodane diterpenes,^[10] hydroazulene,^[11] himachalene,^[11] α -cedrane,^[11] and grayanotoxin sesquiterpenes,^[11, 12] bridged steroids,^[13] and aconitine-type alkaloids.^[14] The combination of our new cyclodialkylation reaction and the new palladium-catalyzed rearrangement constitutes a significant expansion of the methods known today for the synthesis of functionalized bicyclo[3.2.1]octanes^[8f] which are of pharmacological significance and of importance for natural product syntheses.

Our first attempts to induce a cyclization reaction of the dianion of ethyl acetoacetate **1a** with 1,4-dibromo-2-butene (**2**) were unsuccessful. Addition of a solution of **2** in THF to a solution of the dianion at 0 °C gave a complex reaction mixture. Much to our satisfaction, addition of dibromide **2** to a solution of the dianion in THF at –78 °C resulted in formation of the cyclization product **3a** in 41 % yield (Scheme 1).

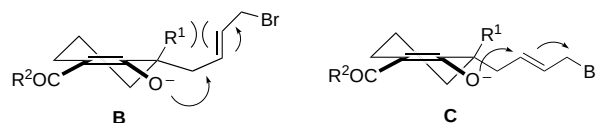


Scheme 1. LDA = lithium diisopropylamide.

Inspection of the crude product mixture showed that significant amounts of the 2:1 condensation product^[15] had been formed. Therefore, an inverse–addition protocol was employed: slow addition of a solution of the dianion in THF to a solution of **2** afforded the 2-alkylidene-5-vinyltetrahydrofuran **3a** in 61 % yield with very good regio- and stereoselectivity. Formation of **3a** can be explained by a domino-S_N-S_N' displacement reaction involving regioselective attack of the terminal carbon atom of the dianion on the dielectrophile and subsequent regioselective cyclization through the oxygen atom.^[16] The stereoselectivity in favor of the *E*-configured exocyclic double bond can be explained by the W-shaped configuration of intermediate **A** which allows a minimization of the dipole–dipole repulsion of the oxygen atoms.^[17]

To investigate the preparative scope of the new cyclization reaction, the substituents of the 1,3-dicarbonyl compounds were systematically varied (Table 1). Reaction of dibromide **2** with the dianions of *tert*-butyl acetoacetate, acetylacetone, 5,5-dimethylhexane-2,4-dione, and *N,N*-diethylacetylacetamide afforded the *E*-configured 2-alkylidene-5-vinyltetrahydrofurans **3b–e** in good yields and with very good stereoselectivities. Reaction of 1,4-dibromo-2-butene with the dianions of the ethyl acetoacetates **1f–h**, containing a substituent at their central carbon atom, resulted in formation of the tetrahydrofurans **3f–h**. Reaction of **2** with the dianion of 2-acetyl- γ -butyrolactone (**1i**) afforded the interesting 2-alkylidene-5-vinyltetrahydrofuran **3i**. Despite the fact that the products **3f–i** contain additional substituents at their exocyclic double bonds, very good *E*-selectivities were observed in all cases. The reaction of 1,4-dibromo-2-butene with the dianions of methyl 3-oxopentanoate and ethyl 3-oxohexanoate afforded the 2-alkylidene-5-vinyltetrahydrofurans **3j–k** in good yields and with very good *Z*-selectivities. The change from *E*- to *Z*-configuration can be explained by the steric influence of the substituents R¹.

Reaction of the dianion of ethyl cyclohexanone-2-carboxylate (**1l**) with dibromide **2** afforded the 5,6-bicyclic product **3l** in good yield and with very good 1,3-diastereoselectivity. Similarly, reaction of the dianions of isopropyl and methoxyethyl cyclohexanone-2-carboxylate (**1m** and **1n**) afforded the bicyclic 2-alkylidene-5-vinyltetrahydrofurans **3m** and **3n** in good yields and with very good stereoselectivities. Treatment of dibromide **2** with the dianion of aldehyde **1o** afforded the bicyclic tetrahydrofuran **3o** with very good stereoselectivity. Reaction of **2** with the dilithiated ester **1p**, containing a benzyl group at the 6-position of the cyclohexane ring, resulted in stereoselective formation of the 2-alkylidene-5-vinyltetrahydrofuran **3p**. The 1,3-diastereoselectivity can be explained by destabilization of transition state **B** (relative to **C**) due to a 1,3-interaction between the substituent R¹ (H, Bn) and the allyl group.



Reaction of **2** with the dianions of ethyl 5-methylcyclohexanone-2-carboxylate **1q** and methyl 5-ethylcyclohexanone-2-

Table 1. Synthesis of 2-alkylidene-5-vinyltetrahydrofurans **3**.

	1	3	R ¹	R ²	Yield [%] ^[a]	E:Z	d.r. ^[b]
a			H	OEt	61	> 98:2	–
b			H	O <i>t</i> Bu	64	> 98:2	–
c			H	Me	48	> 98:2	–
d			H	<i>t</i> Bu	46	> 98:2	–
e			H	NEt ₂	58	> 98:2	–
f			Me	OEt	66	> 98:2	–
g			Et	OEt	63	> 98:2	–
h			Bu	OEt	36	> 98:2	–
i			–	–	37	> 98:2	–
j			Me	OMe	65	< 2:98	45:55
k			Et	OEt	60	< 2:98	65:35
l			H	OEt	81	–	> 98:2
m			H	O <i>t</i> Pr	77	–	> 98:2
n			H	O(CH ₂) ₂ OMe	78	–	> 98:2
o			H	H	37	–	> 98:2
p			Bn	OEt	48	–	> 98:2
q			Me	OEt	74	–	> 98:2
r			Et	OMe	72	–	> 98:2
s			<i>t</i> Bu	–	53	–	> 98:2
t			Ph	–	65	–	> 98:2
u			H	–	42	–	> 98:2
v			Me	–	37	–	> 98:2
w			H	OEt	66	–	70:30
x			H	<i>t</i> Bu	51	–	90:10

[a] Yield of isolated product. [b] Diastereomeric ratio in favor of the drawn isomer.

carboxylate **1r** afforded the bicyclic 2-alkylidene-5-vinyltetrahydrofurans **3q** and **3r** in good yields. In these reactions, three stereocenters are formed with complete selectivity. The initial attack of the dianion onto the dielectrophile proceeded with very good 1,2-diastereocontrol giving rise to a *cis* configuration between the substituents R¹ and the bridgehead hydrogen atom. The subsequent cyclization proceeded with very good 1,3-diastereoselectivity. The stereochemical effect of the presence of a substituent at the 4-position of the cyclohexane moiety was studied next: reaction of dibromide **2** with the dilithiated ethyl cyclohexanone-2-carboxylates **1s** and **1t** afforded the tetrahydrofurans **3s** and **3t**, respectively, containing three stereocenters, in good yields. Both the initial attack of the dianion onto the dielectrophile and the subsequent cyclization proceeded with very good 1,3-diastereoselectivities. Owing to steric reasons, the *tert*-butyl and the phenyl group are located *trans* to the bridgehead hydrogen

atom. The reaction of 1,4-dibromo-2-butene with the dilithiated cyclohexane-1,3-dione derivatives **1u** and **1v** afforded the bicyclic products **3u** and **3v**, respectively, with very good 1,3-stereoselectivities.

Reaction of 1,4-dibromo-2-butene with the dianions of the cycloheptanones **1w** and **1x** afforded the 5,7-bicyclic products **3w** and **3x**, respectively, in good yields with moderate to good stereoselectivities. For entropic reasons, the destabilization of intermediate **B** is less important in more flexible fused seven-membered rings than in more rigid six-membered homologues. In open-chain substrates, the conformational flexibility is even higher. Therefore, the best 1,3-diastereoselectivities were obtained for the formation of 5,6-bicyclic 2-alkylidene-5-vinyltetrahydrofurans **3l–v**.

Our first attempts to induce a palladium-catalyzed rearrangement of the 2-alkylidene-5-vinyltetrahydrofuran **3l** were unsuccessful. No conversion was observed when the reaction was carried out at 20 °C using Pd(OAc)₂/PPh₃ or [Pd(PPh₃)₄]. At elevated temperatures, complex mixtures were formed. Fortunately, employment of [Pd(dppe)₂] (dppe = 1,2-bis(diphenylphosphanyl)ethane) resulted in a clean rearrangement reaction which afforded the functionalized bicyclo[3.2.1]octan-8-one **4a** in 95 % yield (*endo:exo* = 1.2:1). The diastereomers could be readily separated to give *endo*-**4a** and *exo*-**4a** in 45 and 36 % yields, respectively. The formation of **4a** can be explained by initial ring-opening, formation of a π -allyl palladium complex,^[18a] and recyclization by nucleophilic attack of the carbon atom of the enolate onto the π -allylpalladium complex (Table 2). This palladium-catalyzed rearrangement leading to bicyclo[3.2.1]octan-8-ones has, to the best of our knowledge, not

been previously noted.^[18b] The reaction proceeded with complete regioselectivity; no formation of any seven-membered ring product was observed. The low stereoselectivity can be explained by a π - σ - π -isomerization of the π -allylpalladium complex. Rearrangement of the esters **3m** and **3n** afforded the bicyclo[3.2.1]octan-8-ones **4b** and **4c** in high yields. The Pd-catalyzed rearrangement of the independently prepared 2-alkylidenetetrahydrofuran **3y**, containing an ester group at the bridgehead position,^[6b] gave the bicyclo[3.2.1]octan-8-one **4d** in 95 % yield. The 2-alkylidenetetrahydrofuran **3q**, containing a methyl group at the 5-position of the cyclohexane moiety, was efficiently transformed into the bicyclo[3.2.1]octan-8-one **4e** in 94 % yield.

The combination of our new cyclodialkylation reaction and the new palladium-catalyzed rearrangement constitutes a significant expansion of the methods known today for the synthesis of functionalized bicyclo[3.2.1]octanes which are of

Table 2. Synthesis of bicyclo[3.2.1]octan-8-ones **4**.

4	R ¹	R ²	R ³	Catalyst	Solvent	T [°C]	t [h]	Yield ^[a] [%]
a	H	H	OEt	Pd(OAc) ₂ /PPh ₃	CH ₃ CN	80	24	0
a	H	H	OEt	[Pd(PPh ₃) ₄]	DME or THF	60	24	0
a	H	H	OEt	[Pd(dppe) ₂]	DMSO	20	24	0
a	H	H	OEt	[Pd(dppe) ₂]	DMSO	80	6	95
b	H	H	O <i>i</i> Pr	[Pd(dppe) ₂]	DMSO	80	6	92
c	H	H	O(CH ₂) ₂ OMe	[Pd(dppe) ₂]	DMSO	80	6	91
d	CO ₂ Et	H	OEt	[Pd(dppe) ₂]	DMSO	80	6	95
e	H	Me	OEt	[Pd(dppe) ₂]	DMSO	80	6	93

[a] Yield of isolated product. In all reactions the *endo:exo* selectivities were in the

pharmacological significance and of importance for natural product syntheses.^[8–14]

Experimental Section

31: A solution of lithium diisopropylamide (LDA) (4.7 mmol) in THF was prepared by addition of *n*BuLi (2 mL, 2.35 M) to a solution of diisopropylamine (0.65 mL) in THF (30 mL) at 0 °C. After the mixture had been stirred for 20 min, ethyl cyclohexanone-2-carboxylate (340 mg, 2 mmol) was added at 0 °C. The mixture was stirred for 60 min, and then this solution was slowly added to a solution of **2** in THF (15 mL) at –78 °C. The temperature was allowed to rise to 20 °C over 4 h and the solution was stirred at this temperature for 2 h. The reaction mixture was poured into an aqueous solution of hydrochloric acid (0.1 M) and extracted with diethyl ether. The combined organic layers were dried (MgSO₄), filtered, and the solvent of the filtrate was removed in vacuo. The residue was purified by column chromatography (silica gel, ether:petroleum ether 1:10 → 1:3) to give **31** as a colorless oil. ¹H NMR (CDCl₃, 250 MHz): δ = 1.10–1.55 (m, 3 H; CH₂), 1.25 (t, 3 H, *J* = 7 Hz; CH₃), 1.80–2.40 (m, 5 H; CH₂), 2.68 (m, 1 H; CH), 4.15 (m, 2 H; OCH₂), 4.77 (m, 1 H; CH=CH=CH₂), 5.19–5.42 (2 × dd, 2 × 1 H; CH=CH₂), 5.91 (ddd, 1 H; CH=CH₂); ¹³C NMR (CDCl₃, 62.5 MHz): δ_c = 13.91, 21.73, 23.49, 26.98, 36.78, 41.25, 58.80, 82.93, 96.25, 116.60, 135.80, 166.36, 166.99; MS (70 eV): 222 (37, [M⁺]), 177 (34), 168 (100), 122 (73); elemental analysis calcd for C₁₃H₁₈O₃ (%): C 70.24, H 8.16; found: C 70.42, H 8.02.

4a: A solution of **31** (200 mg, 0.9 mmol) in dimethyl sulfoxide (2.4 mL) was degassed and [Pd(dppe)₂] (40 mg, 5.0 mol %) was added. The red solution was stirred at 80 °C for 6 h. To the mixture was added diethyl ether (20 mL), the suspension was filtered through a pad of Celite and the Celite was washed with diethyl ether (200 mL). The solvent of the filtrate was removed in vacuo and the residue was purified by filtration through a short pad of silica gel to give pure **4a** as a mixture of two diastereomers (190 mg, 95%, *endo:exo* = 1.2:1). The diastereomers were separated by column chromatography (silica gel, ether:petroleum ether 1:20 → 1:3) to give *endo*-**4a** (90 mg, 45%) and *exo*-**4a** (72 mg, 36%) as colorless oils. The assignment of the diastereomers is based on analogy to the chemical shifts of 6-*endo*- and 6-*exo*-vinyl-bicyclo[3.2.1]octan-8-one: *endo*-**4a**: ¹H NMR (CDCl₃, 250 MHz): δ = 1.20 (t, 3 H, *J* = 7 Hz; CH₃), 1.42–1.90 (m, 3 H; CH₂), 1.95 (m, 2 H; CH₂), 2.10–2.30 (m, 3 H; CH₂), 2.43 (m, 1 H; CHCH=CH₂), 3.48 (m, 1 H; CHCO), 4.18 (m, 2 H; OCH₂), 5.10–5.20 (2 × dd, 2 × 1 H; CH=CH₂), 5.92 (ddd, 1 H; CH=CH₂); ¹³C NMR (CDCl₃, 62.5 MHz): δ_c = 14.11, 17.15, 27.34, 33.82, 36.60, 41.47, 46.01, 60.99, 61.61, 117.53, 135.40,

170.63, 214.90. MS (70 eV): 222 (52, [M⁺]); elemental analysis calcd for C₁₃H₁₈O₃ (%): C 70.24, H 8.16; found: C 70.36, H 8.10; *exo*-**4a**: ¹H NMR (CDCl₃, 250 MHz): δ = 1.22 (t, 3 H, *J* = 7 Hz; CH₃), 1.60–2.10 (m, 7 H; CH₂), 2.42 (m, 2 H; CHCH=CH₂), 2.90 (m, 1 H; CHCO), 4.10 (m, 2 H; OCH₂), 4.80–5.00 (2 × dd, 2 × 1 H; CH=CH₂), 5.70 (ddd, 1 H; CH=CH₂); ¹³C NMR (CDCl₃, 62.5 MHz): δ_c = 14.01, 17.42, 30.44, 36.85, 39.99, 46.57, 60.41, 60.63, 114.23, 139.98, 169.69, 215.01; MS (70 eV): 222 (46, [M⁺]); elemental analysis calcd for C₁₃H₁₈O₃ (%): C 70.24, H 8.16; found: C 70.40, H 8.06. All compounds were characterized by spectroscopic methods and gave correct elemental analyses and/or high-resolution mass spectra.

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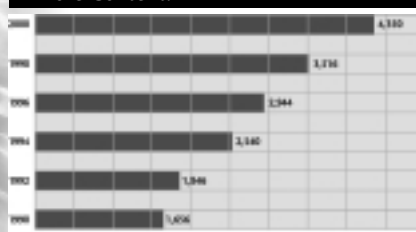
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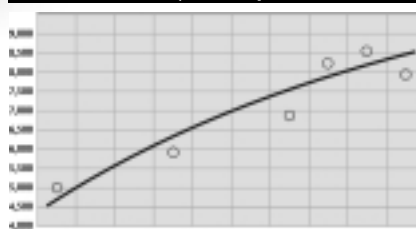
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An Unusual Open-Framework Cobalt(II) Phosphate with a Channel Structure That Exhibits Structural and Magnetic Transitions**

Amitava Choudhury, S. Neeraj, Srinivasan Natarajan, and C. N. R. Rao*

Among the wide variety of open-framework materials, metal phosphates constitute an important family,^[1–4] and interest in these materials is mainly because of potential uses associated with their microporosity. Open-framework transition metal phosphates are of additional interest because of possible magnetism.^[5–12] Magnetic channel structures could have many useful applications, including the separation of oxygen and nitrogen from air. The most common transition metal phosphates are those of iron^[5–8], and only a few derivatives of cobalt^[9–11] and nickel^[12] are known. However, very few open-framework structures with magnetic channels are known to date. Ferri- and ferromagnetic behavior was observed in certain iron phosphates^[5–7] and Ni^{II} diphosphonates^[13] at low temperatures (less than 10 K). Magnetism in the Ni^{II} diphosphonate arises from a change in the dimensionality and the coordination of the Ni^{II} center on dehydration. Ferrimagnetism was reported in a three-dimensional cobalt succinate^[14, 15] at about 10 K, and a metamagnetlike behavior in an organically pillared Co^{II} sulfate.^[16] In our pursuit of open-framework metal phosphates with magnetic channels we employed novel synthetic routes to obtain an unusual cobalt(II) phosphate possessing a 12-membered channel with novel structural features and magnetic properties. Interestingly, the cobalt phosphate undergoes a structural phase transition around 170 K followed by the emergence of ferrimagnetism around 16 K. We believe that this is the first observation of such a temperature-induced change in the channel structure of an open-framework metal phosphate.

Open-framework cobalt phosphates are difficult to prepare by the conventional hydrothermal procedure.^[17, 18] We therefore employed two routes: with an amine phosphate^[19] or a metal amine complex as starting material. Thus, the reaction of Co^{II} ions with diethylenetriamine phosphate (detap) at 180 °C gave [enH₂][Co_{3.5}(PO₄)₃] (**I**) along with a mixture of a few other open-framework cobalt phosphates; the triamine deta decomposed to ethylenediamine (en). The reaction of a mixture with the composition [Co(en)₃Cl₃] · 2H₂O/2H₃PO₄/piperazine/150H₂O at 165 °C for 3 d gave **I** in pure form.^[20a] The asymmetric unit of **I** (Figure 1 a) contains four crystallographically distinct Co and three P atoms. The final atomic

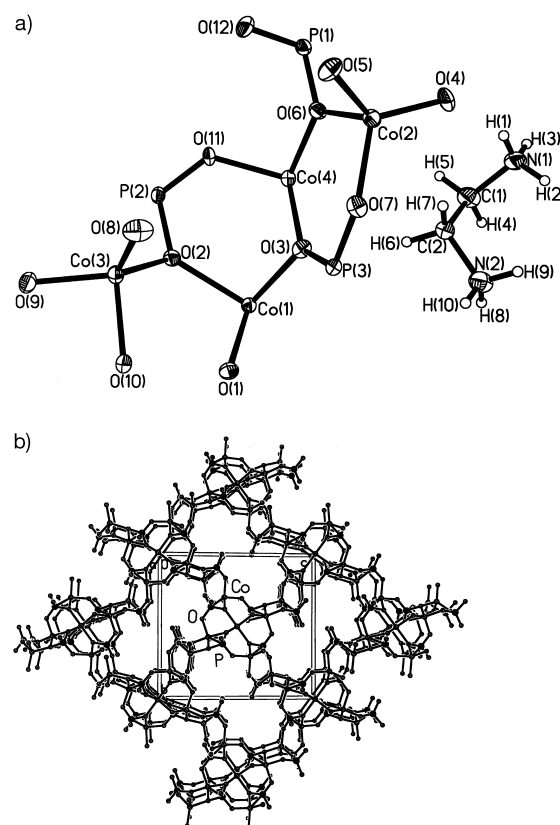


Figure 1. a) Asymmetric unit of **I** at room temperature (50 % probability thermal ellipsoids). b) Structure of **I** along the [100] direction at room temperature showing the 12-membered elliptical channels. The enH₂ cations are omitted for clarity.

coordinates of **I** at 298 K are given in Table 1. The room-temperature structure consists of a network of CoO₄ [Co(2)], CoO₅ [Co(3), Co(4)], and CoO₆ [Co(1)] polyhedra that are interconnected by PO₄ tetrahedra to form a three-dimensional architecture (Figure 1 b). The Co–O and P–O distances are in the ranges 1.931–2.427 and 1.499–1.573 Å, respectively. The polyhedral connectivity between the various cobalt species in **I** (Figure 2) is such that Co(1) is linked to Co(3) and Co(4) while Co(4) is linked to Co(2) through oxygen atoms to form a secondary building unit (SBU) of composition Co₄O₈. The SBUs are interconnected by phosphate tetrahedra to form a one-dimensional 12-membered elliptical channel of width 6.5 × 14.2 Å (the longest atom–atom distance not including the van der Waals radii), as shown in Figures 1 b and 2 a.

A preliminary magnetic susceptibility investigation of **I** in the range 30–300 K showed a Curie–Weiss behavior, where in the slope exhibited a definite change around 200 K. Differential scanning calorimetry (DSC) indicated a phase transition with a small enthalpy change ($\Delta H \approx 20$ cal mol^{–1}) around this temperature. A crystallographic study of **I** at 140 K showed no change in the space group (*P*2₁/*n*), but changes in the lattice parameters.^[20b] The atomic coordinates at 140 K are presented in Table 1. The unit-cell volume at 140 K was significantly larger (ca. 1 %) than at 298 K—a behavior reminiscent of materials with negative coefficients of thermal expansion.^[21, 22] The main changes in the structure of **I**

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Table 1. Atomic coordinates [$\times 10^4$] and equivalent isotropic displacement parameters [$\text{\AA}^2 \times 10^3$] for **I** at 298 and 140 K.

Atom	298 K				140 K			
	x	y	z	$U(\text{eq})^{[a]}$	x	y	z	$U(\text{eq})^{[a]}$
Co(1)	−10 000	0	0	14(1)	−10 000	0	0	7(1)
Co(2)	−4 655(2)	1 771(1)	2 836(1)	16(1)	−4 654(2)	1 771(1)	2 835(1)	9(1)
Co(3)	−6 543(2)	−1 969(1)	815(1)	15(1)	−6 567(2)	−1 974(1)	822(1)	9(1)
Co(4)	−10 085(2)	710(1)	1 802(1)	15(1)	−10 104(2)	715(1)	1 796(1)	8(1)
P(1)	−9 154(4)	1 008(1)	3 699(1)	14(1)	−9 151(4)	999(1)	3 695(1)	7(1)
P(2)	−10 754(4)	−1 306(1)	1 622(1)	14(1)	−10 756(4)	−1 305(1)	1 622(1)	7(1)
P(3)	−4 748(4)	1 140(1)	882(1)	18(1)	−4 761(4)	1 141(1)	875(1)	7(1)
O(1)	−7 102(10)	−402(3)	−663(3)	17(1)	−7 095(9)	−403(3)	−668(3)	9(1)
O(2)	−9 702(10)	−1 148(3)	773(3)	17(1)	−9 706(10)	−1 148(3)	771(3)	9(1)
O(3)	−7 543(10)	786(3)	950(3)	23(1)	−7 572(10)	794(3)	940(3)	12(1)
O(4)	−6 000(11)	2 942(3)	2 988(3)	22(1)	−6 042(10)	2 949(3)	2 989(3)	13(1)
O(5)	−2 164(10)	1 105(3)	3 572(3)	17(1)	−2 178(10)	1 095(3)	3 572(3)	11(1)
O(6)	−8 059(10)	1 141(3)	2 841(3)	18(1)	−8 065(9)	1 141(3)	2 837(3)	9(1)
O(7)	−3 468(10)	1 451(3)	1 746(3)	27(1)	−3 483(10)	1 461(3)	1 744(3)	11(1)
O(8)	−3 630(11)	−1 588(4)	1 581(3)	22(1)	−3 628(10)	−1 603(3)	1 585(3)	13(1)
O(9)	−7 057(11)	−3 258(3)	738(3)	17(1)	−7 071(10)	−3 273(3)	735(3)	11(1)
O(10)	−5 229(11)	−1 912(3)	−288(3)	17(1)	−5 210(10)	−1 911(3)	−283(3)	13(1)
O(11)	−10 326(11)	−493(3)	2 171(3)	20(1)	−10 342(10)	−489(3)	2 176(3)	13(1)
O(12)	−8 330(11)	121(3)	4 035(3)	23(1)	−8 341(10)	100(3)	4 026(3)	13(1)
N(1)	−10 214(14)	4 305(4)	1 880(4)	28(12)	−10 167(13)	4 286(4)	1 896(4)	18(2)
N(2)	−8 043(13)	3 522(5)	297(4)	28(2)	−8 023(12)	3 507(4)	286(4)	12(1)
C(1)	−11 296(17)	3 628(6)	1 311(5)	28(2)	−11 348(16)	3 623(5)	1 304(5)	16(2)
C(2)	−9 187(17)	3 077(5)	984(5)	27(2)	−9 211(16)	3 065(5)	974(5)	16(2)

[a] $U(\text{eq})$ is defined as one third of the trace of the orthogonalized U_{ij} tensor.

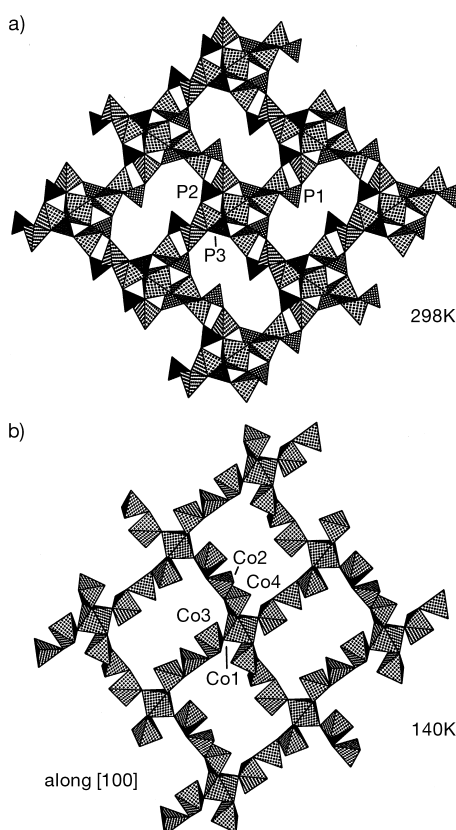


Figure 2. a) The polyhedral connectivity in **I** showing the channels along the a axis at 298 K. b) The cobalt-oxygen polyhedral connectivity showing the channels at 140 K.

on cooling occurs around Co(3), especially for the bonds to O(10) and O(4) (Table 2). The Co(3)–O(4) distance is rather long in the room-temperature phase. If we ignore the Co(3)–O(4) bond in the room-temperature phase, the coor-

Table 2. Some interatomic distances of **I** at 298 and 140 K

Moiety	Bond length [$\text{\AA}$]	
	298 K	140 K
Co(3)–O(8)	1.930(5) [0.526] ^[a]	1.940(5) [0.512] ^[a]
Co(3)–O(9)	1.980(5) [0.460]	1.999(5) [0.436]
Co(3)–O(10)	1.987(5) [0.452]	2.007(5) [0.427]
Co(3)–O(2)	2.029(5) [0.402]	2.031(5) [0.400]
Co(3)–O(4)	2.427(5) [0.137]	2.405(5) [0.146]
Co(3)–P(2)	2.809(2)	2.807(2)
Co(2)–P(2)	3.056(2)	3.065(2)
Co(3)–Co(2)	3.620(2)	3.616(2)
Co(2)–O(4)	1.932(5)	1.955(5)
P(2)–O(4)	1.548(6)	1.556(5)

[a] Sum of the bond valences.

dination number of Co(3) would change from four to five. If the Co(3)–O(4) bond is taken into account, Co(3) would be essentially unaffected in that it is a pentacoordinate in both the phases, possibly in a trigonal-bipyramidal environment. However, a slight change in the orientation of the enH_2^+ cations could occur. The Co–Co and Co–P distances in the two phases bear testimony to the structural transition (Table 2). These changes are consistent with the fact that the transition is not truly first order, but is likely to be displacive. Note that the single crystal remains intact across the transition. The face-sharing of the Co(3) and P(2) polyhedra in **I** is rather unusual and is noteworthy. Furthermore, if the Co(3)–O(4) bond is taken into consideration, the structure is three-dimensional. If it is not, the Co network would consist of isolated ribbons shared by PO_4 groups, a situation intermediate between that of magnetic clusters commonly found in porous transition metal phosphates and the Ni phosphates with a three-dimensional Ni network.^[12]

The change in the slope of the Curie–Weiss plot of **I** around the structural transition temperature was mentioned above.

The paramagnetic Curie temperature was calculated as -79 K from susceptibility data in the range 200 – 300 K, and as -45 K for the range 40 – 150 K. These values indicate an increase in the ferromagnetic interaction after the structural transition. We examined the low-temperature magnetic properties of the material with a SQUID magnetometer. Figure 3 shows the zero-field-cooled (ZFC) and field-cooled

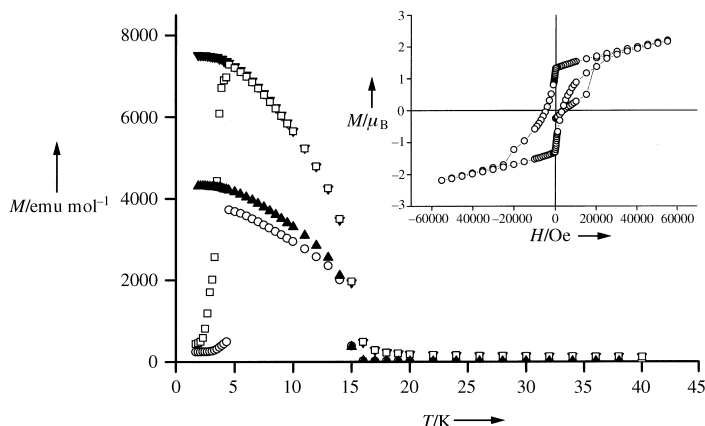


Figure 3. Variation of the magnetic susceptibility of **I** with temperature. The field-cooled (FC) data show a saturation behavior. $\circ$ ZFC, $\blacktriangle$ FC, 50 Oe; $\square$ ZFC, $\blacktriangledown$ FC, 1000 Oe. The inset shows the variation of magnetization with the applied field with hysteresis at 2 K. Note the sudden rise in magnetization around 15 kOe.

(FC) magnetization data of the sample at 50 and 1000 Oe. The ZFC data showed a low magnetization at 1.2 K, an abrupt increase between 2 and 4 K on warming, and attainment of a maximum at 4.2 K. It then shows a sharp drop at 16 K where the magnetism vanishes. The drop in magnetization is not found in the FC samples, which exhibit saturation. Such a ferrimagnetic behavior was found recently in a layered Co^{II} hydroxide.^[23] The Co–O–Co angles in **I** are in the range of 94 – 122.4° , which favors ferromagnetic interaction. The angle of 122.4° corresponds to the shared corner of the Co(1) octahedron and the Co(3) trigonal bipyramid. The magnetization versus field plot of **I** shows hysteresis (inset of Figure 3). The magnetization initially rises with increasing field, followed by a sharp jump around 15 kOe. After the jump, the hysteresis loop is that of a typical ferromagnet. Apparently, a transition from one ferrimagnetic to another ferrimagnetic state occurs in a magnetic field; the magnetic moment is too low for a ferromagnetic state.

The isolation of an open-framework Co^{II} phosphate that exhibits a structural phase transition constitutes a novel finding. It is noteworthy that the change in the structure is accompanied by the evolution of ferrimagnetism at low temperatures. We expect that novel routes such as those employed here will enable the synthesis of open-framework structures which are magnetic at considerably higher temperatures.

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- [20] a) In a typical synthesis $[\text{Co}(\text{en})_3\text{Cl}_2] \cdot 2\text{H}_2\text{O}$ (0.191 g) was dispersed in water (1.35 mL), and H_3PO_4 (85 wt %, 0.07 mL) was added to solution. Then, piperazine (0.043 g) was added with constant stirring to attain a pH of 3.0. The resulting mixture was homogenized for 30 min, transferred into a 23-mL PTFE-lined stainless autoclave, and heated at 165°C for 72 h. After the reaction, the mixture contained purple-blue needle-shaped crystals of **I**, which were collected by filtration, washed with deionized water, and dried under ambient conditions. A suitable single crystal was subjected to X-ray diffraction studies on a Siemens SMART-CCD diffractometer. Crystal data for **I** (298 K): monoclinic, space group $P2_1/n$ (no. 14), $a = 5.0798(1)$, $b = 15.2030(4)$, $c = 16.3535(1)$ Å, $\beta = 95.69(1)^\circ$, $V = 1256.7(3)$ Å³, $Z = 4$, $M_r = 553.3$, $\rho_{\text{calcd}} = 2.895$ g cm⁻³, $\mu(\text{MoK}\alpha) = 4.955$ mm⁻¹; 5228 reflections were collected at $3.5 \leq 2\theta \leq 46.5^\circ$ and merged to give 1808 unique reflections ($R_{\text{int}} = 0.08$), of which 1422 with $I > 2\sigma(I)$ were considered to be observed. The structure was solved and refined by using the SHELXTL-PLUS suite of programs to $R_1 = 0.04$, $wR_2 = 0.1$, and $S = 1.13$ for 206 parameters. b) Crystal data for **I** (140 K): monoclinic, space group $P2_1/n$ (no. 14), $a = 5.0979(1)$, $b = 15.2338(1)$, $c = 16.4247(4)$ Å, $\beta = 95.67(1)^\circ$, $V = 1269.3(3)$ Å³, $Z = 4$, $M_r = 553.3$, $\rho_{\text{calcd}} = 2.895$ g cm⁻³, $\mu(\text{MoK}\alpha) = 4.955$ mm⁻¹; 5193 reflections were collected at $3.5 \leq 2\theta \leq 46.5^\circ$ and merged to give 1818 unique reflections ($R_{\text{int}} = 0.08$), of which 1434 with $I > 2\sigma(I)$ were considered to be observed. The structure was solved and refined by using the SHELXTL-PLUS suite of programs to $R_1 = 0.04$, $wR_2 = 0.08$, and $S = 1.11$ for 206 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 no. CCDC-141075 (298 K) and -141076 (140 K). 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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Formation of Extended Tapes of Cyclic Water Hexamers in an Organic Molecular Crystal Host**

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Water clusters have been extensively studied both theoretically and experimentally, as they can provide insight into the structure and characteristics of liquid water or ice.^[1] Among the clusters, the water hexamer is particularly interesting. Recent studies indicated that this cluster is the smallest possible unit that can exhibit some of the properties found in bulk water.^[2] Theoretical calculations found several different isomers for the water hexamer, of which the five lowest energy ones, that is, cage, prism, book, boat, and cyclic, are almost isoenergetic.^[3] The cage structure, however, was predicted to be the most stable isomer at very low temperatures, and its existence was confirmed experimentally.^[4] Recently, the less-stable cyclic hexamer was isolated, and its structure characterized by IR spectroscopy.^[5] Fast cooling in liquid helium allowed its growth under kinetic control and prevented its transformation into more-stable three-dimensional structures. This isomer is particularly significant, as it is the building block of ice I_h ,^[6] and it appears to be relevant to liquid water as well.^[7] It was also described as the simplest supramolecular analogue of cyclohexane.^[1]

The lattice of a crystal host may offer an alternative environment for stabilizing various topologies of water clusters.^[8] Herein we describe the formation of cyclic water hexamers with an icelike chair conformation and their self-assembly into unprecedented one-dimensional tapes with spectroscopic features similar to those of liquid water, along the channels of an organic molecular crystal host.

2,4-Dimethyl-5-aminobenzo[*b*]-1,8-naphthyridine^[9] (**1**) crystallizes from MeOH or EtOH with 2.5 mol of H₂O, as revealed by X-ray crystallography.^[10] Two of the five unique water molecules interconnect crystallographically different benzonaphthyridine units in hydrogen-bonded dimers (Figure 1 a). The dimers further associate by N(3)–H...O(2) and N(6)–H...O(1) hydrogen bonds into one-dimensional chains (Figure 1 b), which π stack along the *a* axis to generate two-dimensional extended layers. A closer examination of the packing reveals the presence of extended channels, approximately 9 Å × 6 Å wide, through the crystal (Figure 2). Their existence seems to originate in the highly asymmetric shape of

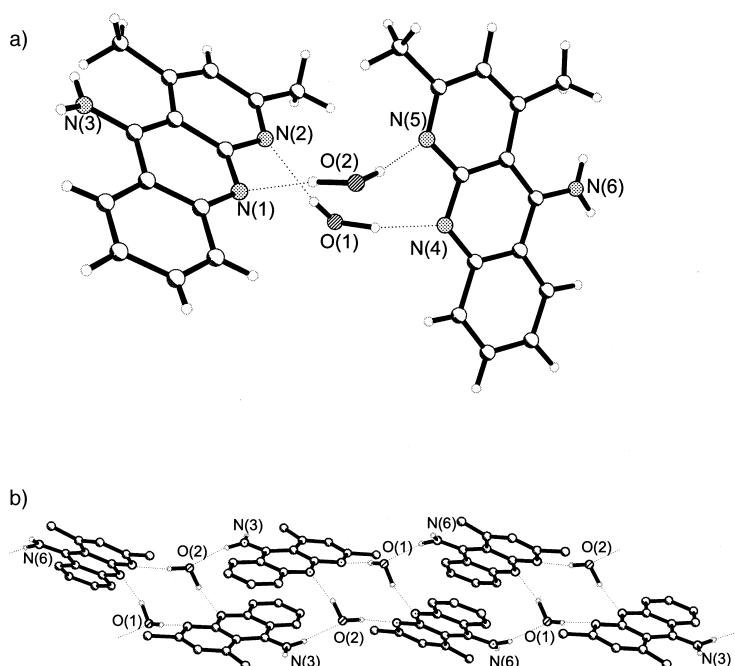


Figure 1. X-ray crystal structure of **1**. a) Hydrogen-bonded dimers. b) Self-assembly of the dimers into chains.

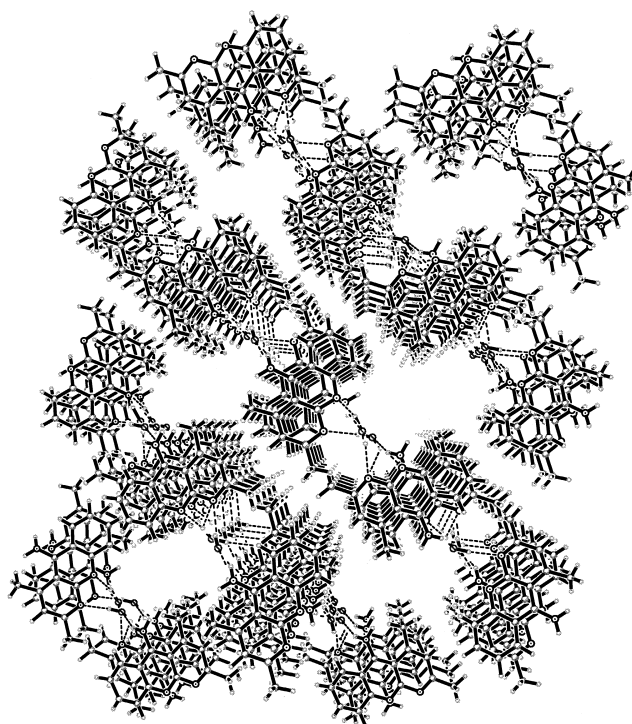


Figure 2. Crystal packing of **1**, viewed along the *a* axis. Water molecules from the channels are omitted for clarity.

the hydrogen-bonded building block dimers, which precludes efficient packing of the layers.

The channels are occupied by the remaining water molecules, which are associated by strong O–H...O hydrogen bonds into cyclic centrosymmetric hexamers that adopt an icelike chair conformation (Figure 3). The geometric parameters of the hexamers are summarized in Table 1. The average O–O distance is 2.776 Å. For comparison, the corresponding

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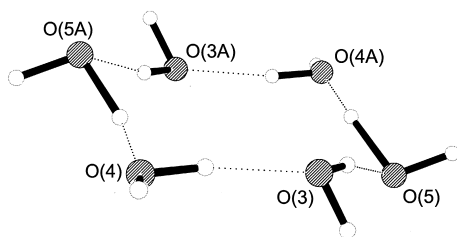


Figure 3. Representation of the chair conformation of the cyclic water hexamers found in the channels of **1**.

Table 1. Geometrical parameters [$\text{\AA}$, $^\circ$] for the cyclic water hexamers in **1**·2.5H₂O.

O—H...O	O—H	O...O	H...O	O—H...O
O(4)—H...O(3)	0.960 ^[a]	2.711	1.758	171.3
O(3)—H...O(5)	0.956	2.785	1.844	167.6
O(5)—H...O(4A)	1.264	2.833	1.703	145.0
O...O...O				
O(3)...O(5)...O(4A)	113.2			
O(5A)...O(4)...O(3)	96.0			
O(4)...O(3)...O(5)	140.2			
O...O...O...O				
O(5)...O(3)...O(4)...O(3A)	106.2			
O(5)...O(4A)...O(3A)...O(4)	105.9			

[a] Restrained.

value in ice I_h is 2.759 $\text{\AA}$ at -90°C .^[6a] However, there is a wide variation in the O—O—O angles, with an average of 116.5° . This value deviates considerably from the corresponding value of 109.3° in hexagonal ice. The hexamers are self-assembled along the channels by O(5)—H...O(4) hydrogen bonds into extended tapes consisting of fused four- and six-membered water rings (Figure 4). The interhexamer O(5)—O(4) distance of 2.854 $\text{\AA}$ is very similar to the O—O distance of 2.85 $\text{\AA}$ in liquid water.^[11] This supramolecular association of water molecules in tapes is presumably enforced by the shape of the host's channels, whose relatively narrow openings preclude the formation of more-stable three-dimensional clusters. Additional O(4)—H...O(2) hydrogen bonds anchor the water

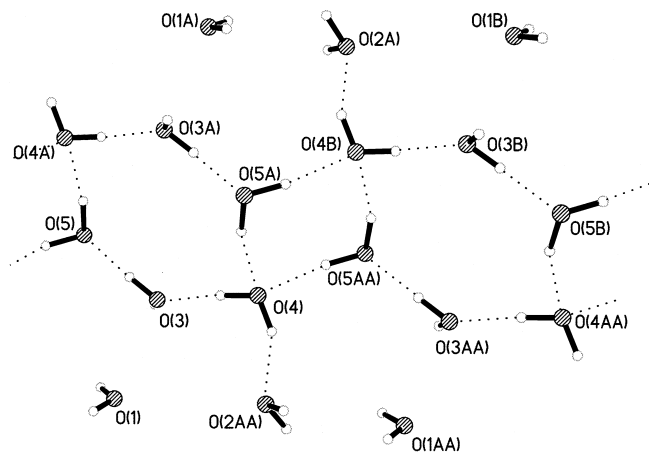


Figure 4. Self-assembly of the water hexamers into extended tapes. Hydrogen-bond lengths [$\text{\AA}$] and angles [$^\circ$]: O(5A)—H...O(4B): O...O 2.854, H...O 1.848; O—H...O 169.1; O(4)—H...O(2): O...O 2.743, H...O 1.879; O—H...O 148.3.

tapes to the organic framework. Although the O(3)...O(1) distance of 2.696 $\text{\AA}$ suggests strong hydrogen bonding between these oxygen atoms, the positions of the O(3)—H dangling protons, as found by X-ray diffraction, do not indicate any attractive interaction. Notably, there are no O—H...N hydrogen bonds between the water tapes and the organic host. This structure appears therefore to be unique, in the sense that the water molecules in the cluster experience a molecular surrounding that resembles more closely the environment found in liquid water or ice, as opposed to the previously described inclusion complexes of water clusters,^[8] in which strong interactions with the host were present.^[12]

The relatively weak interactions with the host framework suggested that the water tapes are fairly loose inside the channels. Therefore, facile removal of this water was anticipated. Indeed, thermogravimetric analysis (TGA) showed an 11.65 % weight loss between room temperature and 70°C , which corresponds to 1.73 of the 2.5 water molecules. Moreover, when a crystal of **1**·2.5H₂O was examined with a hot-stage microscope, loss of liquid water and concomitant loss of crystal transparency were observed on heating. The remaining water is retained up to 166°C and eventually lost before the melting point of **1** (227°C) is reached. The water from the channels is also slowly released at room temperature, as indicated by a TGA taken on a five-month-old sample. It appears that the structure of the organic host is maintained after water release, as demonstrated by powder X-ray diffraction, which shows almost identical patterns for the initial and dehydrated compounds. However, N₂ absorption isotherms obtained under different conditions indicated no porosity for the latter, and this suggests that the channels become inaccessible after removal of the water.

We attempted to assign the O—H stretching vibrations of the water molecules of the tapes on the basis of the difference between the FT-IR spectrum of the initial complex and that of the solid obtained after removing the water from the channels by heating at 120°C for 10 min. As illustrated in Figure 5, the bands centered around 3495 and 3359 cm^{-1} and the analogous bands at 2614 and 2469 cm^{-1} in the deuterated complex (obtained by recrystallization from deuterated methanol) disappear upon heating. We therefore assign these bands to the O—H and O—D bonds of the water tapes. These peaks do not reappear when the dehydrated complex is exposed to water vapor or stirred in liquid water for 24 h, and this suggests that the loss of water is irreversible, as is also supported by TGA. However, the IR spectrum of ice is significantly different, with the O—H (O—D) stretching vibration at 3220 (2420) cm^{-1} .^[6a] In this respect, the water tapes described here appear to have more similarities with liquid H₂O (D₂O), which exhibits analogous bands at 3490 (2540) and 3280 (2450) cm^{-1} .^[6a] Our measured band at 3359 cm^{-1} is also close to the value of about 3335 cm^{-1} reported for the cyclic water hexamer formed in liquid helium.^[5]

In summary, we reported the supramolecular association of icelike cyclic water hexamers into one-dimensional tapes in an organic host. Further investigation of this unprecedented manifestation of H₂O, with characteristics reminiscent of both

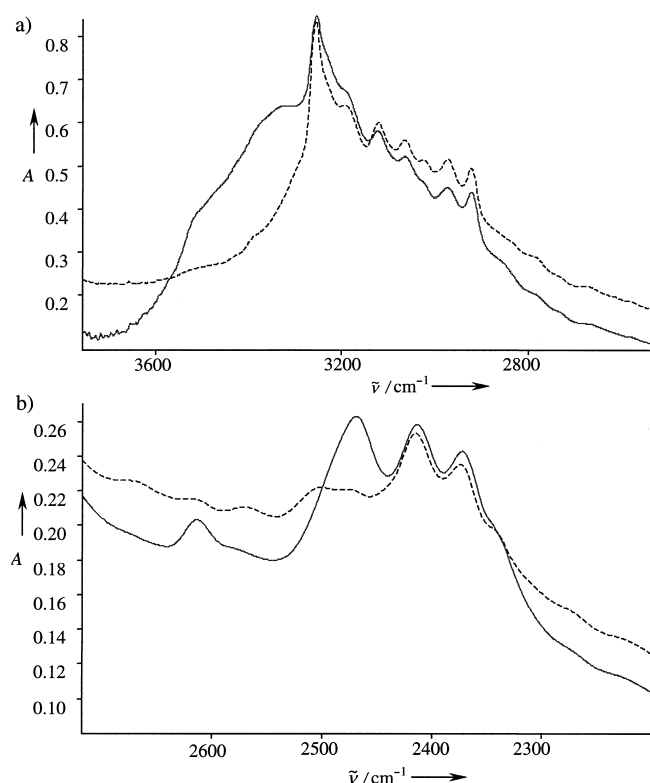


Figure 5. a) FT-IR spectra of the initial $1 \cdot 2.5\text{H}_2\text{O}$ (solid line) and after evacuation of water from the channels (dotted line); b) analogous spectra of $1(\text{ND}_2) \cdot 2.5\text{D}_2\text{O}$. A = absorbance.

liquid water and ice, offers the prospect of improving our understanding of this fundamental, yet complex substance.

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- [10] Crystal data for $1 \cdot 2.5\text{H}_2\text{O}$: A Siemens SMART CCD diffractometer with MoK_α radiation ($\lambda = 0.71073$) was used for data collection. The structure was solved by direct methods and refined by full-matrix least-squares methods on F^2 by using the SHELXTL package (Structure Analysis Program 5.1, Bruker AXS, Inc., Madison, WI,

1997). Absorption corrections were applied by using SADABS. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located from difference Fourier maps and refined isotropically. However, the two hydrogen atoms on O(4) and the dangling hydrogen atom of O(3) became unstable during refinement, and therefore the corresponding O–H distances were constrained at 0.96 Å. Crystal dimensions: $0.41 \times 0.10 \times 0.05$ mm; $T = 173$ K; triclinic, space group $P\bar{1}$; $a = 7.4737(9)$, $b = 12.5474(15)$, $c = 14.5261(18)$ Å, $\alpha = 82.315(3)$, $\beta = 84.305(2)$, $\gamma = 80.020(2)^\circ$, $V = 1325.5(3)$ Å³, $Z = 4$; $\rho_{\text{calc}} = 1.345$ g cm^{−3}; $2\theta_{\text{max}} = 50^\circ$; 9901 reflections collected, of which 4624 were unique; 497 parameters, three restraints; $R_1 = 0.0656$, $wR_2 = 0.1387$ for $I > 2\sigma(I)$; residual electron density: 0.256 e Å^{−3}. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-142220. 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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The First Solid-Phase Synthesis of a Peptide-Tethered Platinum(II) Complex**

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It is well established that removal of cisplatin- and carboplatin-induced DNA adducts contributes to tumor resistance to these drugs.^[1] To overcome this problem, a plethora of platinum(II) complexes which interact with DNA in a manner distinct from that of the parent drugs have been designed and evaluated with respect to the distortion of DNA, as well as the interaction with proteins and antitumor activity.^[2] In addition, amino acid residues, peptides, and polyamides have been successfully employed as site-specific DNA-interacting elements conjugated to metal complexes.^[3a–h] For example, Kelland et al.^[4] reported minor-groove-directed platinum complexes derived from the polyamides netropsin and distamycin. Despite these efforts, only a few

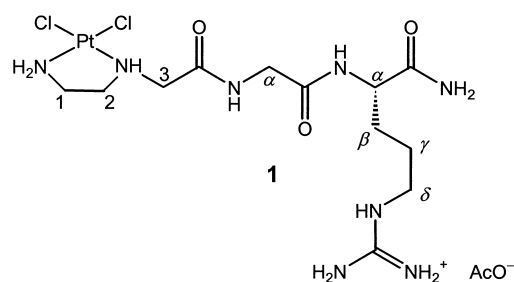
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successful antitumor candidates have emerged.^[1–5] The latter observations, together with the fact that efficient screening protocols are now available,^[6] strongly underline the need to develop a methodology which allows a rapid synthesis (e.g. solid-phase) of a wide variety of platinum(II) complexes.

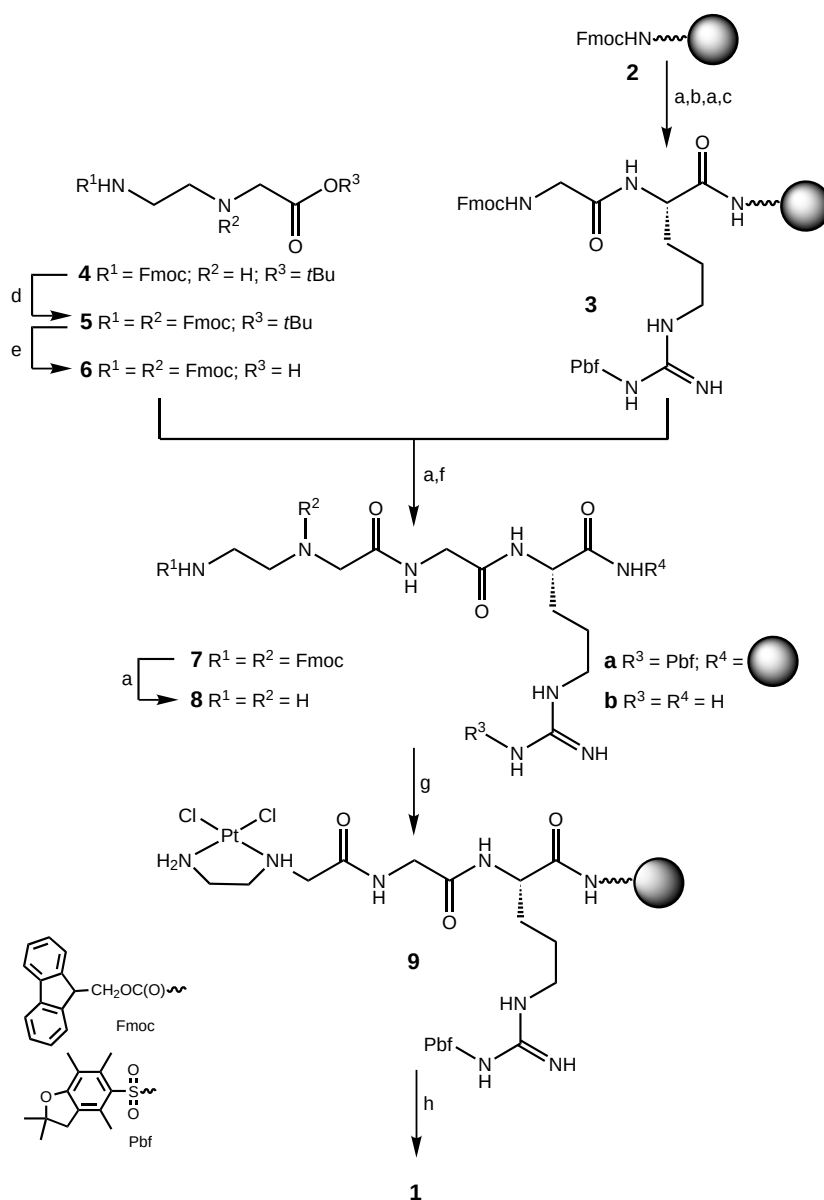
Thus far, only a few metal–peptide conjugates (rhodium, ruthenium, and rhenium) have been prepared by solid-phase peptide synthesis.^[3b,d,g] However, the general usefulness of this approach has remained largely unexplored. It has also been shown that constructs containing guanidinium functions exhibit an interaction with the phosphate backbone and guanine bases in DNA.^[3e, 7]

Herein we report for the first time the construction of a potentially active trimeric arginine-containing peptide–dichloroplatinum(II) complex (**1**) by a solid-phase approach.^[8] The target molecule **1** encompasses an arginine–glycine dipeptide tethered to an ethylenediamine moiety, which—in turn—serves as a



platinum-chelating ligand. The route to the target compound **1** is presented in Scheme 1 and comprises the assembly of the immobilized and functionalized tripeptide derivative **8a**, platination of the ethylenediamine moiety in **8a**, and subsequent deprotection and release from the solid support. The synthesis of the functionalized tripeptide **8a** could be effected by condensing the immobilized dipeptide **3** with Fmoc-protected *N*-2-aminoethyl-glycine derivative **6**. Thus, stepwise elongation of the Fmoc-protected Rink amide resin (**2**) with the commercially available protected amino acids Fmoc-Arg(Pbf)-OH and Fmoc-Gly-OH following a standard Fmoc protocol^[9] gave immobilized dipeptide **3**. Condensation of the latter with the platinum-binding unit **6**, obtained by the reaction of the known^[10] partially protected compound **4** with Fmoc-Cl, and subsection of the resulting bis-Fmoc-protected derivative **5** to acidolysis gave immobilized trimer **7a**. Removal of both Fmoc-protecting groups in **7a** led to the isolation of the partially protected tripeptide **8a**.

At this stage, the crucial platination step of the immobilized peptide trimer **8a** was explored. Preliminary experiments indicated that platination of **8a** with excess K_2PtCl_4 (i.e.



Scheme 1. Solid-phase synthesis of platinum complex **1**. Reagents and conditions: a) piperidine; b) Fmoc-Arg(Pbf)-OH, PyBOP, DiPEA, NMP; c) Fmoc-Gly-OH, PyBOP, DiPEA, NMP; d) DiPEA (2 equiv), Fmoc-Cl (1.15 equiv), CH_2Cl_2 , 1 d, 80% of **5**; e) TFA, CH_2Cl_2 , 2 h, 100% of **6**; f) PyBOP, DiPEA, NMP; g) K_2PtCl_4 (10 equiv, 0.05 M), DMF/ H_2O (9/1, v/v), 24 h in the dark; h) TFA/ H_2O (95/5, v/v), 2 h, 56% (based on **8a**). DiPEA = diisopropylethylamine; NMP = *N*-methylpyrrolidone, PyBOP = benzotriazol-1-yl-oxytris(pyrrolidino)-phosphonium hexafluorophosphate.

20 equiv, 0.1 M) in DMF/water (4/1) for 24 h was promising, gauged on the results of atomic absorption spectroscopy. Unfortunately, cleavage of the resin and removal of the protecting group Pbf with trifluoroacetic acid (TFA)/water/triisopropylsilane (TIS) (95/2.5/2.5) led to metallic platinum, as well as free ligand **8b**.^[11] The latter is probably formed by reduction of the coordinated $PtCl_2$ moiety by the scavenger TIS. Indeed, executing the cleavage step in the absence of TIS in TFA/water (95/5) afforded the crude peptide–platinum complex **1**, as evidenced by ^1H and ^{195}Pt NMR spectroscopy and electrospray-ionization mass spectrometry (ESI-MS), in an encouraging 87% conversion. However, quantitative

transformation of the immobilized ligand **8a** into the platinum complex **9** is a prerequisite for a rapid purification of an array of closely related platinum complexes. To achieve this goal, additional platination experiments in DMF/water and THF/water mixtures were performed. The results of this study are summarized in Table 1. Perusal of the data in Table 1 clearly

Table 1. Results of platination experiments on the solid support in various media.^[a]

Entry	K ₂ PtCl ₄ [equiv, M] ^[b]	Solvent	Conversion [%] ^[c]
1	10, 0.05	DMF/water (1/1)	0
2	20, 0.10	DMF/water (1/1)	0
3	10, 0.05	THF/water (1/1)	63
4	20, 0.10	THF/water (1/1)	64
5	10, 0.05	DMF/water (9/1)	96

[a] Experiments were performed for 24 h at room temperature in the dark. [b] Amount of equivalents with respect to the loading of the immobilized trimer **8a**, and molar concentration. [c] Conversion of unplatinated **8a** to platinated trimer **9** as calculated from ¹H NMR spectra of crude products deprotected and cleaved from the resin (characterized by ¹H and ¹⁹⁵Pt NMR spectroscopy).

indicates that the amount and concentration of platinum does not significantly influence the effectivity of the complexation. It is also evident (entries 1–4) that poor or virtually no complexation occurs by executing the platination of the immobilized peptide in 50% aqueous solutions; this may be due to insufficient swelling of the resin. In these cases, reduction to metallic platinum was observed. However, nearly quantitative conversion of **8a** was observed in DMF/water (9/1) (entry 5). Thus, treatment of **8a** with excess K₂PtCl₄ (10 equiv, 0.05 M) in DMF/water (9/1) for 24 h, followed by concomitant cleavage of the rather acid-stable Pbf group and release of **9** from the solid support with TFA/water (95/5) for 2 h, yielded crude **1**. Analysis of the reaction mixture showed nearly quantitative conversion of **8a** into **1** (Figure 1). Purification of the product by gel permeation chromatogra-

phy (HW-40, 1% acetic acid) gave homogeneous **1** (in 56% yield), as evidenced by capillary electrophoresis (CE). The identity of **1** was fully confirmed by NMR spectroscopy (¹H, ¹³C, ¹⁹⁵Pt) and ESI-MS.

The results presented herein clearly show that platinum complexes of high quality are readily accessible using standard solid-phase peptide synthesis, thus circumventing laborious liquid-phase synthesis. In addition, it was firmly established that the reactive coordinated dichloroplatinum moiety is compatible with the deprotection and cleavage conditions. The latter is imperative for the facile synthesis and purification of a plethora of peptide–platinum complexes. We therefore believe that this new methodology can be adopted for the preparation of a library of platinum antitumor compounds by a combinatorial synthesis approach. This aspect is currently under investigation and will be published in due course.

Experimental Section

6: To a solution of **4** (10 mmol) in CH₂Cl₂ (50 mL) was added DiPEA (20 mmol) and the reaction mixture was cooled to 0 °C. Fmoc-Cl (11.5 mmol) was then added, and the reaction mixture was stirred overnight. After the mixture was concentrated in vacuo, purification by silica gel column chromatography (diethyl ether/petroleum ether 40–60 (1/1, v/v)) gave **5** in 80% yield. To a solution of **5** (8 mmol) in CH₂Cl₂ (5 mL) at 0 °C was added TFA (30 mL), and the reaction mixture was stirred for 2 h at room temperature. The solution was concentrated in vacuo and coevaporated with toluene (3 × 100 mL). Recrystallization from diethyl ether/petroleum ether 40–60 (1/1, v/v) afforded pure **6** in 100% yield, based on **5**.

Solid-phase synthesis of 1: Peptide **8** was assembled on an ABI 433A (Applied Biosystems, division of Perkin-Elmer) peptide synthesizer employing a Fastmoc peptide synthesis protocol, treated with K₂PtCl₄ (10 equiv, 0.05 M) in DMF/H₂O (9/1, v/v) in the dark for 24 h, washed with H₂O, DMF, and CH₂Cl₂, and dried in a vacuum oven (12 h, 37 °C). Cleavage of the Pbf group and the solid support was effected by treatment of **9** with TFA/H₂O (95/5, v/v) for 2 h, after which the reaction mixture was precipitated with diethyl ether. The resulting solid was washed with diethyl ether, water was added, the mixture was filtered, and the resin was washed with water. Subsequently, the combined water fractions were lyophilized, yielding crude **1** as a yellow powder. Purification by gel permeation chromatography (HW-40, 1% acetic acid) afforded **1** in 56%, based on **8a**. The complete assignment of the nonexchangeable protons was confirmed by a ¹H, ¹H COSY, and NOESY experiment. ESI-MS: *m/z*: 597 [M+H]⁺, 619 [M+Na]⁺; ¹⁹⁵Pt NMR (D₂O): δ = –2382.

Free-zone high-performance capillary electrophoresis (CE) analysis was performed on an Applied Biosystems 270A instrument (Applied Biosystems Inc., Foster City, CA) at 20 kV with 50 μm ID capillaries (effective length 72 cm). Sodium citrate (20 mM, pH 2.5, Applied Biosystems) was employed as a running buffer. Gel permeation chromatography was executed on a HW-40 column (26 mm × 600 mm) at 1.5 mL min^{–1}. NMR spectra were recorded by using a Bruker DPX 300 and DMX 600 spectrometer. ¹H NMR and ¹³C NMR spectra were determined with respect to external TMS. ¹⁹⁵Pt spectra were calibrated by using K₂PtCl₄ as an external reference at δ = –1614. Electrospray mass spectra were recorded on a Finnegan MAT TSQ-70 instrument with a custom-made electrospray interface (ESI).

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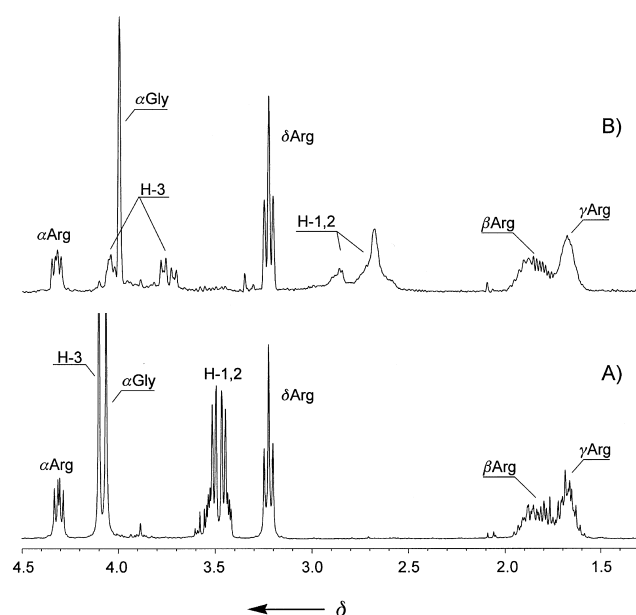


Figure 1. ¹H NMR spectrum (300 MHz, D₂O) of the crude TFA salts of ligand **8b** (A) and platinum complex **1** (B) obtained after deprotection and cleavage from the resin.

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- [11] To corroborate the identity of trimer **8b**, a small sample of **7a** was withdrawn and subjected to the following conditions. Treatment of **7a** with piperidine, followed by acidic treatment afforded crude trimer **8b**, which was purified by gel permeation chromatography (HW-40, 1% acetic acid). Trimer **8b** was characterized by ^1H and ^{13}C NMR spectroscopy, ESI mass spectrometry, and capillary electrophoresis.

The First Structurally Characterized Aluminum Compounds with Terminal Acetylide Groups**

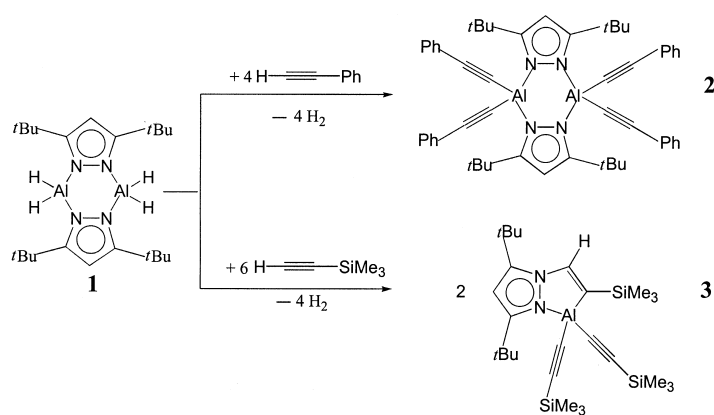
Wenjun Zheng, Nadia C. Mösch-Zanetti, Herbert W. Roesky,* Manuel Hewitt, Fanica Cimpoesu, Thomas R. Schneider, Andreas Stasch, and Jörg Prust

Aluminum compounds containing acetylide groups have been known since 1960.^[1] All the published aluminum acetylides feature a dimeric structure with a bridging acetylide ligand between two aluminum centers^[2] or between one

aluminum atom and one early transition metal atom.^[3] Since the addition of aluminum acetylides to unsaturated hydrocarbons is of interest in organic synthesis,^[4] it is surprising that only few structurally characterized aluminum acetylides have been reported.^[2, 3] Therefore, we set out to synthesize such complexes starting from an aluminum dihydride precursor with bulky pyrazolato ligands.^[5] Herein, we present the preparation and molecular structures of **2** and **3** (see Scheme 1), which to the best of our knowledge, represent the first structurally characterized terminal acetylide complexes of aluminum.

The starting material for **2** and **3**, the aluminum dihydride **1**, is synthesized from $\text{H}[\text{tBu}_2\text{pz}]$ (tBu_2pz = 3,5-di-*tert*-butylpyrazolate)^[6] and $\text{AlH}_3 \cdot \text{NMe}_3$ ^[7] in high yield.^[8]

Compound **2** is prepared from **1** and an excess of $\text{HC}\equiv\text{CPh}$ (Scheme 1). The X-ray structure analysis of **2** shows a dimeric species with some interesting features (Figure 1).^[9] Most



Scheme 1.

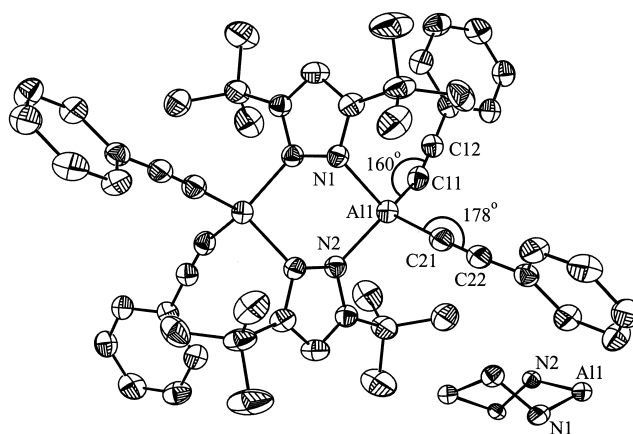


Figure 1. Molecular structure of **2**. Selected bond lengths [Å] and angles [°]: Al1–C11 1.929, Al1–C21 1.913(3), C11–C12 1.211(4), C21–C22 1.218(4); C12–C11–Al1 160.2(3), C22–C21–Al1 178.3(3), C21–Al1–C11 116.99(12), N2–Al1–N1 102.88(10). Bottom right: the twisted core of **2**.

surprisingly, the six-membered Al_2N_4 ring is in a twisted conformation. This is in sharp contrast to other bridged bispyrazolate compounds.^[5c] Another interesting finding is the marked deviation of the two $\text{Al}-\text{C}\equiv\text{C}$ backbones from linearity ($\text{Al1}-\text{C11}-\text{C12}$ $160.2(3)^\circ$ vs. $\text{Al1}-\text{C21}-\text{C22}$ $178.3(3)^\circ$; the $\text{C11}-\text{C12}$ and $\text{C21}-\text{C22}$ bond lengths are 1.211(4) and 1.218(4) Å, respectively). Whereas some small angles in $\text{M}-\text{C}\equiv\text{C}$ units in transition metal complexes have been

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reported,^[10] only two examples^[11] are known in main group chemistry (160.5° for M = Bi^[11a] and 166.4° for M = Ge^[11b]).

Ab initio calculations were carried out to gain a better understanding of the geometry of **2**.^[12] We considered an idealized molecule with D_{2h} symmetry^[12a] and subsequently analyzed the forces causing its distortion.^[12b] We were able to identify four instability coordinates of the reference backbone^[12c] with A_u , B_{1u} , B_{2g} , and B_{3g} symmetries.^[12d] These correspond to vibrations with computed imaginary frequency and are manifestations of the pseudo-Jahn–Teller effect.^[12b] The experimentally found geometry may be described as a superposition of 93.2% A_u and 6.8% B_{1u} . The contributions of B_{2g} and B_{3g} are negligible. The distortion of A_u type is in good agreement with the results of the X-ray structure determination.

The analysis of the different contributions to the vibronic curvature of the A_u nuclear coordinate $K_v(A_u)$ gives an insight into the intimate stereochemical mechanism.^[12e] The driving force seems to be the strong reluctance of Al atoms for π bonding, thereby converting these contributions to a higher σ character. Two possible $\pi \rightarrow \sigma^*$ electron promotions associated with this effect are shown in Figure 2. The depicted unoccupied states of the σ type contain aluminum atomic orbitals (AOs), while the π significant states are located on the ligands. The vibronic energy gain accompanying such excitations^[12f] determines the above-mentioned distortion. Therefore, the observed bending and twisted character in **2** seems to be caused by inner features of

the pyrazolato ligands rather than by the electronic structure of the acetylide ligands themselves.

Reaction of **1** with an excess of $\text{HC}\equiv\text{CSiMe}_3$ affords compound **3** (Scheme 1). The X-ray structure analysis of **3** reveals a mononuclear aluminum compound with an aluminum atom coordinated by the [*t*Bu₂-*N*-CH=C(SiMe₃)-pz] chelating unit and two $\text{C}\equiv\text{CSiMe}_3$ groups (Figure 3).^[9] The chelating unit consists of a pyrazolato ligand which has

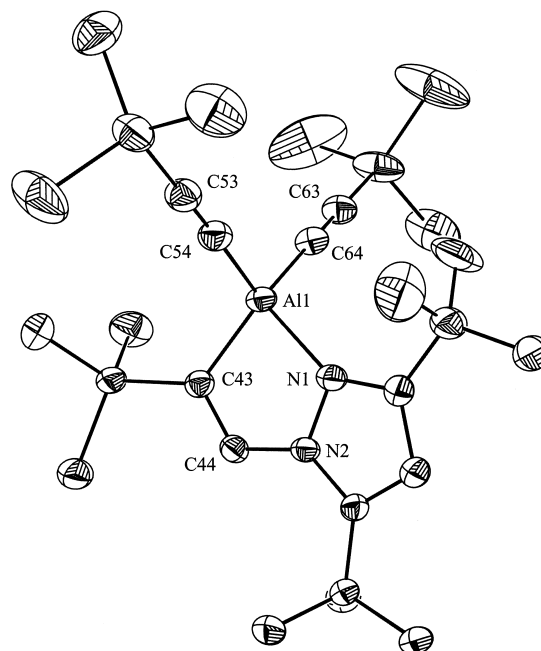


Figure 3. Molecular structure of **3**. Selected bond lengths [Å] and angles [°]: C44–C43 1.337(4), C53–C54 1.223(4); C54–Al1–C64 113.42(12), N1–Al1–C43 87.31(10), C53–C54–Al1 178.2(2).

formally added to a $\text{C}\equiv\text{C}$ bond, thereby forming a five-membered metallacycle with the aluminum center. The two fused five-membered rings, AlN_2C_2 and pyrazolate, are close to coplanar. The $\text{C}\equiv\text{C}$ bond length of the inserted acetylene molecule is significantly elongated (C43–C44 1.337(4) vs. C63–C64 1.219(4) Å) indicating double-bond character. The geometry of the aluminum atom is distorted tetrahedral (N1–Al1–C43 87.31(10)° and C54–Al1–C64 113.42(12)°) due to the constraint imposed by the chelate ring. The angles of the $\text{Al}-\text{C}\equiv\text{C}$ bonds (178.2(2)° and 175.5(2)°) are in the normal range of other known aluminum acetylides.^[2a, 3b]

The chelating unit in **3** is unusual and its formation shows that this should be a promising new ligand for other metal ions.

Experimental Section

2: $\text{HC}\equiv\text{CPh}$ (1.8 mL, 16 mmol) was added in excess to a solution of **1** (0.83 g, 2.0 mmol) of in toluene (50 mL). The mixture was stirred for 5 h (50 °C) and then for 2 h at room temperature. After the solvent was removed, the residue was washed with cold pentane (3 × 7 mL) to yield **2** (0.96 g, 59%). Single crystals suitable for X-ray diffraction analysis were obtained from toluene/hexane (5:1) at –26 °C. M.p. 212 °C. ¹H NMR (200 MHz, C_6D_6): δ = 1.72 (s, 18H; *t*Bu), 6.44 (s, 1H; CH), 6.91–7.40 (m, 10H; Ph); ¹³C NMR (125 MHz, C_6D_6): δ = 31.46 (s; Me), 33.38 (s; CMe_3), 102.9 (br; Al–C), 108.42 (s; CH), 174.54 (s; C-*t*Bu), 108.31 (s; C-Ph) 125.35,

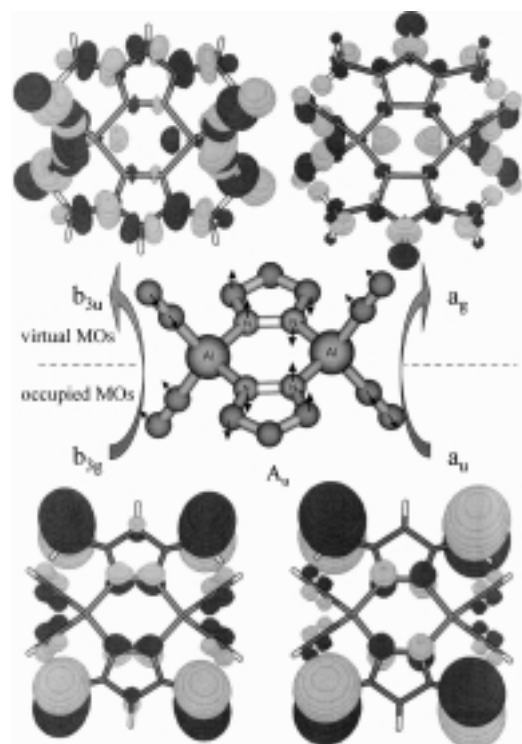


Figure 2. Two pairs of the transformed orbitals are shown, which are vibronically coupled through the normal coordinate of the A_u distortion. The notion of transformed orbitals refers to the states whose coupling leads to transition densities that concentrate the electron density flow associated with the distortion in a maximal way.^[12f] The represented excitations, $a_u \rightarrow a_g$ and $b_{3g} \rightarrow b_{3u}$, are matching 25% of the vibronic curvature.

127.78, 128.31, 132.04 (s, Ph); MS(EI): m/z (%): 816 ($[M^+]$ 100); IR: $\tilde{\nu}$ = 2143, 2129, 1596, 756, 691 cm^{-1} ; elemental analysis (C H N) is correct for $\text{C}_{34}\text{H}_{58}\text{Al}_2\text{N}_4$.

3: $\text{HC}\equiv\text{CSiMe}_3$ (2.0 mL, 14 mmol) was added in excess to a solution of **1** (0.83 g, 2.0 mmol) in toluene (50 mL). The mixture was stirred under reflux for 1.5 h and then for 2 h at room temperature. The solvent was removed and **3** was isolated in hexane as white crystals at -26°C (1.0 g, 51 %). Single crystals suitable for X-ray diffraction analysis were obtained from THF at -26°C . M.p. 133°C . ^1H NMR (200 MHz, C_6D_6): δ = 0.13 (s, 18H; $\equiv\text{CSiMe}_3$), 0.44 (s, 9H; SiMe_3), 0.81 (s, 9H; C3-*t*Bu), 1.56 (s, 9H; C1-*t*Bu), 5.79 (s, 1H; C2-H), 7.42 (s, 1H; C44-H); ^{13}C NMR (125 MHz, C_6D_6): δ = -0.08 (s; Si1- Me_3), 0.29 (s; Si2(3)- Me_3), 32.91 (s; C10), 31.34 (s; C30), 30.79 (s; C31(32,33)), 29.52 (s; C11(12,13)), 103.80 (s; C44), 116.35 (s; Si2-C $\equiv$), 125.40 (br; Al-C $\equiv$), 141.80 (br; Al-C $\equiv$), 134.62 (s; C2), 152.00 (s; C1), 163.08 (s; C3); ^{29}Si NMR (99 MHz, C_6D_6): δ = -21.51 (s; Si2(3)), -5.74 (s; Si1); IR: $\tilde{\nu}$ = 3041, 2075, 1941, 1079, 955, 857, 618 cm^{-1} ; MS(EI): m/z (%): 498 ($[M^+]$, 20), 441 ($[M^+ - \text{AlMe}_2]$, 100); elemental analysis (C H N) is correct for $\text{C}_{26}\text{H}_{47}\text{AlN}_2\text{Si}_3$.

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total number of reflections measured was 6909 in the range $4.34 \leq 2\theta \leq 49.46^\circ$, of which 6650 were unique. 5289 with $F > 4\sigma(F)$, 277 parameters. Final R indices: $R_1 = 0.0530$ ($I > 2\sigma(I)$) and $wR_2 = 0.1351$ (all data). Residual electron density, max./min. $312/-326 \text{ e nm}^{-3}$. The THF molecule was modeled as threefold disordered. Owing to this disorder, the oxygen atom of THF could not be localized and was modeled as a CH_2 group. 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-143738 (**2**) and CCDC-143739 (**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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Ruthenium-Catalyzed Enyne Metathesis of Acetylenic Boronates: A Concise Route for the Construction of Cyclic 1,3-Dienylboronic Esters

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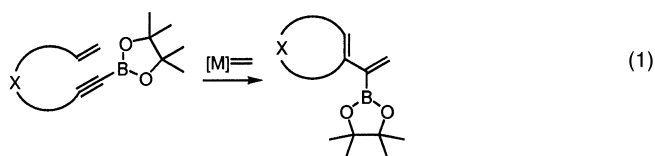
Since its inception in the late 1980s, the ring-closing metathesis (RCM) reaction of dienes has inspired a plethora of exciting studies.^[1] In comparison, the enyne ring-closing metathesis reaction is less well documented. Most reports on this topic are confined to the assembly of compounds containing an unsubstituted or an alkyl-substituted 1,3-dienyl

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[†] NMR analyses.

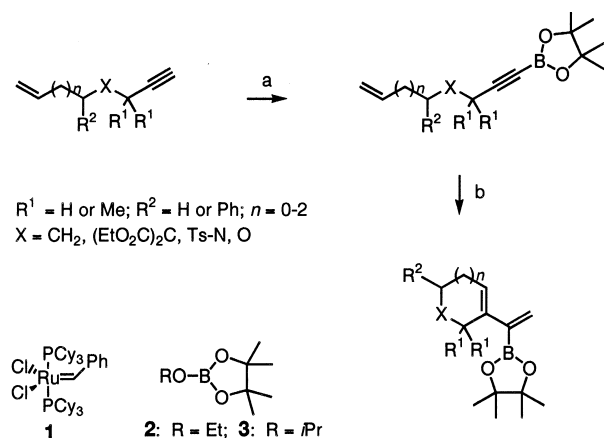
Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

motif.^[2, 3] We recently demonstrated that cyclic alkenylboronates can be obtained by RCM of dienyboronic esters.^[4] In connection with these studies, we considered the enyne ring-closing metathesis reaction as a practical and concise strategy for the construction of cyclic 1,3-dienylboronates [Eq. (1)]. In



addition to being amenable to further functionalization by manipulation of the vinylboronate moiety,^[5] 1,3-dialkenylboronic esters are well poised to enter into Diels–Alder cycloaddition reactions and therefore constitute highly versatile synthetic intermediates. Here we disclose the first examples of conversion of en-1-ynylboronic esters into five- to seven-membered carbocyclic and heterocyclic 1,3-dialkenyl-2-boronates by enyne metathesis. Additionally, we report that these dienes readily undergo cycloaddition reactions with high regio- and stereoselectivity.

Our synthetic route towards the preparation of the metathesis substrates is summarized in Scheme 1. The critical alkynyl-1-ylboronate moiety was introduced by trapping an alkynyllithium intermediate with a 2-alkoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**2** or **3**) followed by treatment with anhydrous ethereal HCl, according to a procedure described by Brown et al.^[6, 7]



Scheme 1. Synthesis and enyne metathesis of en-1-ynylboronates. a) Procedure A: LDA, Et₂O or THF, –78 °C, 5–15 min; **2** or **3**, 1–3 h; HCl in Et₂O, –78 °C to RT; Procedure B: LDA was replaced by *n*BuLi. b) [(Cy₃P)₂Cl₂Ru=CHPh] (**1**, 5–10 mol %), benzene or CH₂Cl₂, RT. Ts = *p*-toluenesulfonyl; LDA = lithium diisopropylamide.

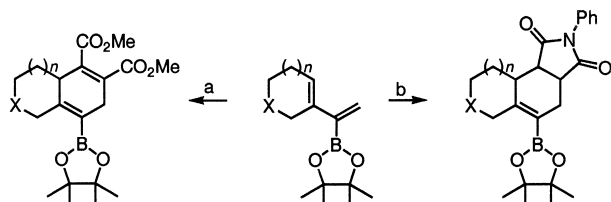
With the alkynylboronic esters in hand, we next established the experimental conditions for the ring-closing enyne metathesis (Scheme 1). The reactions were routinely carried out in the presence of 5–10 mol % of Grubbs' catalyst (**1**)^[8] in CH₂Cl₂ at room temperature.^[9] As shown in Table 1, the ruthenium-promoted enyne metathesis efficiently converts a range of substrates into five- and six-membered carbocyclic and heterocyclic 1,3-dienylboronates. The yields are excellent, ranging from 87 to 95%, and the reactions are generally complete within 1–14 h (Table 1, entries 1, 3, 5, 6, and 9).

Table 1. Synthesis of 1,3-dialkenylboronates by enyne metathesis.^[a]

Entry	Enyne	Product	Yield ^[b] [%]	Time [h]	Solvent ^[a]
1		13 	87 ^[c]	14	A
2		14 	86 ^[d, e]	1	A
3		15 	93	5	A
4		16 	94 ^[d, f]	3	B
5		17 	93	2	A
6		18 	95	6	A
7		19 	65 ^[d, f]	12	B
8		20 	81 ^[d, f, g]	12	B
9		21 	91	3	A

[a] Metathesis conditions: 5–10 mol % catalyst **1**, 0.06 M, RT. Solvent A: CH₂Cl₂; solvent B: benzene. [b] Yields refer to isolated products. [c] Ethylene atmosphere. [d] Overall yield from the alkynylboronate. [e] Diels–Alder conditions: MeO₂CC≡CCO₂Me, EtAlCl₂, CH₂Cl₂, –78 to 0 °C, 2 h. [f] Diels–Alder conditions: MeO₂CC≡CCO₂Me or *N*-phenylmaleimide, benzene, 80 °C, 1 h. [g] Two diastereoisomers (*dr* = 7:1). B(OR)₂ = B(OCMe₂)₂; Ts = *p*-toluenesulfonyl. Further details on the experimental procedure can be found in the Supporting Information.

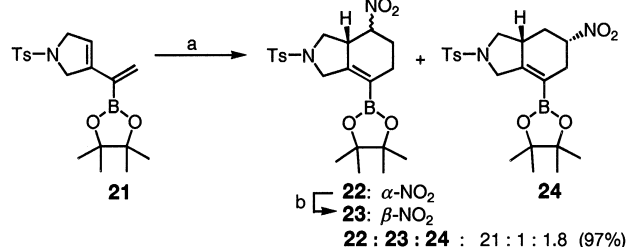
Whilst five of the 1,3-dialkenylboronic esters synthesized were isolated in good yield after chromatography (Table 1, **13**, **15**, **17**, **18**, and **21**), the four containing unsubstituted five- or seven-membered rings proved to be unstable upon concentration, most likely because of dimerization.^[10] To circumvent this problem, these dienyboronic esters were directly transformed into Diels–Alder adducts (Scheme 2). Thus, reaction of the intermediate 1,3-dialkenylboronates with *N*-phenylmaleimide or with dimethyl acetylenedicarboxylate under thermal or Lewis acid catalyzed conditions^[11] led cleanly to the expected cycloaddition products (Table 1, entries 2, 4, 7, and 8). The tandem enyne metathesis/Diels–Alder cyclo-



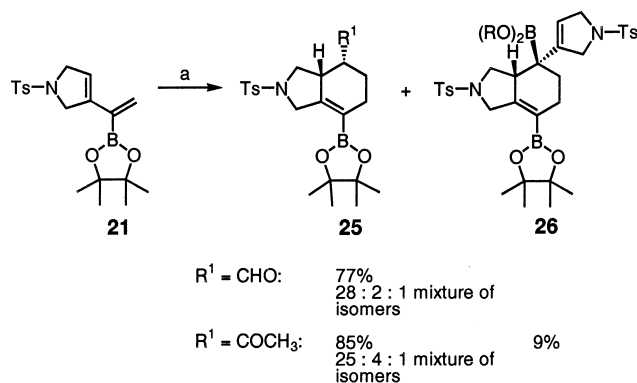
Scheme 2. Diels–Alder cycloaddition reaction of 1,3-dialkenylboronates. a) $\text{MeO}_2\text{CC}\equiv\text{CCO}_2\text{Me}$, EtAlCl_2 , CH_2Cl_2 , -78 to 0°C , 2 h or $\text{MeO}_2\text{CC}\equiv\text{CCO}_2\text{Me}$, benzene, 80°C , 1 h. b) *N*-Phenylmaleimide, benzene, 80°C , 1 h.

addition sequence was accomplished efficiently without removal of the ruthenium catalyst in yields of 65 to 94%. Although **16** resulted from the exclusive *endo* addition of the dienophile, adduct **20** was obtained as a 7:1 mixture of the *endo:exo* isomers.^[12]

The ability of cyclic 1,3-dialkenylboronic esters to undergo Diels–Alder reactions in a regio-^[13] and stereoselective fashion was substantiated by examining the cycloaddition reactions of dialkenylboronate **21** (Schemes 3 and 4). Treatment of **21** with an excess of nitroethylene at 40°C for 2 h furnished one major product (**22**) in 89% yield, along with a



Scheme 3. Selectivity of the Diels–Alder reaction with nitroethylene. a) CH_2CHNO_2 (10 equiv), benzene, 40°C , 2 h. b) NaOMe (1.2 equiv), $\text{THF}:\text{MeOH}$ (3:1), RT, 2 h, 73%.



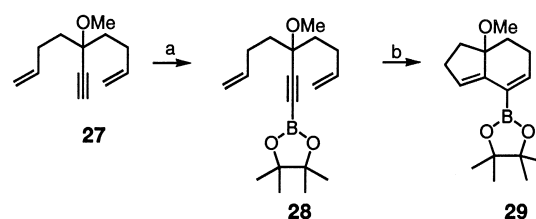
Scheme 4. Selectivity of the Diels–Alder cycloaddition reaction. a) For $\text{R}^1 = \text{CHO}$: CH_2CHCHO (10 equiv), benzene, 65°C , 3 h. For $\text{R}^1 = \text{COCH}_3$: $\text{CH}_2\text{CHCOCH}_3$ (10 equiv), benzene, 70°C , 4 h.

mixture of isomers **23** and **24** (1:1.8; 8% yield), which accounted for most of the remaining material (Scheme 3). The identity of the major compound **22** was ascertained by X-ray crystallography, and the structures of **23** and **24** were assigned on the basis of NMR experiments.^[12] The structure of **23** was also confirmed independently by epimerization of **22** under basic conditions.^[14]

Likewise, the reaction of **21** with acrolein or methyl vinyl ketone was highly regio- and stereoselective and produced

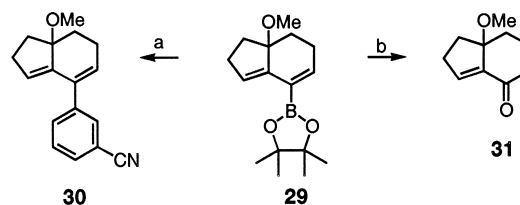
one predominant isomer (Scheme 4). A minor component of the mixture was shown to be the β -epimer of **25**.^[14] By analogy with the results obtained in the cycloaddition reaction with nitroethylene, the other minor product was assigned the structure corresponding to the *endo* regioisomer. In the reaction of **21** with methyl vinyl ketone, by-product **26** resulting from dimerization of **21** was isolated in 9% yield.^[12]

To broaden the utility of our synthetic route to cyclic 1,3-dienyl-2-boronic esters, we tackled the conversion of higher molecular weight alkynylboronic esters into their cyclic counterparts, with the aim of circumventing the purification step. We examined the transformation of diyne **27** into the bicyclic vinylboronate **29** (Scheme 5). We were gratified to find that the crude alkynylboronate **28** cleanly underwent



Scheme 5. Synthesis of bicyclic dienylboronate **29**. a) LDA , THF , -78°C , 20 min; 2-ethoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**2**), 2 h; HCl in Et_2O , -78°C to RT; removal of solvents and filtration. b) $[(\text{Cy}_3\text{P})_2\text{-Cl}_2\text{Ru}=\text{CHPh}]$ (**1**, 5 mol %), CH_2Cl_2 , RT, 3 h, 70%.

ruthenium-promoted metathesis in 70% yield.^[15] The resultant bicyclic dialkenylboronic ester **29** was efficiently oxidized to the corresponding enone **31** by treatment with anhydrous Me_3NO in refluxing THF (Scheme 6). Alternatively, reaction of **29** with 3-bromobenzonitrile in the presence of CsF and a catalytic amount of $[\text{PdCl}_2(\text{dppf})] \cdot \text{CH}_2\text{Cl}_2$ in refluxing DME furnished the cross-coupling product **30**.



Scheme 6. Conversion of dienylboronate **29** to **30** and **31**. a) 3-Bromobenzonitrile, CsF , $[\text{PdCl}_2(\text{dppf})] \cdot \text{CH}_2\text{Cl}_2$, DME, reflux, 14 h, 88%. b) Me_3NO (anhydrous), THF , 70°C , 1.5 h, 76%. $\text{dppf} = 1,1'$ -bis(diphenylphosphanyl)-ferrocene.

In summary, we have developed an unprecedented enyne metathesis-based approach for the concise construction of cyclic 1,3-dialkenylboronates. We have further demonstrated that these 1,3-dialkenylboronic esters undergo Diels–Alder reactions with electron-deficient dienophiles with excellent regio- and stereoselectivity to give vinylboronates of higher molecular complexity in a short sequence of steps. Further investigations to broaden the scope of this enyne metathesis and to explore asymmetric Diels–Alder cycloadditions by using chiral, enantiomerically pure 1,3-dienyl-2-boronic esters are in progress.

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- [14] Epimerization of the Diels–Alder adducts with NaOMe (1.2 equiv) in THF:MeOH (3:1) at room temperature afforded quantitatively the β epimers; this allowed identification of one of the minor isomers.
- [15] The LiCl or *i*Pr₂NH₂Cl resulting from the preparation of the intermediate alkynylboronate **28** was removed by filtration through Whatman glass fiber filters before the metathesis reaction.

A Light-Modulated Sequence-Specific DNA-Binding Peptide**

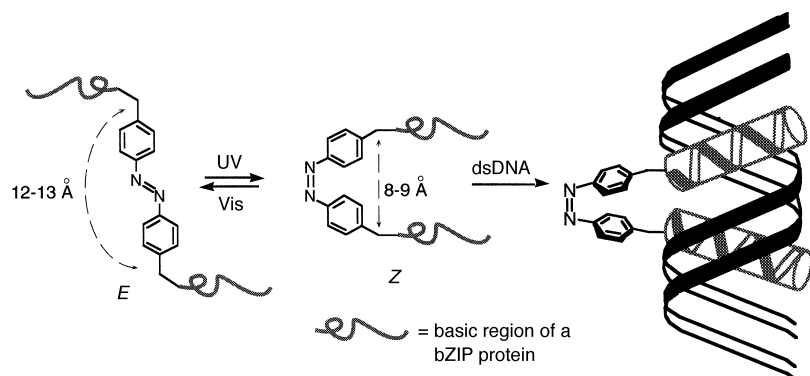
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In eukaryotes, gene expression is primarily regulated at the transcription level through the action of a host of sequence-specific DNA-binding proteins known as transcription factors.^[1] At any given time, most transcription factors are present in the cell in an inactive form and they become activated upon sensing and responding to specific external signals.^[2] In a number of cases, it has been shown that activation involves the transcription factor undergoing a stimulus-induced conformational change that converts it from a non DNA binding protein into a form capable of recognizing the appropriate binding site at the promotor.^[3] We reasoned that molecules mimicking this natural activation mechanism, which are capable of binding to specific DNA sequences with high affinity only after receiving an external stimulus, might offer a promising potential for applications in cell biology and molecular medicine^[4] and could provide new insights into the mechanisms underlying DNA recognition. As a first step towards this goal, we have designed a peptide whose sequence-specific DNA-binding affinity can be modulated by light.^[5]

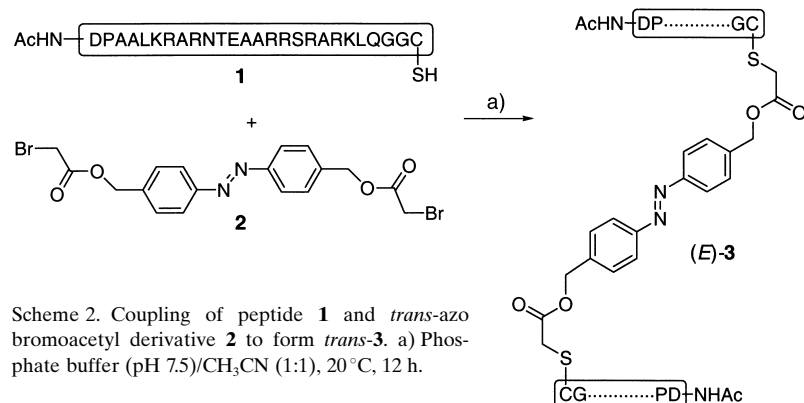
Our design is inspired by the well-known ability of artificially dimerized basic regions (BRs) of bZIP proteins to recognize DNA sites.^[6,7] We envisaged that appropriate linking of these BRs through a rigid photoresponsive device such as an azobenzene moiety, capable of undergoing a substantial geometrical change upon irradiation,^[8] might allow modulation of the site-specific DNA affinity of the resulting dimer. In designing the system we sought to favor the DNA-binding ability of the *cis* over the *trans* isomer by taking advantage of the considerably shorter distance between the benzylic carbon atoms of the *cis*-azobenzene template and the favorable preorientation of its recognition arms to grip the major groove of the DNA (Scheme 1). Molecular modeling corroborated this idea for hybrid **3** (Scheme 2); each of the peptide segments of **3** consists of amino acids 226–248 from the BR of the yeast transcription

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Scheme 1. It is known that sequence-specific DNA binding by bZIP proteins or synthetic minimized versions of these proteins is accompanied by folding to an α helix.^[6, 7]



Scheme 2. Coupling of peptide **1** and *trans*-azo bromoacetyl derivative **2** to form *trans*-**3**. a) Phosphate buffer (pH 7.5)/CH₃CN (1:1), 20 °C, 12 h.

factor GCN4 together with an additional Gly-Gly-Cys linker at the C terminus.^[9]

The azo compound **3** was synthesized by coupling peptide **1** with the *trans*-azo bromoacetyl derivative **2**.^[10] Irradiation of

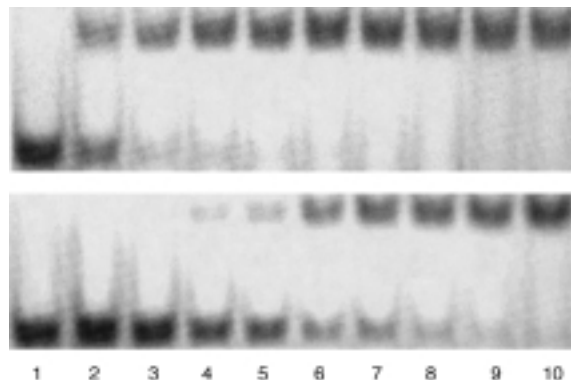


Figure 1. Autoradiograms showing the titration of ³²P end-labelled CREB20 with increasing amounts of *cis*-**3** (top) and *trans*-**3** (bottom). Peptide concentrations in lanes 1–10, respectively, were: 0, 1, 5, 10, 30, 60, 100, 200, 500, and 1000 nM. Binding reactions were performed over 45 min at 4 °C using labeled CREB20 (<1 nM) in a binding mixture (20 μ L) containing Tris (20 mM, pH 7.5), KCl (100 mM), MgCl₂ (2 mM), EDTA (2 mM), glycerol (10%), BSA (0.3 mg mL⁻¹), and NP-40 (82%). Products were resolved by polyacrylamide gel electrophoresis using a 10% non-denaturing acrylamide gel and 0.5X TBE buffer. Duplex oligonucleotide CREB20 (containing a consensus ATF/CREB site):

5'-d(TGGAGATGACGTCATCTCGT)-3'
3'-d(ACCTCTACTGCAGTAGAGCA)-5'

an aqueous solution of *trans*-**3** at 365 nm^[11] led to a mixture containing a 95:5 ratio of *cis*:*trans* isomers, with photostationary equilibrium being reached after approximately 60 min.^[12] As expected, the isomerization process was completely reversed by irradiating the previous *cis*:*trans* mixture at 430 nm,^[13] or by thermal activation.^[14]

The DNA affinities of *trans*-**3** and *cis*-**3** were assessed by gel mobility shift analysis, by titrating a 20 base-pair duplex DNA containing a consensus ATF/CREB recognition site (CREB20) with increasing concentrations of the peptides.^[15] As is shown in Figure 1, *cis*-**3** bound this DNA with very high affinity, and approximately 60–70 times more efficiently than the *trans* isomer. The difference in affinity can be easily visualized by observing that, at a peptide concentration of 5 nM (lane 3) the *cis* isomer was completely bound while the *trans* isomer remained unbound. Thus, as hypothesized, the geometric alteration that occurs upon irradiation brings about a substantial change in the sequence-specific DNA affinity of the azobenzene–peptide hybrid.^[16]

DNA binding was further characterized by circular dichroism, a technique that is particularly useful for studying DNA–bZIP interactions owing to the well-established folding transition of the bZIP BR from random coil

to α helix upon specific DNA binding.^[6, 7] Addition of CREB20 to *cis*-**3** at 4 °C brought about a significant increase in the magnitude of the signal at 222 nm (Figure 2 A, spectra a and e), whereas for *trans*-**3** the ellipticity increase was smaller (Figure 2 A, spectra c and d). To check whether this relatively small change of ellipticity might result from *trans*-**3** binding specifically with just one of its BR arms, with the other arm remaining unstructured and non-specifically bound, this peptide was incubated with duplex oligonucleotides containing just half of the dimer recognition site. Since no significant helicity changes were observed (Figure 2 B),^[17] it can be inferred that *trans*-**3** binds specifically CREB20 with both BR arms.

Interestingly, the *trans* $\rightarrow$ *cis* isomerization can be carried out in the presence of DNA, although it is about eight times slower than in the absence of DNA and leads to a slightly lower proportion of the *cis* isomer at the equilibrium (inset to Figure 2 A, curve 1). This rate decrease can be attributed to partial stabilization of the *trans* conformation by nonspecific electrostatic peptide–DNA interactions. Indeed, increasing the ionic strength of the medium by adding MgCl₂ slightly accelerates the isomerization process and restores the *cis*:*trans* ratio to the value it has in the absence of DNA (inset to Figure 2 A, curve 2). Remarkably, the stability of the DNA–*cis*-**3** complex completely inhibited the *cis* $\rightarrow$ *trans* reverse process, at least under the present photoreaction conditions.^[18]

In summary, linking two bZIP basic regions through an azobenzene group produces a peptide whose sequence-

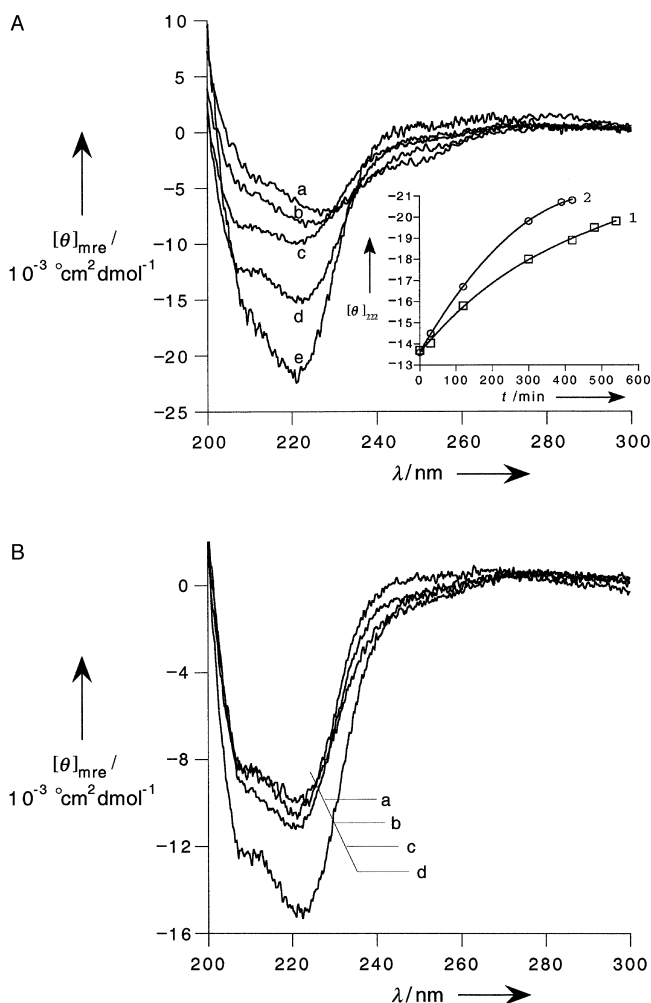


Figure 2. CD spectra of peptides in the presence or absence of various double-stranded (ds) oligonucleotides. CD measurements were performed at 4°C on a JASCO J-715 apparatus in a 2 mm cell. Samples contained phosphate buffer (10 mM, pH 7.0), NaCl (100 mM), peptide (2 μM), and oligonucleotide (2 μM) when present. The spectra are averages of 10 scans and were neither smoothed nor noise reduced. Spectra of the peptides in the presence of DNA were calculated as the difference between the spectra of the peptide–DNA mixture and the spectrum of free DNA. It should be noted that *cis*-3 is contaminated by a small proportion of the *trans* isomer (5%).

NON20 (random): 5'-d(TGGAGTATGCGTCGATTTCGT)-3'
3'-d(ACCTCATACGCAGCTAAGCA)-5'
CREB20-half1: 5'-d(TGGAGATGACGTTGTCTCGT)-3'
3'-d(ACCTCTACTGCAACAGAGCA)-5'
CREB18-half2: 5'-d(AGGATTTTATGACGTTTCG)-3'
3'-d(TCCTAAAATACTGCAAGC)-5'

A) Curve a: *cis*-3 without DNA; curve b: *cis*-3 with NON20; curve c: *trans*-3 without DNA; curve d: *trans*-3 with CREB20; curve e: *cis*-3 with CREB20. B) Spectra of *trans*-3: Curve a: without DNA; curve b: with CREB18-half2; curve c: with CREB20-half1; curve d: with CREB20. At 20°C, the ellipticity of all the complexes with CREB20 is about 12% less. Inset: Time evolution of the CD difference spectrum of a mixture of duplex CREB20 and *trans*-3 upon irradiation at 365 nm. Curve 1: in a buffer containing NaCl (100 mM); HPLC analysis of the final mixture showed a 85:15 *cis:trans* ratio. Curve 2: in a buffer containing MgCl₂ (10 mM) in addition to the NaCl (100 mM); HPLC analysis of the final mixture indicated a 95:5 *cis:trans* ratio.

specific DNA-binding affinity can be modulated by light. Although further development is needed, in particular to allow external induction of the dissociation of the DNA–

peptide complex, we envisage that this kind of externally regulatable peptide system will eventually prove invaluable for in situ control of gene expression.

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- The syntheses of **1** and **2** are described in the Supporting Information. The coupling reaction required the use of an excess of peptide **1** (approximately 3 equivalents). Azopeptide *trans*-3 was isolated by RP-HPLC and identified by ES-MS: *m/z* [*M*+*H*⁺] found: 6110.2; calculated: 6110.3.
- Irradiation at 365 nm was performed with a 8 W UV lamp, at a concentration of 2 μM.

- [12] The isomer ratio was calculated by integration of the HPLC peaks corrected by the extinction coefficients. Remarkably, the equilibrium *cis:trans* ratio is slightly higher than for the azobenzene precursor **2** (90:10).
- [13] Irradiation was performed in a spectrofluorometer (Fluorometer-2; light source: 150 W xenon lamp; 5 nm path). At a concentration of 2 μM , isomer *trans*-**3** was obtained after 15 min.
- [14] In the absence of visible light, the isomerization of *cis*-**3** to *trans*-**3** at 35 °C had a half-life of approximately 65 h.
- [15] It should be borne in mind that *cis*-**3** is contaminated by 5 % of the *trans* isomer.
- [16] An azobenzene-peptide hybrid related to **3** but lacking the two glycines at the C terminus is unable to bind to the CREB site in either the *cis* or *trans* form (results not shown).
- [17] The absence of specific binding was also corroborated by PAGE using ^{32}P end-labelled CREB18-half2 as the DNA probe.
- [18] The UV spectrum of the peptide-DNA complex after 4 h irradiation with visible light is identical to that obtained before irradiation, suggesting that the *cis*-azobenzene unit was unable to switch to the *trans* form (see the Supporting Information).

TEM Studies of Platinum Nanowires Fabricated in Mesoporous Silica MCM-41**

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Porous materials attract much attention as catalysts, catalyst supports, and shape/size-selective adsorbents, as well as containers for making new materials. According to the definition of IUPAC,^[1] porous materials can be divided into three types according to their pore diameters: microporous (<2 nm), mesoporous (2–50 nm), and macroporous (>50 nm). Although zeolites, which belong to the microporous group, provide excellent catalytic properties as a result of their crystalline aluminosilicate networks, their applications are limited by the relatively small internal spaces and pore openings. Therefore, an expansion of the spaces is one of the main targets in the synthesis of porous materials.

In 1992, researchers at Mobil Corporation used a liquid crystal template to synthesize a new family of mesoporous

silicates and aluminosilicates known as the M41S family.^[2, 3] These ordered mesoporous materials have since attracted much attention. Silica MCM-41 is a member of the M41S family with a hexagonal array of one-dimensional pores. The diameter of the pores can be varied from 1.6 to 10 nm by using surfactants of different chain lengths or organic molecules for expanding the surfactant micelles.^[4–11] Numerous studies have been reported on the synthesis of high-quality MCM-41, especially with pore diameters below 4.5 nm.^[2–5, 12–14] Recently, the synthesis of very high quality MCM-41 was achieved by controlling the micelle packing parameter with two kinds of surfactant.^[15] These MCM-41 molecular sieves attract considerable and growing attention because of their remarkable properties. The spaces of the mesoporous materials can be used as containers or templates for the synthesis of other new materials. Here we report on the structural characterization by transmission electron microscopy (TEM) and thermal stability of Pt nanowires as synthesized in the MCM-41 channels and of Pt nanowires removed from the silica template.

We synthesized MCM-41 silica by following a previously reported procedure;^[15] platinum nanowires were prepared in the MCM-41 channels in a manner similar to that reported previously;^[16] and unsupported Pt nanowires were obtained by removing the silica framework from the Pt/MCM-41 sample with an aqueous solution of hydrofluoric acid (see Experimental Section).

Figures 1a and 1b show high-resolution electron microscopy (HREM) images of the calcined MCM-41 sample. Figure 1a shows a uniform hexagonal arrangement of bright dots corresponding to the straight channels of MCM-41. The diffraction pattern in the inset of Figure 1a clearly shows that the incident beam is along the [001] direction. The brightness and the shape of the channels seem to differ slightly from

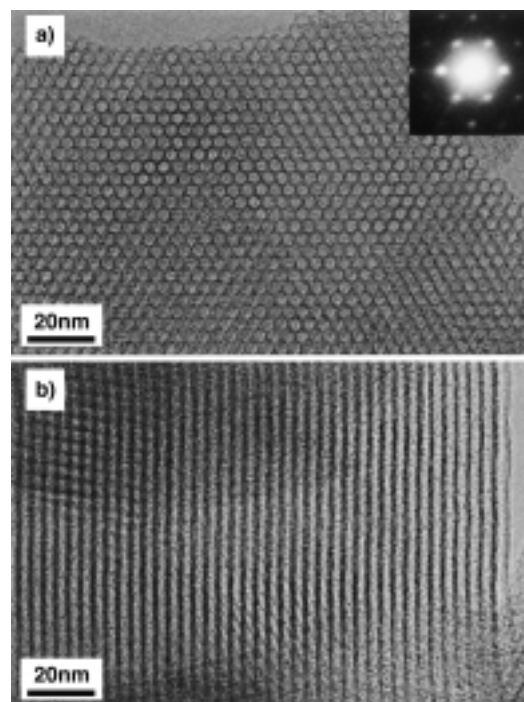


Figure 1. TEM images of calcined mesoporous MCM-41 along the channel direction (c axis; a) and perpendicular to it (b).

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place to place. This is a result of slight changes in diffraction conditions, which show a domain character. Note that all the HREM images are projected along the incident beam direction; therefore, the value of pore diameter from Figure 1a is the projection of the pores along the [001] direction and may be somewhat less than the actual value. Nevertheless, in principle, the pore diameter which is calculated from Figure 1b may give more reliable value than that obtained from other methods, on the assumption that the channels are cylindrical. The HREM method requires appropriate methods of data analysis to avoid artifacts that can lead to an incorrect result.^[17, 18] The diameters of the channels are uniformly about 3.2 nm.

When Pt metal was supported on the MCM-41 silica, most of the Pt clusters were obtained in the form of nanowires. These nanowires followed the bending of the channels, although some Pt particles were observed on the external surfaces of the MCM-41 (Figure 2). The lengths of the Pt nanowires differed from place to place. The lengths ranged from several tens of nanometers to several hundreds of nanometers.

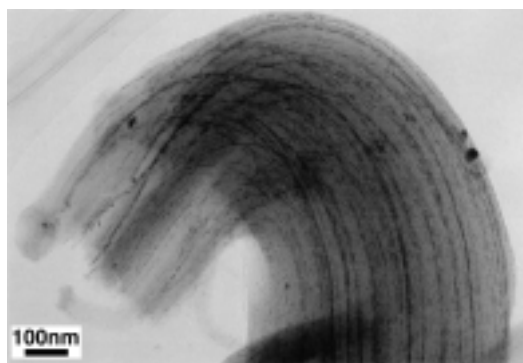


Figure 2. TEM image of Pt nanowires in the channels of calcined mesoporous MCM-41 (Pt/MCM-41).

Figure 3 shows an HREM image of Pt/MCM-41 taken in the [001] direction, which gives the cross-sections of the MCM-41 channels. The channels are parallel to the incident

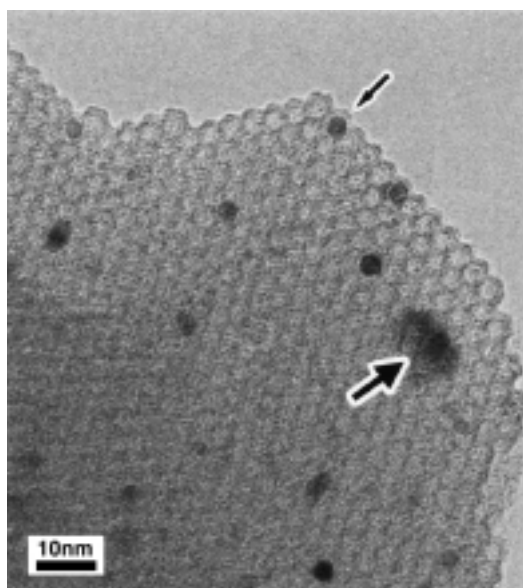


Figure 3. TEM image of a Pt/MCM-41 sample taken in the [001] direction.

beams and arranged in a honeycomb fashion. Two different types of dark contrast from Pt particles are apparent. The smaller, darker areas correspond to Pt nanowires located inside the honeycomb channels. The others give a smeared contrast over the channels, which corresponds to a Pt particle on the external surface. They are indicated by small and large arrows, respectively. The result suggests that the Pt nanowires are well confined in the channels.

Figures 4a and 4b show a low-magnification TEM image and an HREM image, respectively, of the Pt nanowires extracted from Pt/MCM-41 by dissolution of the silica frameworks with HF. The diameter of the Pt nanowires was

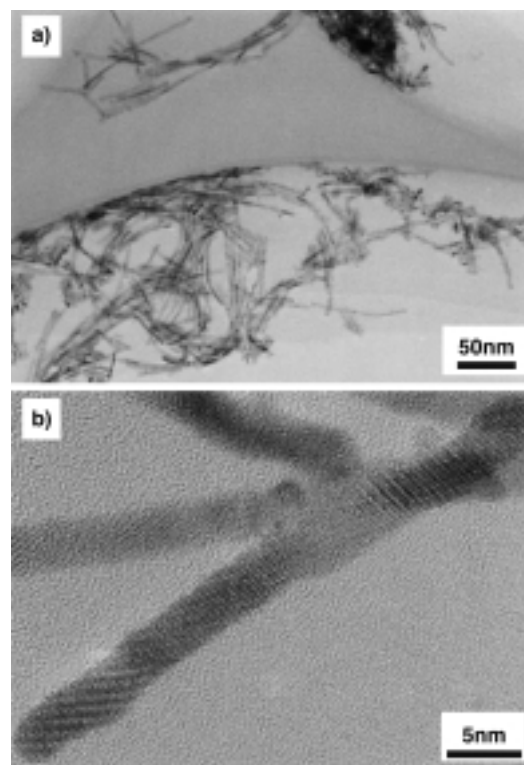


Figure 4. a) Low-magnification TEM image and b) HREM image of Pt nanowires extracted from the Pt/MCM-41 samples.

calculated from many HREM images that were similar to Figure 4b to be 3.0 nm. This is almost the same value as that of the channels. The Pt nanowires have good crystallinity, each can be regarded as a single crystal, and the wires have fairly smooth surfaces with specific indices (see below). It was reported that the shape of the pores of MCM-41 is hexagonal rather than circular.^[19] However, it is difficult to discern whether the cross-section of the Pt nanowires is hexagonal or circular from Figures 3 and 4b.

To determine whether certain directions are preferred during the growth of Pt nanowires, we studied which planes of the Pt nanowires make contact with the channels. Figures 5a and 5b are typical HREM images of Pt nanowires showing the planes parallel to the channel walls. The insets are magnified images of the areas indicated by rectangles. The values of the *d* spacings between the planes shown in the images indicate that they are coincident with the {111} and {200} planes of Pt.

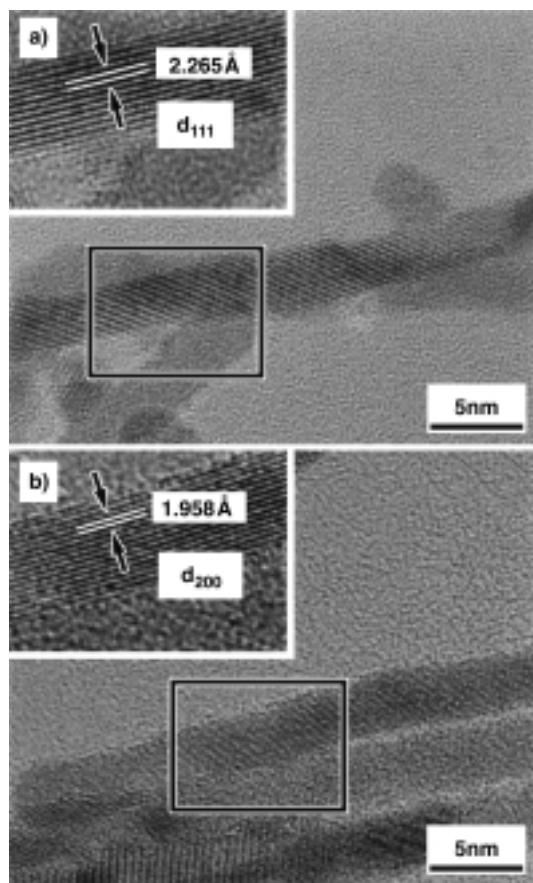


Figure 5. HREM images of Pt nanowires showing the planes parallel to the wall of channels. a) {111} planes; b) {200} planes.

Since the channels are not completely straight, steps are present on the atomic scale. Ignoring the inclination, it can be concluded that the indices of the planes that are parallel to the channel walls are {111} and {200} in Figures 5a and 5b, respectively. We can also deduce the same result from the other HREM images. Hence, the only possibilities for the indices of the planes parallel to channel walls are {111} and {200}. If the planes parallel to channel walls in one Pt nanowire are {111} and {200}, then the axis of preferential growth should contain both {111} and {200} planes. For platinum, which has a face-centered cubic structure, it can be concluded that the $\langle 110 \rangle$ axes are the preferential growth directions of the Pt nanowires.

From the viewpoint of synthesis and applications, the thermal stability of the Pt nanowires is important. Figure 6 shows a series of TEM images of the Pt/MCM-41 sample observed in situ by using heated-stage TEM. The Pt nanowires in the channels of MCM-41 were stable and remained unchanged up to 500 °C. The Pt nanowires did not agglomerate or diffuse to the outer surface.

The Pt nanowires that were extracted from the Pt/MCM-41 samples were also observed in situ by heated-stage TEM under the same condition as for the Pt/MCM-41 samples. Figure 7 shows the TEM images taken at various temperatures. The Pt nanowires began to agglomerate at around 300 °C and broke below 400 °C, as indicated by the arrows in Figure 7. Hence, the Pt nanowires in the channels of MCM-41 were much more stable than those outside the channels.

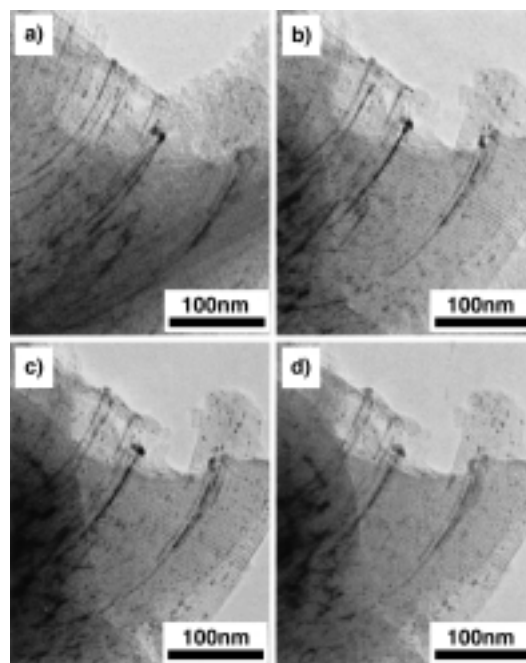


Figure 6. TEM images of a Pt/MCM-41 sample observed in situ by heated-stage TEM. a) Room temperature; b) 300 °C; c) 400 °C; d) 500 °C.

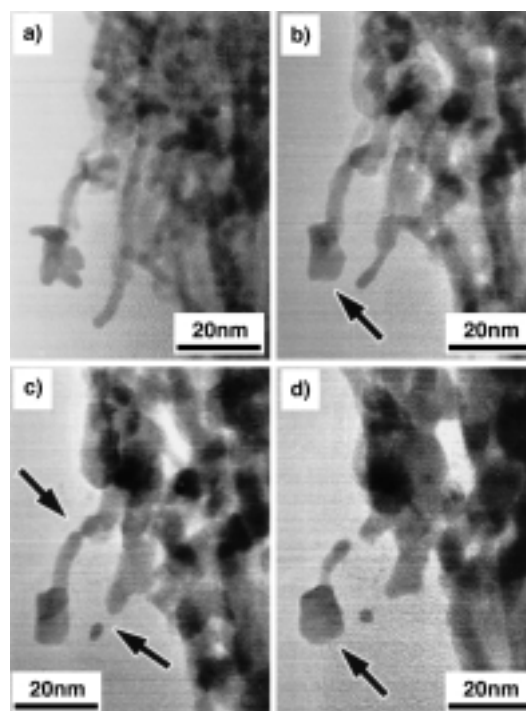


Figure 7. TEM images of Pt nanowires observed in situ by heated-stage TEM. a) Room temperature; b) 300 °C; c) 400 °C; d) 500 °C.

Experimental Section

MCM-41: An aqueous solution of sodium tetrasilicate ($\text{Si}/\text{Na} = 2$) was used as the silica source and added drop by drop to an aqueous solution of tetradecyltrimethylammonium bromide (TTMABr, Aldrich) surfactant at room temperature while the solution was stirred vigorously. After the mixture had been continuously stirring for 1 h, the resultant gel mixture was heated for 24 h at 100 °C. The molar composition of the gel mixture was $3.0\text{SiO}_2:0.75\text{Na}_2\text{O}:1.0\text{TTMABr}:400\text{H}_2\text{O}$. The mixture was cooled to room temperature, and the pH was adjusted to 10 with acetic acid. The mixture

was heated again for 48 h at 100 °C. The pH adjustment and subsequent heating were repeated, and the precipitated MCM-41 product was collected by filtration. The product was washed with EtOH/HCl and calcined in air at 550 °C.

Pt/MCM-41: The calcined MCM-41 sample was slurried in a 3×10^{-3} wt % aqueous solution of $[\text{Pt}(\text{NH}_3)_4](\text{NO}_3)_2$ (Aldrich) for 3 h at room temperature to incorporate $[\text{Pt}(\text{NH}_3)_4]^{2+}$ ions into the channels of MCM-41 by exploiting the weak ion-exchange capability of the silica material. The solution-to-silica ratio was 100 mL g^{-1} . After this treatment, the sample was collected by filtration and subsequently dried in a vacuum oven at room temperature. The ion-exchanged Pt precursor was activated while being slowly heated to 320 °C in a stream of O_2 and then reduced with a stream of H_2 at 300 °C. The resultant sample contained 0.1 wt % Pt seed. This sample was immersed in 1.5×10^{-1} wt % aqueous solution of $[\text{Pt}(\text{NH}_3)_4](\text{NO}_3)_2$, which corresponded to 5 wt % of the MCM-41 silica on the basis of Pt. After the solvent was completely evaporated in a rotary evaporator, the sample was further dehydrated in a vacuum oven at room temperature. The impregnated Pt-metal precursor was finally reduced in a stream of H_2 while the temperature was increased from room temperature to 300 °C over 4 h and maintained at 300 °C for 2 h. These treatments gave Pt nanowires inside the MCM-41 channels.

Unsupported Pt nanowires: A small amount of Pt/MCM-41 was added to a 10 wt % aqueous solution of HF diluted with ethanol. The silica was completely dissolved after stirring this solution gently at room temperature. The Pt nanowires were collected by filtration, washed with ethanol, and stored in ethanol until used.

TEM measurements: All samples (calcined MCM-41, Pt/MCM-41 and extracted Pt nanowires) were suspended in ethanol (99.9 vol %) by ultrasonication. The suspension was deposited on a carbon microgrid for TEM observation. The HREM observations were performed with a 400 kV electron microscope (JEM-4000EX) at room temperature. High-temperature observations were carried out by 1250 kV EM (JEM-1250) with a top-entry high-temperature stage.

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Network Polysilanes: Synthesis, Electrical Conductivity, Charge-Transfer Interaction, and Photoconductivity

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Much attention has been paid to polysilanes because of their unique and versatile properties resulting from extensive delocalization of the σ electrons. These properties have made polysilanes useful as photoresists, ceramic precursors, photoconductors, semiconductors, and nonlinear optical materials in high technology fields.

With MeO-functionalized polysilanes as the starting material,^[1] we recently obtained a new type of network polysilane, $[\text{MeSi}(\text{OMe})_x(\text{R})_y]_n$, with various R groups (alkyl, aralkyl, alkenyl, and aryl) as the side chains by the action of the corresponding Grignard reagents RMgX ($\text{X} = \text{Cl}, \text{Br}$).^[2] Among these polysilanes, those having *N,N*-dialkylaminophenyl groups as side chains ($p\text{-R}'_2\text{NC}_6\text{H}_4$; $\text{R}' = \text{Me}, \text{Et}, \text{Pr}$) exhibited electrical conductivities on the order of 10^{-3} Scm^{-1} upon iodine doping; they are essentially stable under oxidative conditions because of their network structures.^[2] Although linear polysilanes generally show high hole mobility or photorecepting ability due to their electrical characteristics,^[3,4] no studies regarding such properties for network polysilanes have appeared so far. This led us to study network polysilanes for applications as conducting materials when combined with organic electron acceptors such as 9,10-dicyanoanthracene (DCA), *p*-chloranil, and tetracyanoquinodimethane (TCNQ).

Studies on the preparation and properties of network polysilanes arising from Wurtz-type coupling reactions with alkylated trichlorosilanes as starting materials have appeared,^[5] whereas there are only limited reports on arylated network polysilanes, which are accessible using the corresponding trichlorosilanes.^[6] As part of a continuing study on network polysilanes, we now report on the properties, charge-transfer interactions with organic electron acceptors, photo-carrier generation as well as electrical conductivity of two types of network polysilanes. The network polysilanes were prepared by homo- or copolymerization using arylated

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trichlorosilanes (WZ-polysilanes) and the introduction of aryl groups into MeO-functionalized polysilanes (FR-polysilanes).

In contrast to the alkyltrichlorosilanes previously used,^[5] the Wurtz coupling polymerization of arylated trichlorosilanes **1** proceeded readily with sodium under the usual conditions to give the corresponding soluble WZ-polysilanes **2** in reasonable to high yields. On the other hand, substituted MeO-functionalized network polysilanes **3** (FR-polysilanes) were obtained according to a previously reported method^[2] (Table 1).

It was of interest to investigate the conductivity of polysilanes **2**, which have various groups as side chains, and to elucidate their spectral behavior by comparison with other polysilanes, since there have been only few reports describing the electrical conductivities of polysilanes.^[2, 7] Thus, film samples of WZ-polysilanes **2a–i** and linear polysilanes **4a, b** were prepared on glass plates, and their conductivities were measured by the four-probe method using iodine as the dopant. Before doping, the values for all the polysilanes were on the order of 10^{-9} – 10^{-7} S cm⁻¹. The values for the polysilanes **2c–h** bearing *N,N*-dialkylaminophenyl groups (*p*-R₂NC₆H₄; R' = Me, Et) significantly increased upon doping to around 10^{-3} and then leveled off; these values remained unchanged for at least 15 h. This is comparable with the behavior of network FR-polysilanes bearing the same groups (**3c,d**).^[2] In contrast, for the linear polysilanes **4a, b** and network polysilanes **2a, b, i** and **3b**, the conductivities only increased slightly upon doping (Table 1). The remarkable increase and the conductivities of **2c–h** may be due to the increased concentration of positive carriers, which are stabilized by the strong electron-donating nature of the *p*-R₂NC₆H₄ groups.

The enhanced conductivities are further supported by the spectral behavior in CH₂Cl₂ of the film samples doped with iodine: Polysilanes **2c–h** and **3c, d**, which gave the higher conductivities, formed the corresponding charge transfer complexes (λ_{max} = 365 and 610(sh) nm, respectively; Table 2). Interestingly, the band in the 610 nm region, arising from the WZ-polysilanes doped with iodine, is in good agreement with

Table 2. Charge-transfer interactions between network polysilanes and organic electron acceptors and resulting absorption bands in CH₂Cl₂.

Polysilane (λ_{max} [nm]) ^[a]	DCA	λ_{max} [nm] ^[b]			iodine ^[c]
		chloranil	TCNQ		
2b (none)	— ^[d]	— ^[d]	— ^[d]		none ^[e]
2c (287)	none ^[e]	none ^[e]	750, 850		362, 610(sh)
2d (288)	none ^[e]	none ^[e]	750, 850		364, 602(sh)
2e (280)	none ^[e]	none ^[e]	750, 850		362, 610(sh)
2f (285)	none ^[e]	none ^[e]	750, 850		360, 605(sh)
2g (273)	none ^[e]	none ^[e]	750, 850		363, 612(sh)
2h (278)	none ^[e]	none ^[e]	750, 850		362, 605(sh)
2i (none)	none ^[e]	none ^[e]	620, 750, 850		none ^[e]
3a (none)	none ^[e]	none ^[e]	600, 760, 850		none ^[e]
3b (none)	none ^[e]	none ^[e]	620, 750, 850		none ^[e]
3c (276)	none ^[e]	585	620, 750, 850		364, 602(sh)
3d (287)	none ^[e]	none ^[e]	620, 750, 850		364, 602(sh)
4a (335)	none ^[e]	— ^[d]	— ^[d]		— ^[d]
4b (330)	none ^[e]	— ^[d]	none ^[e]		362

[a] All the network polysilanes (**2b–3d**) exhibit, besides the peak noted, an absorption band rising near 400 nm, which increases in intensity with shorter wavelength. [b] The absorption intensities for these bands are fairly weak compared with those of the starting materials. [c] Absorption bands of solutions in dichloromethane prepared using the film samples after iodine doping. [d] Not determined. [e] Neither a color change nor an absorption peak were observed.

that from the FR-polysilane **3c** and iodine (λ = 615 nm region),^[2] which indicates that the two types of polysilanes have very similar structures. Therefore, the bands at λ = 610 nm from **2c–h** may correlate to the charge transfer complexes associated with their conductivities, as described for the FR-polysilanes.^[2] Furthermore, it is likely that the smaller molecular weights do not significantly affect their conductivities and spectral properties,^[8] particularly for **2d, i** compared with the other polysilanes (Table 1; see also Table 2).

Generally, the electrical conductivity of the polysilanes was determined by iodine doping because of the simple procedure. We have shown the first examples for the spectral observation of a charge-transfer interaction between network polysilanes having alkyl or aryl substituents and iodine.^[2] We also found that in the DCA-photosensitized reaction of cyclopolysilanes

Table 1. Electrical conductivities of network polysilanes upon doping with iodine.

Starting materials	Polysilane	Yield [%]	$M_w \times 10^{-3}$ ^[a]	σ [S cm ⁻¹] ^[b]	
				before doping	after doping
C ₆ H ₁₃ SiCl ₃ (1a)	2a	89	20.0	5×10^{-9}	8×10^{-9}
C ₆ H ₅ SiCl ₃ (1b)	2b	60	5.4	3×10^{-8}	4×10^{-8}
<i>p</i> -Me ₂ NC ₆ H ₄ SiCl ₃ (1c)	2c	46	5.6	8×10^{-7}	5×10^{-3}
<i>p</i> -Et ₂ NC ₆ H ₄ SiCl ₃ (1d)	2d	53	3.3	5×10^{-7}	4×10^{-3}
1c + 1a	2e	30	9.1	1×10^{-7}	9×10^{-4}
1d + 1a	2f	22	12.4	2×10^{-7}	4×10^{-3}
1c + 1e	2g	20	17.5	1×10^{-7}	1×10^{-3}
1d + 1e	2h	20	9.7	4×10^{-7}	1×10^{-3}
1c + 1e	2i	52	3.9	6×10^{-8}	4×10^{-7}
[MeSi(OMe)] _n (1f) ^[2] + C ₆ H ₁₃ MgCl	3a	—	20.0	—	—
1f + 2-SC ₄ H ₉ MgBr	3b	—	19.5	8×10^{-8} ^[c]	5×10^{-7} ^[c]
1f + <i>p</i> -Me ₂ NC ₆ H ₄ MgBr	3c	—	18.5	2×10^{-7} ^[c]	1×10^{-3} ^[c]
1f + <i>p</i> -Et ₂ NC ₆ H ₄ MgBr	3d	—	10.8	8×10^{-8} ^[c]	1×10^{-3} ^[c]
C ₆ H ₅ MeSiCl ₂ (1e)	4a ^[d]	—	24.0	4×10^{-8}	1×10^{-6}
Cl(C ₆ H ₅ MeSiSiMe ₂)Cl (1g) ^[14]	4b ^[d,e]	—	348.0	1×10^{-7}	3×10^{-6}

[a] Determined by gel permeation chromatography (GPC) with polystyrene standards. [b] The electrical conductivity σ was measured with a film on a glass plate by the four-probe method at room temperature. [c] Taken from ref. [2]. [d] Prepared according to the present method (see Experimental Section). [e] Available from Yuki Gosei Kogyo Co. Ltd.; prepared according to ref. [14] using monomer ClMePhSiSiMe₂Cl.

$[R_2Si]_n$ ($n=3-7$, R = alkyl; $\lambda_{\max}=250-300$ nm) electron transfer only occurs from the cyclosilanes to DCA (the longest wavelength absorption band is at $\lambda_{\max}=425$ nm) upon irradiation with light ($\lambda > 390$ nm), producing the DCA anion radical as the intermediate.^[9] Thus, to obtain information about the charge-transfer interactions between network polysilanes and organic electron acceptors such as DCA ($E_{1/2} = -0.97$ V),^[10] *p*-chloranil ($E_{1/2} = -0.92$ V),^[10] and TCNQ ($E_{1/2} = -0.45$ V; all values with respect to the standard calomel electrode (SCE)),^[10] we carried out a series of spectral measurements, because this would disclose a useful property of the network polysilanes as photoconducting materials.

Upon mixing network polysilanes **2c–h** and **3a–d** with DCA or chloranil in CH_2Cl_2 , the solutions showed, based on their absorption spectra, neither an interaction nor formation of a charge-transfer complex, except in the case of **3c** with chloranil, where the charge-transfer complex was formed ($\lambda_{\max}=585$ nm, blue color; Table 2). Equally, in experiments using TCNQ, WZ-polysilanes **2c–h** only formed the TCNQ anion radical^[11] ($\lambda_{\max}=750, 850$ nm; green), whereas with the FR-polysilanes **3a–d** both the complex ($\lambda_{\max}=600-620$ nm) and the TCNQ anion radical resulted ($\lambda_{\max}=750, 850$ nm; blue-green). However, the combination of the two types of network polysilanes and iodine ($E_{1/2} = -0.57$ V)^[10] resulted in the formation of only charge-transfer complexes ($\lambda_{\max}=362-364, 602-610$ nm (sh)) except for **2b**, **i** and **3a**, **b** (Table 2). In the ESR spectral analysis in CH_2Cl_2 , a solution ($\lambda_{\max}=364, 602$ nm (sh)) consisting of polysilane **3c** and iodine as well as DCA or chloranil exhibited no signal, while similar experiments for a sample from **3c** and TCNQ afforded a signal due to the TCNQ anion radical. These results clearly show that in almost all cases the network polysilanes **2c–h** and **3a–d** do not interact with DCA or chloranil, which have higher reduction potentials. In contrast, with iodine or TCNQ, which have lower reduction potentials or stronger electron-accepting abilities, they form the corresponding charge-transfer complexes and/or TCNQ anion radicals. Thus, it can be concluded that the oxidation potentials for the series of polysilanes^[12] decrease in the following order: **2c–2h** < **3c**, **3d** < **3a**, **3b** $\cong$ **2i** < **4a**, **4b**; that is, the reducing ability or electron-donating nature of the polysilanes decreases in this order. These results, together with those of the previous study on the DCA-photosensitized reaction,^[9] provide quite helpful information with respect to the photoconductivity in WZ- and FR-polysilane systems.

To clarify the photoconductivity of the polysilanes, we have qualitatively investigated the generation of the carrier by means of transient photoconductivity analysis using single-layer films consisting of network polysilanes (ca. 42% in weight), DCA (ca. 8%) as a sensitizer, and polybutyral (ca. 50%) as a phototransparent binder. Film samples were irradiated with a field strength on the order of 10^4 V cm⁻¹ using a pulsed laser light (10 ns, $\lambda=425$ nm, 1 mJ cm⁻² per pulse) which coincides with the longest wavelength absorption band of DCA at $\lambda=425$ nm. Irradiation of control samples containing only polysilane (50%) in polybutyral (50%) showed no signal, and samples consisting of DCA (50%) and polybutyral (50%) produced only very weak

signals (mV in intensity) due to carrier generation (Figure 1a). Interestingly enough, upon irradiation samples similarly prepared with selected WZ- and FR-polysilanes produced signals due to the carrier concentration which were enhanced by one order of magnitude compared with the control samples. The intensities of the observed signals remained unchanged during irradiation in each case, but upon conclusion of irradiation the signals immediately

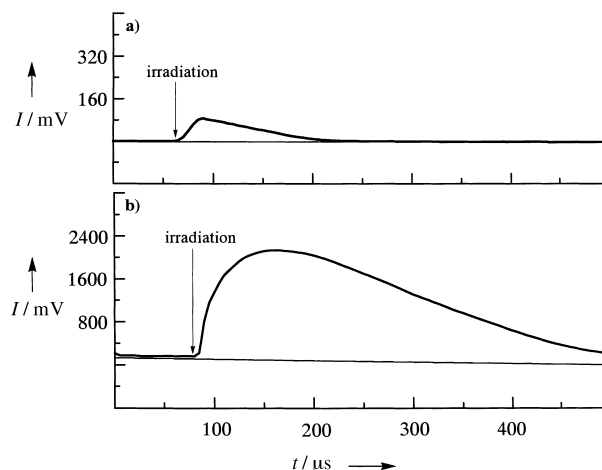


Figure 1. Carrier signals generated by one-time irradiation (10 ns) with pulsed laser light at $\lambda=425$ nm for a) a control sample of DCA (50% by weight) in polybutyral (50%; film thickness 20 μ m) and b) a sample containing polysilane **3c** (42%), DCA (8%), and polybutyral (50%; film thickness 6 μ m). The termination of irradiation results in a base-line signal in each case. I = intensity.

disappeared. The signal for the hole carrier arising from the sample consisting of **3c** and DCA is shown as an example in Figure 1b. Based on these results, it was evident that both the WZ- and FR-polysilanes bearing the aryl group would be useful for use as new photoconducting materials consisting of stable silicon network structures. As has been shown in studies on the photoconductivities of linear polysilanes^[4c,d,e] and silicon-based polymers,^[13] it is likely that generation of the carrier by electron transfer from polysilanes to the photo-excited DCA efficiently results in hole transport between the polysilanes in the present single-layer systems.

Finally, it should be stressed that, as expected from the FR-polysilanes,^[2] the WZ-polysilanes are capable of forming stable films after doping with iodine because of the resulting network structures. Furthermore, polysilanes **2c–h**, bearing the strong electron-donating groups, showed constant and stable electrical conductivities on the order of 10^{-3} S cm⁻¹, even after standing for at least 15 h. From the combination of the WZ- or FR-polysilane donor and DCA acceptor, qualitative evidence for the carrier generation followed by efficient hole transport was obtained for the first time by irradiation at $\lambda=425$ nm with pulsed laser light. Therefore, the present results open up the way for new applications for the two types of network polysilanes as photoconducting materials. In addition, the synthesis of donor–acceptor systems resulting from the interaction between organic electron acceptors such as DCA, chloranil, and TCNQ, which have different reduction potentials, and various polysilanes

provide a convenient method for the estimation of the electron-donating ability of polysilanes using electronic absorption spectroscopy in solution.

Experimental Section

As an example, the synthesis of **2a** is described: A solution containing *p*-*N,N*-dimethylaminophenyltrichlorosilane (**1c**; 1.27 g, 5 mmol) and hexyltrichlorosilane (**1a**; 1.11 g, 5 mmol) in HMPA (2 mL) was added dropwise with high-speed stirring and toluene refluxing within 15 min to a sodium dispersion (0.84 g, 36 mmol) prepared in refluxing toluene (30 mL). The mixture was stirred for 2 h with refluxing, treated with MeLi (ca. 1.2 mL of a 1.1 M solution in diethyl ether) at 50 °C for 1 h, and cooled. The excess Na was quenched with MeOH, and the resulting mixture was washed with water. The toluene solution obtained was dried over MgSO₄ and evaporated under reduced pressure to give the crude products which were dissolved in THF. Fractional precipitation obtained by pouring the THF solution onto *i*PrOH gave a precipitate which was collected on a filter and dried to give **2c** as a pale yellow powder (0.373 g; 30 % Si yield based on the starting materials) as a methanol-insoluble and toluene-soluble material ($[p\text{-}(\text{Me}_2\text{NC}_6\text{H}_4\text{Si})_{0.48}(\text{C}_6\text{H}_{13}\text{Si})_{0.52}]_n$; $M_w = 9100$). The reactions for the other chlorosilanes were carried out in a similar manner to give the corresponding products **2**. The products were characterized by IR, NMR, and UV/VIS spectroscopy, and the relative molecular weights M_w were determined. Polysilanes **2c–h** showed a UV/Vis absorption peak at $\lambda = 280$ nm linked to the curve rising near $\lambda = 400$ nm that increases in intensity with shorter wavelength, suggesting that the structure is a network system^[15] to which the hexyl and aryl side chains are attached. Thermogravimetric analyses of the polysilanes **2a–i** (40–1000 °C, heating rate 10 °C min^{−1}, N₂ stream) were carried out; the weight losses were less than 5 % at 300 °C for all samples tested, except for **2c**. Molecular weight and spectral data for the polysilanes: **2a**: ($\text{C}_6\text{H}_{13}\text{Si}$)_{*n*}, $M_w = 21\,400$, $M_w/M_n = 3.3$; ¹H NMR (CDCl₃): $\delta = 0.5\text{--}2.3$ (SiC₆H₁₃, 13.0 H). **2b**: (PhSi)₃, $M_w = 3200$, $M_w/M_n = 1.8$; ¹H NMR (CDCl₃): $\delta = 5.3\text{--}8.5$ (SiC₆H₅, 5.0 H). **2c**: (*p*-Me₂NC₆H₄Si)_{*n*}, $M_w = 4400$; $M_w/M_n = 1.2$; ¹H NMR (CDCl₃): $\delta = 2.2\text{--}3.3$ (NCH₃, 6.0 H), 5.1–8.0 (C₆H₄, 4.0 H). **2d**: (*p*-Et₂NC₆H₄Si)_{*n*}, $M_w = 3300$, $M_w/M_n = 1.2$; ¹H NMR (CDCl₃): $\delta = 0.6\text{--}1.4$ (NCCH₃, 6.0 H), 2.5–3.6 (NCH₂C, 4.0 H), 5.3–7.9 (C₆H₄, 4.0 H). **2e**: [*p*-Me₂NC₆H₄Si]_{0.35}(Hex-Si)_{0.65}]_{*n*}, $M_w = 14\,300$, $M_w/M_n = 1.6$; ¹H NMR (CDCl₃): $\delta = 0.2\text{--}2.0$ (C₆H₁₃, 25.3 H), 2.5–3.1 (NCH₃, 6.0 H), 6.0–7.7 (C₆H₄, 3.9 H). **2f**: [*p*-Et₂NC₆H₄Si]_{0.30}(HexSi)_{0.70}]_{*n*}, $M_w = 12\,400$, $M_w/M_n = 1.4$; ¹H NMR (CDCl₃): $\delta = 0.1\text{--}2.0$ (C₆H₁₃Si, 27.3 H, NCCH₃, 6.0 H), 2.7–4.2 (NCH₂, 4.0 H), 6.2–8.3 (C₆H₄, 4.0 H). **2g**: [*p*-Me₂NC₆H₄Si]_{0.45}(MePhSi)_{0.55}]_{*n*}, $M_w = 5000$, $M_w/M_n = 1.2$; ¹H NMR (CDCl₃): $\delta = -0.8\text{--}0.8$ (SiCH₃, 4.5 H), 2.4–3.7 (NCH₃, 6.0 H), 6.2–8.1 (C₆H₄, 4.0 H; C₆H₅, 7.0 H). **2h**: [*p*-Et₂NC₆H₄Si]_{0.45}(MePhSi)_{0.55}]_{*n*}, $M_w = 9700$, $M_w/M_n = 1.2$; ¹H NMR (CDCl₃): $\delta = -0.9\text{--}0.8$ (SiCH₃, 4.0 H), 0.8–2.0 (NCCH₃, 6.0 H), 2.7–3.9 (NCH₂C, 4.0 H), 5.9–8.1 (C₆H₄, 4.0 H; C₆H₅, 6.6 H). **2i**: [*p*-Me₂NC₆H₄Si]_{0.04}(MePhSi)_{0.96}]_{*n*}, $M_w = 3900$; ¹H NMR (CDCl₃): $\delta = -1.0\text{--}0.8$ (SiCH₃, 3.0 H), 2.6–3.1 (NCH₃, 0.25 H), 6.1–7.7 (C₆H₄, 0.17 H; C₆H₅, 5.0 H).

The synthesis of **3a** from MeO-functionalized polysilane [MeSi(OMe)]_{*n*} (**1f**; $M_w = 24\,000$, $M_n = 4400$; $l = 0.4$)^[2] by a Grignard alkylation is described as a typical example: A solution of C₆H₁₃MgCl (12.3 mmol) was prepared from magnesium and hexyl chloride in THF (10 mL). The Grignard reagent was added with stirring to a solution containing polysilane **1f** (0.50 g) in THF (10 mL). After a weakly exothermic reaction, the mixture was heated at 40 °C for 20 h with stirring, and then dry methanol (2–3 mL) was added to quench the active species. From the resulting mixture, the solvent was removed completely to give a solid material, which was extracted with toluene (ca. 50 mL). The toluene extracts were concentrated under reduced pressure, and warm methanol (15 mL) was added to precipitate a solid material, which was collected on a filter, washed with methanol, and then dried under reduced pressure. The obtained FR polysilane **3a** was a pale yellow powder ([MeSi(OMe)]_{0.18}(Hex)_{0.23}]_{*n*}, $M_w = 20\,000$, $M_n = 4300$; 0.54 g, 108 % yield based on **1f**).^[2] The other FR-polysilanes (**3b–d**) were prepared in a similar manner (see Table 1). Products **3a–d** were identified and characterized as previously described^[2] (see also the WZ-polysilanes and footnote [b] in Table 2). Molecular weight and spectral data for the four polysilanes: **3a**: [MeSi(OMe)]_{0.18}(Hex)_{0.23}]_{*n*}, $M_w = 20\,000$, $M_w/M_n = 4.6$; ¹H NMR (CDCl₃): $\delta = -0.5\text{--}0.7$ (SiCH₃, 3.0 H; SiCH₂C₅, 0.2 H), 0.8–1.0

(C₄H₉, 0.7 H), 1.1–1.6 (SiC(CH₂)₄C, 1.8 H), 3.2–3.8 (SiOCH₃, 0.6 H). **3b**: [MeSi(OMe)]_{0.21}(SC₄H₉)_{0.08}]_{*n*}, $M_w = 9500$, $M_w/M_n = 3.1$; ¹H NMR (CDCl₃): $\delta = -0.4\text{--}1.0$ (SiCH₃, 3.0 H), 3.0–3.6 (SiOCH₃, 0.6 H), 7.0–7.8 (SC₄H₉, 0.3 H). **3c**: [MeSi(OMe)]_{0.21}(C₆H₄NMe₂)_{0.22}]_{*n*}, $M_w = 8700$, $M_w/M_n = 3.2$; ¹H NMR (CDCl₃): $\delta = -0.2\text{--}1.0$ (SiCH₃, 3.0 H), 2.7–3.1 (NCH₃, 1.3 H, SiOCH₃, 0.6 H), 6.5–7.6 (C₆H₄, 0.9 H). **3d**: [MeSi(OMe)]_{0.23}(C₆H₄NEt₂)_{0.29}]_{*n*}, $M_w = 9900$, $M_w/M_n = 2.9$; ¹H NMR (CDCl₃): $\delta = -0.2\text{--}0.9$ (SiCH₃, 3.0 H), 1.0–1.3 (NCCH₃, 1.8 H), 3.0–3.7 (NCH₂C, 1.2 H; SiOCH₃, 0.7 H), 6.4–7.6 (C₆H₄, 1.2 H).

Preparation of the sample films for measurement of electrical conductivity: A solution containing the polysilane (ca. 50 mg) in solvent (0.5 mL; toluene, xylene, or DME) was prepared. The sample films (thickness 10–40 μm; area size ca. 16 mm × 9–12 mm) were prepared by spin-coating the solution on a glass plate (18 mm × 18 mm) and dried for 1 h at about 60 °C. The glass plate was equipped with four gold-film electrodes at regular intervals of 2 mm prior to the spin-coating, and fine leading wires were attached to the four gold electrodes. For the electrical conductivity measurement, the sample film thus obtained was placed in an apparatus, which was equipped with a programmable DC voltage/current generator TR-6142 (ADVANTEST Co. Ltd.), digital electrometer TR-8652 and computing digital multimeter TR-8651 (Takeda Scientific Laboratories Co. Ltd.), and GP-IB adapter (ADVANTEST Co. Ltd.), and after evacuation the electrical conductivity (σ [S cm^{−1}]) of the sample was measured at 20 °C by a typical four-probe method before and after doping with saturated iodine vapor.

Preparation of sample films for the photoconductivity measurement: The sample films (thickness 5–25 μm; area size ca. 10 × 10 mm) were prepared on a conducting glass plate^[16] by spin-coating with a solution prepared by mixing polysilane in CH₂Cl₂ with DCA and phototransparent polybutylal in CH₂Cl₂, and then drying at about 60 °C for 1 h. Fine leading wires for the three electrodes were attached to the sample film, on which a gold-film electrode was placed by evaporation in vacuo, and on the conducting film of the plate on both sides across the sample film. The measurement for the signals due to the photocarrier generation in the single layer films is described in the text.

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Synthesis, Structure, and Magnetism of a Novel Alkoxide Bridged Nonacopper(II) (Cu_9O_{12}) $[3 \times 3]$ Square Grid Generated by a Strict Self-Assembly Process**

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Sarah L. Heath, David O. Miller, and Masaaki Ohba

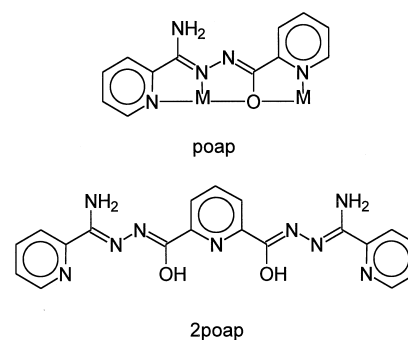
Single ligands with repeating linear arrays of coordination pockets have the potential to create gridlike arrangements of metal centers by self-assembly processes. Linear polytopic ligands of this sort with pyridazine and pyrimidine bridging subunits have produced essentially flat $[2 \times 2]$ homotetranuclear grids with Cu^{I} and Co^{II} salts,^[1, 2] and a flat $[3 \times 3]$ nonanuclear grid with Ag^{I} salts.^[3]

The Co^{II} complex has been shown to exhibit antiferromagnetic coupling within the cluster, despite long distances of separation between the metal centers (6.5 Å).^[4] The ordering of paramagnetic metal ions in gridlike arrays presents the possibility of extended spin communication within a lattice of closely spaced metal ion centers, generated from a single preprogrammed ligand. The nuclearity of these clusters is clearly based on the polytopic nature of the ligand and the ability of the metal to read and interpret the coordination

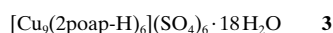
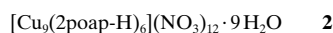
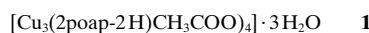
options presented by the ligand in the most efficient way, depending on its own coordination algorithm.^[3]

Tetradentate ligands (e.g. poap) built on a flexible diazine (N–N) backbone have been shown to self-assemble Mn^{II} , Co^{II} , Ni^{II} , and Cu^{II} centers into square, tetranuclear clusters, with just alkoxide bridges between the metal ions.^[5] These systems have close metal–metal spacings (≈ 4 Å) and display dominant antiferromagnetic intramolecular exchange, except in the copper(II) cases where ferromagnetic coupling, associated with orthogonal magnetic connections, prevails. Spin-coupled, homoleptic trigonal-bipyramidal alkoxo-bridged Mn_5^{II} ^[6] and Co_5^{II} ^[7] clusters have also been produced, in which there is an exact match between the ligand donor pocket groupings and the coordination requirements of the six-coordinate metal centers.

Extension of poap using a 2,6-disubstituted pyridine central fragment generates the ligand 2poap, which has an approximately linear array of coordination pockets that produce five-membered chelate rings on coordination. The ligand 2poap behaves as a nonadentate ligand in reaction with



copper(II) acetate, producing a linear trinuclear complex **1**, in which a central copper center is bound to the other copper centers through N–N diazine single bond bridges.^[7] However with copper(II) nitrate and copper(II) sulfate 2poap behaves as a heptadentate ligand, providing donors for nine metal coordination sites through oxygen bridging, and nonanuclear complexes are formed in both cases. Reaction of 2poap with $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ in water produced a brown crystalline product **2** (71 % yield), suitable for structural analysis.^[8] A similar reaction with $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ produced the dark green crystalline product **3** (75 % yield).^[7]



The structure of **2** (Figure 1) involves a novel $[3 \times 3]$ grid of nine pseudo-octahedral Cu^{II} centers coordinated by two groups of three roughly parallel ligands arranged above and below the metal pseudo-plane, with the metals bridged just by alkoxide oxygen atoms. The ligands are roughly eclipsed, but have a slight stagger. The parallel grouping of the ligands is associated with π interactions between the aromatic rings (ring separations ≈ 3.6 – 4.2 Å). Three different pseudo-octahedral copper(II) centers are found; the central Cu atom has a

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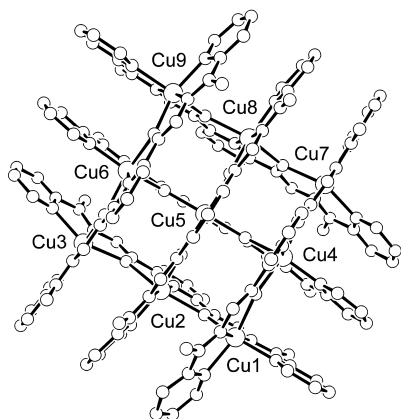


Figure 1. Structural representation of the cation $[\text{Cu}_9(2\text{poap-H})_6]^{12+}$ in **2** (hydrogen atoms omitted for clarity). Selected bond lengths [Å] and angles [°]: Cu5-N14 1.941(10), Cu5-N41 1.912(11), Cu5-O3 2.163(7), Cu5-O4 2.197(7), Cu5-O9 2.210(10), Cu5-O10 2.199(9), Cu1-Cu2 4.148(8), Cu2-Cu3 4.198(7), Cu3-Cu6 4.169(8), Cu6-Cu9 4.085(9), Cu9-Cu8 4.2187, Cu8-Cu7 4.106(7), Cu7-Cu4 4.116(8), Cu4-Cu1 4.227(8), Cu2-Cu5 4.069(7), Cu6-Cu5 4.048(8), Cu8-Cu5 4.065(8), Cu4-Cu5 4.032(6); Cu-O-Cu 136.5–143.6.

trans-N₂O₄ donor arrangement, the corner Cu centers have *cis*-N₄O₂ donor arrangements, and the side Cu centers have *mer*-N₃O₃ donor arrangements.

The Cu₉O₁₂ core (Figure 2) consists of four fused Cu₄O₄ square subunits, each of which has a puckered bridging arrangement with an alternation of oxygen atoms above and

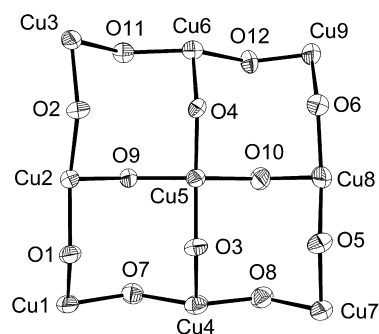


Figure 2. Structural representation of the pseudo-square Cu₉O₁₂ core in $[\text{Cu}_9(2\text{poap-H})_6]^{12+}$.

below the Cu₄ pseudo-plane in a boatlike conformation. Each subunit has a similar structure to known square, tetranuclear copper(II) complexes of poap.^[5] Adjacent copper–copper separations fall in the range 4.032–4.227 Å, which generates a roughly square grid with overall core dimensions of 8.2 × 8.3 Å. Cu–O–Cu angles fall in the range 136.5–143.6° and Cu–O and Cu–N distances fall in the ranges 2.061–2.314 Å and 1.858–2.231 Å, respectively. Similar nonanuclear grids of octahedral Mn^{II} centers can also be produced with 2poap and related ligands (e.g. $[\text{Mn}_9(2\text{poap-2H})_6](\text{ClO}_4)_6 \cdot 18\text{H}_2\text{O}$).^[9] The presence of the acetate anion in **1** causes the ligand to lose two protons, and the acetates also act as ligands leading to the trinuclear structure.^[7] However in **2** and **3** the ligands appear to lose just one proton each, and are involved in oxygen bridging only. The formation of the homoleptic grid structure in **2** is a clear consequence of the exact matching of the nine coordination pockets to the coordination require-

ments of the nine metal centers. However, the formation of a complex with nine pseudo-octahedral copper(II) centers is rather unusual, since copper(II) generally prefers to adopt square or square-pyramidal geometries. This leads to a complicated situation with regard to the magnetic ground states of each copper center. The central copper atom (Cu5) has a compressed tetragonal geometry (*d*_{z²} ground state), with four long equatorial Cu–O contacts to O3, O4, O9, and O10 (2.163–2.210 Å), and two short Cu–N bonds to N14 and N41 (1.941(10) and 1.912(11) Å, respectively). Cu3 and Cu7 have similar distorted geometries, while the other copper centers are best described as elongated tetragonal (*d*_{x²–y²} ground state). However in all cases connections between copper centers through the alkoxide bridges appear to be “orthogonal”, based on the ground-state assessment.

Variable-temperature magnetic data^[10] were obtained for **2** in a 0.1 T field in the temperature range 2–300 K. The magnetic moment (per mole) drops slightly from 6.3 μ_B at 300 K to 5.5 μ_B at 30 K, followed by a rise below this temperature to a value of 6.9 μ_B at 2.0 K (Figure 3). The room-temperature value is consistent with the presence of

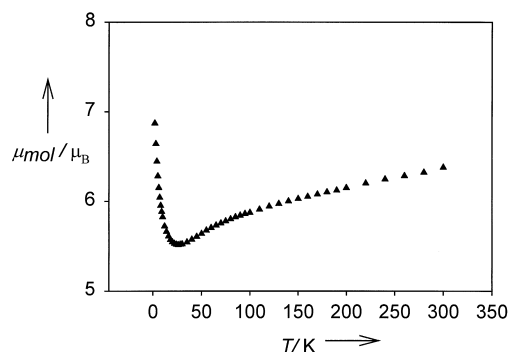


Figure 3. Variable-temperature magnetism for **2** plotted as magnetic moment (μ_B) per mole versus temperature (0.1 T field).

nine Cu^{II} centers, and the small drop towards 30 K suggests the possible presence of a weak antiferromagnetic exchange interaction within the cluster. The rise in μ_B at temperatures below 30 K is associated with a ferromagnetic component, which appears to be dominating the exchange process at low temperatures. Interestingly, for the Cu₄ complexes of poap, which involve orthogonal alkoxide linkages between all copper centers, ferromagnetic coupling dominates the exchange situation.^[5] The analogous complex **3** reveals an almost identical profile of magnetic moment versus temperature, indicating a comparable [3 × 3] grid structure.^[7]

A preliminary fit of the magnetic susceptibility data was carried out to an exchange expression for an assembly of nine *S* = 1/2 centers with a single exchange integral (*J*) between each spin center, according to a Hamiltonian expression ($H = -2J(\sum S_i S_j)$) based on the Cu₉O₁₂ grid structure (Figure 2). Eigenvalues and energy levels were evaluated and substituted into the van Vleck equation. A reasonable data fit was obtained for *g* = 2.23(2), *J* = 3.5(2) cm^{−1}, corrected for 0.5 % paramagnetic impurity, with a Weiss-like temperature correction (*θ*) of −9.6 K (TIP = 800 × 10^{−6} cm³ mol^{−1}). The dominant exchange term is ferromagnetic and comparable with

that for the Cu₄ complexes,^[5] thus supporting the orbital ground state analysis. The requirement of a significant negative Weiss-like (θ) correction indicates the presence of an antiferromagnetic term as well, which is likely to be intramolecular in nature (no viable intermolecular pathways were identified in the structure).

A magnetization study (Figure 4) at 2 K in the range 0–5 T shows that the system approaches saturation at 5.0 T, but appears not to be fully saturated. The magnetization data compare closely with the theoretical line for an $S = 7/2$ system

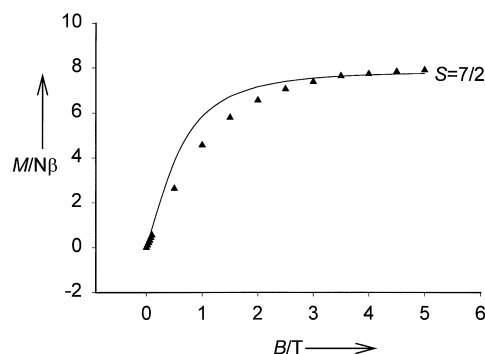


Figure 4. Magnetization data at 2.0 K for **2** in $N\beta$ units (0–5.0 T). ▲ (**2**); theoretical line ($S = 7/2$, $g = 2.23$).

($g = 2.23$), although the lower portion of the profile does not match the theoretical data well. This result confirms the dominant ferromagnetic behavior of **2** at low temperature, but indicates that the magnetic ground state situation within the cluster is complicated, and involves a weak antiferromagnetic component, in agreement with the previous analysis. Significantly, at 30 K a magnetization versus field profile (0–5.0 T) is a straight line, with a constant magnetic moment of $5.5 \mu_B$. This clearly corresponds to a Curie-like situation, but may reflect a small intramolecular antiferromagnetic exchange component. Compound **3** exhibits an almost identical magnetization profile at 2 K. Further magnetic studies on these complicated systems are underway, including single-crystal measurements.

This unique self-assembly of nine pseudo-octahedral copper(II) centers into an oxygen-bridged, square $[3 \times 3]$ grid is, to our knowledge, the first strict self-assembly process to achieve this in copper(II) coordination chemistry. The combined coordination algorithm of the assembly of nine pseudo-octahedral metal centers (fifty-four coordination positions) is exactly matched by the aligned arrangement of the two sets of three “parallel” ligands above and below the metal grid pseudo-plane (fifty-four donor positions). The oxygen-bridging arrangement fixes the metals in an almost completely orthogonal arrangement in the closely spaced grid leading to a complex spin-coupled structure dominated by ferromagnetic exchange. The synthetic strategy has more general application, and antiferromagnetically coupled Mn^{II}^[9] and Ni^{II}^[7] analogues have also been prepared.

The generation of predetermined, large gridlike architectures by self-assembly processes, using preprogrammed ligands, provides a unique opportunity to produce complex nanostructures with the potential for information storage. The

recent report of a hexadecanuclear $[4 \times 4]$ -Pb^{II} pyrimidine-bridged grid complex, based on spectroscopic evidence, indicates the viability of such an approach for larger grids.^[11] Efforts are currently underway in our laboratory to extend ligands like 2poap with additional five-membered chelate ring compartments to produce $[4 \times 4]$ and $[5 \times 5]$ grids, and even higher homologues.

Experimental Section

2-poap: The ligand 2poap was prepared in high yield (85 %) by reaction of the hydrazide of 2,6-pyridine dicarboxylic acid with the methyl ester of imino-picolinic acid, generated in situ from the reaction of 2-cyanopyridine with sodium methoxide in methanol.

2: 2poap (0.40 g, 1.0 mmol) was added to a hot solution of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (1.44 g, 6.00 mmol) in water (15 mL) and the mixture was stirred for 30 min, filtered, and the yellowish solution allowed to stand at room temperature for several days, during which time brown crystals suitable for X-ray structural determination were deposited (Yield 0.65 g, 71 %). Elemental analysis calcd for $[\text{Cu}_9(\text{C}_{19}\text{H}_{16}\text{N}_9\text{O}_2)_6](\text{NO}_3)_{12} \cdot 9\text{H}_2\text{O}$ (%): C 35.21, H 2.96, N 23.76; found (vacuum dried sample): C 35.33, H 3.05, N 23.31 (repeated on X-ray sample; found: C 35.46, H 2.83, N 23.31); IR (Nujol mull, cm^{-1}): $\tilde{\nu} = 3450$ (sh; $\nu_{\text{NH}/\text{H}_2\text{O}}$), 3293 ($\nu_{\text{NH}/\text{H}_2\text{O}}$), 1670 (ν_{CN}); UV/Vis (Nujol mull): 810 nm; $\mu_{\text{RT}} = 6.3 \mu_B$ (mol); $2.1 \mu_B$ (Cu).

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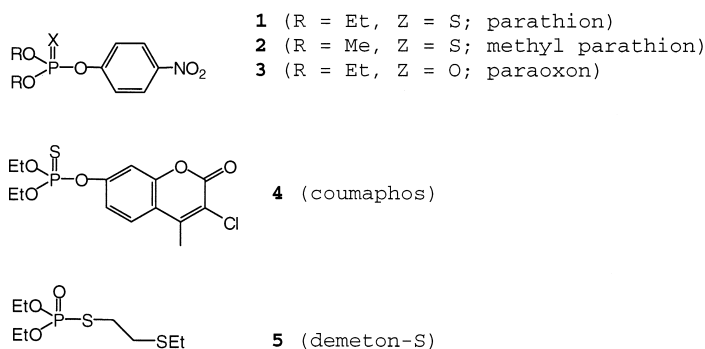
with $I > 2.0\sigma(I)$ (28 848 independent reflections, $R_{int} = 0.0922$), for 2θ in the range 1.56 to 50.0° ($GOF = 0.900$). Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-140825. 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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Highly Efficient Degradation of Thiophosphate Pesticides Catalyzed by Platinum and Palladium Aryl Oxime Metallacycles**

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Sergey D. Varfolomeev, and Alexander D. Ryabov*

Thiophosphoric acid esters such as **1** and **4**, pesticides with an annual world production of hundred thousands of tons, are strong pollutants and their accumulation in the environment is a recognized ecological threat.^[1] Some neurotoxins and V-type chemical warfare agents are chemically similar, and a potential danger is emerging from their aging stockpiles.^[2]

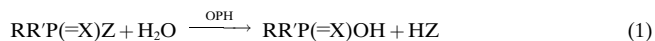


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Needless to say, creation of “green” catalytic processes for degradation of thiophosphoric acid esters is an urgent task of contemporary chemical technology and biotechnology. Significant progress has recently been made by the use of organophosphate hydrolases (OPH), a class of metal-dependent enzymes whose vital virtue is in the detoxification of organophosphoric species by reaction (1).^[3] These enzymes



are superior to their low molecular weight analogues in terms of catalyst specificity and activity, but the latter are often also efficient, more stable, and less subject to inactivation. Chemical catalysts with the features of enzyme active sites are believed to be particularly promising.^[4]

Recently, we introduced orthometalated complexes of Pd^{II} and Pt^{II} as mimetics of metal-dependent esterases. The catalysts display regio- and stereoselectivity as well as reasonable rate accelerations at neutral pH values.^[5] However, the rates of hydrolysis of amino acid esters were not very striking, although the catalysts had features typical of metallohydrolases.^[5b] It could be argued that a square-planar metal configuration, which is not reported in enzymes, is less favorable for catalysis, and that Pd^{II} and Pt^{II} are “soft” acids which require “softer” bases for efficient catalysis. The thiophosphoric acid esters **1–5** are examples of such bases, and therefore metallacycles **6** and **7** (dmsO = dimethyl sulfide, py = pyridine) have been tested as catalysts for the hydrolysis of pesticides **1–5**. Here we show that **6** and **7**^[6] as well as the Pt^{IV} complex **8**^[6] are excellent catalysts for the degradation of thiophosphate pesticides and neurotoxins.

Parathion (**1**) is spontaneously hydrolyzed at pH 8.5 at an immeasurably slow rate. Addition of a catalytic amount of the Pt^{II} complex **6a** enhances the rate of hydrolysis of **1** (by reaction (1)), which proceeds to completion and follows first-order kinetics.^[7] Methyl parathion (**2**) and coumaphos (**4**) behave similarly (Figure 1). No intercept is observed in the

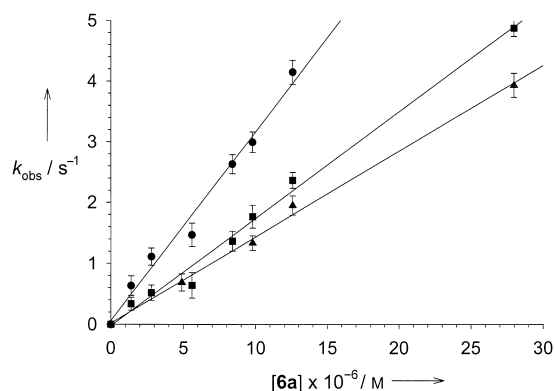
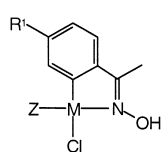


Figure 1. Dependencies of k_{obs} for the hydrolysis of parathion (**1**, ●), methyl parathion (**2**, ■), and coumaphos (**4**, ▲) catalyzed by Pt^{II} complex **6a** at different concentrations. Conditions: [ester] = 1×10^{-4} M, 0.005 M Na₂B₄O₇/NaOH buffer, pH 8.5, 0.01 M NaClO₄, 25 °C.

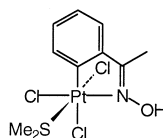
plot of the observed rate constant versus the concentration of **6a**, indicating good catalysis according to the rate law (2).

$$k_{\text{obs}} = k_2[\text{catalyst}] \quad (2)$$

Kinetic data were obtained for all the esters **1–5** in combination with complex **6a**, and for parathion (**1**) with complexes **6–8**. The rate law (2) holds throughout. The values



	M	R ¹	Z
6a	Pt	H	dmsO
6b	Pt	MeO	dmsO
6c	Pt	Me	dmsO
6d	Pt	F	dmsO
6e	Pt	Cl	dmsO
6f	Pt	H	pY
7	Pd	H	pY



8

of k_2 are summarized in Table 1; the rates of the hydroxide-catalyzed hydrolysis of several esters were also measured at 0.001–1.0 M NaOH, and the k_2 values are included for comparison. The catalytic activity of **6** in the hydrolysis of **1**

Table 1. Observed second-order rate constants k_2 [$\text{M}^{-1}\text{s}^{-1}$] for the catalyzed hydrolysis of phosphoric acid triesters **1–5**.^[a]

Catalyst	1	2	Ester 3	4	5
6a	310 ± 17	175 ± 7	10.2 ± 0.1	141 ± 5	27.8 ± 0.3
6b	914 ± 21				
6c	773 ± 29				
6d	429 ± 8				
6e	452 ± 17				
6f	230 ± 8				
7	54 ± 3				
8	10.9 ± 0.4				
OH [−]	$(9.5 \pm 0.6) \times 10^{-5}$	$(2.6 \pm 0.4) \times 10^{-4}$	7.5×10^{-2} ^[b]		

[a] Conditions: pH 8.5, 25 °C, 0.01 M NaClO₄. [b] Reference [3].

is 10^6 – 10^7 times higher than that of hydroxide. Assuming that only the OH[−]-catalyzed pathway is operative in the absence of a metal catalyst at pH 8.0, the metal-catalyzed hydrolysis is 10^9 times more efficient than the spontaneous reaction for a **6b** concentration as low as 10^{-4} M! This is in fact a significant number. In other systems where the hydrolysis of nitrophenyl phosphates catalyzed by biomimetically relevant transition metal catalysts was investigated,^[8] the catalytic effects were usually in the range of 10^4 – 10^6 . However, these estimates should be treated with care, since they often refer to the promoted hydrolysis rather than the catalyzed reaction, and the kinetic data are sometimes not used carefully enough. For example, the estimate of 10^{11} reported by Williams et al. corresponds to the *promoted* cleavage of 4-nitrophenyl methyl phosphate coordinated to a dinuclear Co^{III} complex.^[9]

A comparison of the catalytic activity of complexes **6** and OPH in the hydrolysis of **1** shows that the low molecular weight catalyst is fairly competitive, although OPH is a very reactive enzyme ($k_{\text{cat}} = 600 \text{ s}^{-1}$ and $K_{\text{M}} = 2.8 \times 10^{-4} \text{ M}$).^[3] Recalculation for a gram of catalyst provides $k_2 \approx 1$ and $k_{\text{cat}}/K_{\text{M}} \approx 60 \text{ g}^{-1} \text{ s}^{-1}$, which nicely advertises for the Pt^{II} catalysts taking into account that the rate of the enzymatic reaction levels off at high concentrations of **1**.

The data in Table 1 are indicative of several remarkable features. First, the catalysis of paraoxon (**3**) hydrolysis by **6a** is 30 times less efficient than that of parathion (**1**) hydrolysis. Thus, a soft sulfur donor center is more favorable than the harder oxygen. Interestingly, the base hydrolysis of paraoxon is about 1000 times faster than that of parathion. Second, the square-planar Pt^{II} complexes **6** are 30-fold more catalytically active than the structurally similar octahedral Pt^{IV} species **8**, presumably due to steric effects imposed by a more crowded coordination sphere. Third, the catalytic activity of **6** can be increased threefold by introducing substituents at the benzene ring of the cyclometalated ligand. Remarkably, both electron-donating and electron-withdrawing groups favor the catalysis. Fourth, comparison of the “twin” Pt^{II} and Pd^{II} complexes **6f** and **7** shows that the former is about five times more catalytically active. The hard–soft principle seems to hold here as well.

The hydrolysis of **1** catalyzed by **6a** is pH-dependent in the range 6–10 (Figure 2). The profile suggests two catalytically

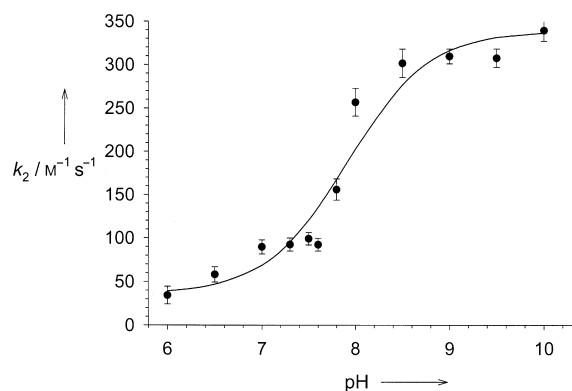
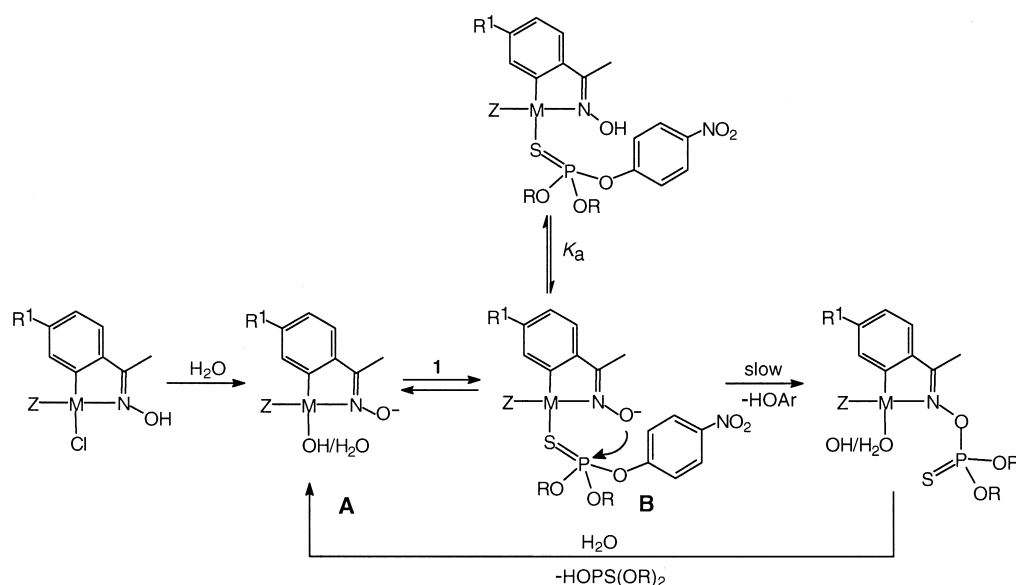


Figure 2. The pH profile for **6a**-catalyzed hydrolysis of parathion (**1**). Conditions: [**1**] = 1×10^{-4} M, 0.01 M NaClO₄, 25 °C. The buffer components were 0.005 M Na₂B₄O₇ (pH 8–10) and 5,5'-diethylbarbituric acid (pH 6–7.8).

active species. Fitting the data to Equation (3) leads to the rate constants $k_{\text{AH}} = 40 \pm 20 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{\text{A}} = 340 \pm 20 \text{ M}^{-1} \text{ s}^{-1}$, and a K_{a} value of $(1.2 \pm 0.5) \times 10^{-8} \text{ M}^{-1}$ ($\text{p}K_{\text{a}} = 7.9$). As in our previous work,^[10] the acid–base equilibrium is ascribed to the deprotonation of the NOH group of the orthometalated oxime, a key nucleophilic center in the reaction.

$$k_2 = \frac{k_{\text{AH}}[\text{H}^+] + k_{\text{A}}K_{\text{a}}}{[\text{H}^+] + K_{\text{a}}} \quad (3)$$

It is well documented by us^[10] and others^[11] that the coordinated halides *trans* to the σ -bound phenyl carbon in **6** or **7** are rapidly and completely hydrolyzed in water. Therefore, the reaction mechanism shown in Scheme 1 can be envisioned. The esters form sulfur-bonded intermediates **B** with aqua/hydroxo species **A**. Subsequently intramolecular nucleophilic attack of the oximate oxygen at P^V takes place.^[12] Thus, Pd^{II} or Pt^{II} play a dual role in the catalysis. The metal centers are binding sites for the substrate in close proximity to the coordinated oxime, the $\text{p}K_{\text{a}}$ of which is substantially lowered



Scheme 1. Plausible reaction mechanism for the hydrolysis of **1–5** catalyzed by complexes **6** and **7**.

due to coordination with the metal. As a result, a strong nucleophile is generated at neutral pH values.

Coordination of the esters and the metals should also cause an increase in the effective positive charge at P^V , and thus facilitate the intramolecular nucleophilic attack by the oximate. The replacement of DMSO or pyridine ligands at Pt^{II} in **6** and **7** is unlikely since no characteristic bands for free pyridine were observed in the UV/Vis spectrum of a mixture of **5** and **7**. The mechanism in Scheme 1 is supported by the UV/Vis and ^{31}P NMR study of the **6a**-promoted hydrolysis of neurotoxin demeton-S (**5**). The ^{31}P NMR resonance for intact **5** is at $\delta = 30.005$ in aqueous $1 \times 10^{-4} M$ $NaClO_4$. Addition of **6a** generates a new signal at $\delta = 0.655$ for the reaction product $(EtO)_2P(=O)OH$.^[13] Although the rate of hydrolysis of **5** increases tremendously in the presence of **6a**, there is no true catalysis and a stoichiometric amount of **6a** is necessary. Cleavage of **5** liberates the potentially good ligand $HSCH_2CH_2SEt$, which on interaction with the Pt^{II} C,N-metallacycle blocks the substrate binding site, making intramolecular nucleophilic attack impossible.

In conclusion, cyclometalated Pt^{II} complexes efficiently catalyze the degradation of thiophosphoric acid pesticides (**1–3**). Comparison with related Pd^{II} and Pt^{IV} complexes indicates that the Pt^{II} catalysts are more active and exhibit an increased selectivity towards sulfur-containing triesters. It has been previously noticed that Pd^{II} complexes could be promising catalysts in reaction (1).^[14] Cycloplatinated compounds with coordinated oxime could be used to create even more specific and reactive catalysts.

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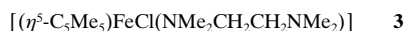
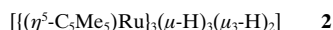
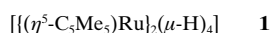
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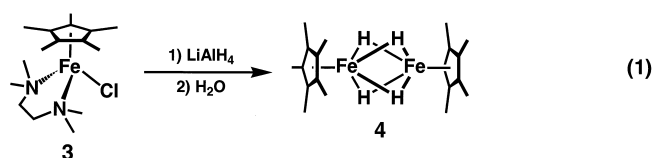
$[(\eta^5\text{-C}_5\text{Me}_5)\text{Fe}]_2(\mu\text{-H})_4$: A Novel Dinuclear Iron Tetrahydrido Complex**

Yasuhiro Ohki and Hiroharu Suzuki*

Transition metal cluster complexes have attracted attention because of their potential ability for cooperative and effective activation of substrates in a multimetallic site.^[1] We have used the term “*multimetallic activation*” to refer to a mode of activation achieved as a result of cooperative action of many metal centers. Our research on multimetallic activation began as an attempt to prepare a cluster with bridging hydrido ligands. As a result, we synthesized a new class of ruthenium polyhydrido clusters, **1** and **2**, and demonstrated many examples of *multimetallic activation* through the reaction with unsaturated hydrocarbons, silanes, and phosphanes as well as alkanes.^[2] According to the vertical trends associated with the transition elements, the metal–metal and metal–ligand bond enthalpies increase down a column and the complexes become more substitutionally inert. The iron analogues of these ruthenium polyhydrido clusters are, therefore, expected to be much more reactive. So far there have been no examples of an iron polyhydrido cluster which has only cyclopentadienyl derivatives ($\eta^5\text{-C}_5\text{R}_5$) as ancillary ligands. Here we report the first synthesis and brief reaction chemistry of the highly reactive and unstable dinuclear iron tetrahydrido complex $[(\eta^5\text{-C}_5\text{Me}_5)\text{Fe}]_2(\mu\text{-H})_4$ (**4**).



Treatment of **3**^[3] with LiAlH_4 followed by work-up with H_2O gave the dinuclear iron tetrahydride **4** [Eq. (1)]. The



tetrahydride **4** was isolated in 41 % yield after purification by column chromatography on neutral alumina and was characterized on the basis of the ^1H and ^{13}C NMR data. The ^1H NMR of complex **4** in C_6D_6 exhibited two resonance signals attributable to the C_5Me_5 group and hydrido ligands at $\delta =$

1.96 and $\delta = -22.12$, respectively. The inversion–recovery T_1 measurement for the hydrido ligands was performed at 400 MHz at various temperatures. The observed T_1 value of 0.63 s at -80°C is deemed to be sufficient to characterize the complex as a classical metal hydride with no bonding interaction between the H atoms.^[4]

The molecular structure of **4** was determined by an X-ray diffraction study (Figure 1).^[5] The two iron atoms are bridged by four hydrido ligands. The long H–H distance (av 1.60 (3) Å) is consistent with the results obtained based on the T_1 value. A noteworthy aspect of the structure is the very short

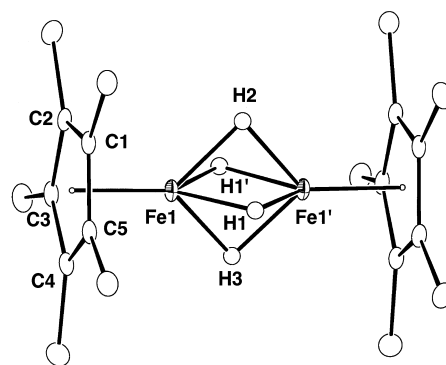
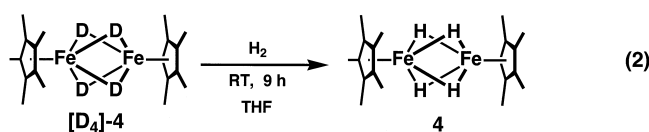


Figure 1. Molecular structure of **4** (thermal ellipsoids at 30 % probability level). Selected bond lengths [Å] and angles [°]: Fe1–Fe1' 2.202(2), Fe1–C1 2.054(2), Fe1–C2 2.058(2), Fe1–C3 2.059(2), Fe1–C4 2.058(2), Fe1–C5 2.056(2), Fe1–H1 1.57(2), Fe1–H2 1.60(2), Fe1–H3 1.56(2), H1–H2 1.60(3), H1–H3 1.60(3); Fe1–H1–Fe1 88(1), Fe1–H2–Fe1 86(1), Fe1–H3–Fe1 89(1).

Fe–Fe distance of 2.202(2) Å, which is shorter than those in any other organometallic iron complexes that have appeared so far in the Cambridge structural database. Since **4** is a 30-electron species, the noble gas rule requires a triple bond between the two iron centers. However, we suggest that there is no metal–metal bond since a theoretical study of dinuclear ruthenium tetrahydride **1** performed by Morokuma and Koga using an ab initio molecular orbital method showed the absence of a metal–metal bond between the two ruthenium centers in this molecule.^[6]

The iron tetrahydride **4** has been shown to undergo intermolecular ligand exchange between hydrides and added dihydrogen. Exposure of $[\text{D}_4]\text{-4}$ to an atmospheric pressure of dihydrogen in $[\text{D}_8]\text{THF}$ at room temperature caused a gradual intensification of the hydrido resonance due to H/D exchange [Eq. (2)]. This must be an associative process because tetrahydride **4** is stable enough in solution not to release dihydrogen even under reduced pressure. The reaction proceeded much faster than the H/D exchange of the corresponding ruthenium analogue,^[2e] and was complete after 9 h.



The iron tetrahydride **4** has proven to be a suitable precursor of highly reactive dinuclear species. Whereas the

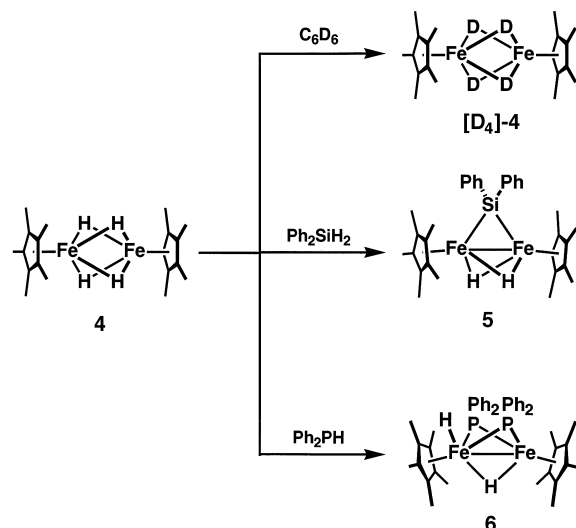
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ruthenium tetrahydride **1** cannot cleave the aromatic C–H bond, **4** smoothly undergoes C–H bond activation of benzene. When the reaction of **4** with C_6D_6 was monitored by ^1H NMR spectroscopy at room temperature, a gradual decrease in the intensity of the hydrido resonance was observed. After 75 h, the hydrido resonance completely disappeared, leaving $[D_4]$ -**4**.

The iron tetrahydride **4** readily reacted with Ph_2SiH_2 in THF below $0^\circ C$ to give a μ -silylene complex **5** by way of Si–H bond activation (Scheme 1). Complex **5** was concluded to be a



Scheme 1.

μ -silylene complex based on the observation of a ^{29}Si resonance signal at $\delta = 293$. The molecular structure of **5** was determined by X-ray diffraction and the results confirm the presence of a diiron framework bridged by the μ -silylene ligand (Figure 2).^[7] The Fe–Si bond lengths of 2.2582(2) and 2.2549(6) Å are comparable to those for the σ bond observed in silyl and silylene complexes of iron.^[8] The Fe–Si–Fe angle is fairly acute ($58.91(2)^\circ$) compared with that observed in $[(\eta^5-C_5Me_5)Ru]_2(\mu-H)_2(\mu-SiPh_2)_2$ ($68.70(3)^\circ$)^[2d] due to the short distance between the iron centers (2.2193(5) Å).

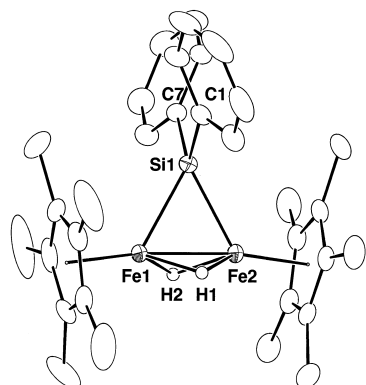


Figure 2. Molecular structure of **5** (thermal ellipsoids at 30% probability level). Selected bond lengths [Å] and angles $^\circ$: Fe1–Fe2 2.2193(5), Fe1–Si1 2.2582(8), Fe2–Si1 2.2549(8), Fe1–H1 1.66(3), Fe1–H2 1.62(3), Fe2–H1 1.66(3), Fe2–H2 1.63(3), Si1–C1 1.884(3), Si1–C7 1.883(3); Fe1–Si1–Fe2 $58.91(2)$, Fe2–Fe1–Si1 $60.47(2)$, Fe1–Fe2–Si1 $60.62(2)$, Fe1–Si1–C1 $118.86(9)$, Fe1–Si1–C7 $123.51(9)$, Fe2–Si1–C1 $123.95(10)$, Fe2–Si1–C7 $117.67(9)$.

Complex **4** also reacted with PPh_2H below $0^\circ C$ to yield the bis- μ -phosphido complex **6** (Scheme 1). The molecular structure of **6** was confirmed by an X-ray diffraction study (Figure 3).^[9] One of the two hydrido ligands was located at the terminal position and the other bridged two iron atoms. This was consistent with the observation that the resonance signals for the hydrido ligands of **6** were inequivalent in the ^1H NMR spectrum. The four Fe–P distances are almost identical and are within the range of those reported for dinuclear iron μ - PPh_2 complexes.^[10]

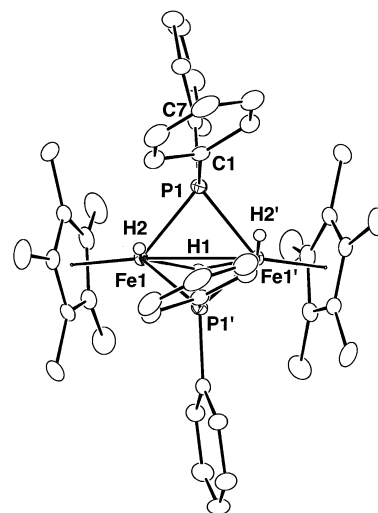
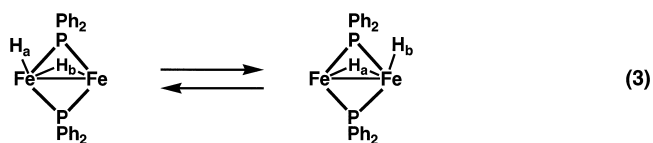


Figure 3. Molecular structure of **6** (thermal ellipsoids at 30% probability level). Selected bond lengths [Å] and angles $^\circ$: Fe1–Fe1' 2.6697(6), Fe1–P1 2.1935(6), Fe1'–P1 2.1989(6), Fe1–H1 1.67(2), Fe1–H2 1.40(6), P1–C1 1.838(2), P1–C7 1.843(2); Fe1–P1–Fe1' $74.86(2)$, Fe1–Fe1'–P1 $52.66(2)$, Fe1'–Fe1–P1 $52.48(2)$, Fe1–P1–C1 $122.03(7)$, Fe1–P1–C7 $117.84(7)$, Fe1'–P1–C1 $121.21(6)$, Fe1'–P1–C7 $121.46(7)$.

The two hydrido ligands in **6** exchange coordination sites with each other [Eq. (3)]; this process was monitored by



variable-temperature ^1H NMR spectroscopy. Two resonance signals attributed to magnetically inequivalent C_5Me_5 groups coalesced at $15^\circ C$. The hydrido resonance signals which appeared at $\delta = -9.72$ and -16.40 at $-50^\circ C$ broadened at room temperature.^[11]

We have prepared a novel dinuclear iron tetrahydrido complex that is more reactive than the ruthenium analogue and effectively activates C–H, Si–H, and P–H bonds under mild conditions. This type of carbonyl-free low-valent cluster is still rare, and the reactivity study on complex **4** provides valuable insight for organometallic chemistry.

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- [11] The free activation energy for this dynamic process was estimated as $\Delta G^{\ddagger}$ (15°C) = 13.5 kcal mol $^{-1}$. This value is comparable to those for site exchange of hydrides in the diruthenium bis- μ -silylene complex $[(\eta^5\text{-C}_5\text{Me}_5)_2\text{Ru}(\mu\text{-H})_2][\mu\text{-SiPh}(\text{OH})](\mu\text{-SiPh}_2)]$ ($\Delta G^{\ddagger}$ (0°C) = 12.6 kcal mol $^{-1}$). T. Takao, S. Yoshida, H. Suzuki, unpublished results.

Asymmetric Alkylation of Nitroalkanes**

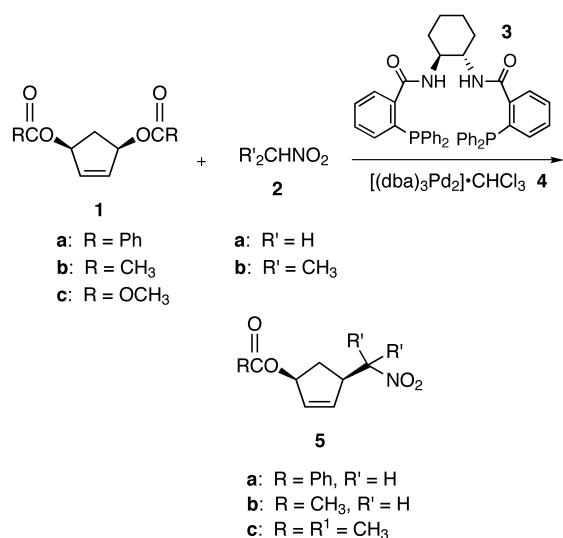
Barry M. Trost* and Jean-Philippe Surivet

The utility of nitro compounds as synthetic intermediates stems from the versatility of the reactivity of the nitro group.^[1] One feature arises from the ease of formation of nitronate anions; however, their low reactivity generally limits the reactions they undergo to carbonyl and conjugate addition.^[2] Alkylations do not normally proceed well. On the other hand, Pd-catalyzed allylic alkylations have had some success.^[3] This success stimulates the search for an asymmetric allylic alkylation (AAA) which has had good results in only one case (the 1,3-diphenylallyl system) and when nitromethane was used as solvent.^[4] We here report that the Pd-catalyzed AAA reaction^[5] of nitroalkanes with cyclic allyl esters can proceed in high yields and enantioselectivities and provide a short asymmetric synthesis of a carbanucleoside.

Our initial studies focused on desymmetrization of *meso* diesters [Eq. (1)].^[6] Our earlier results suggested the diben-

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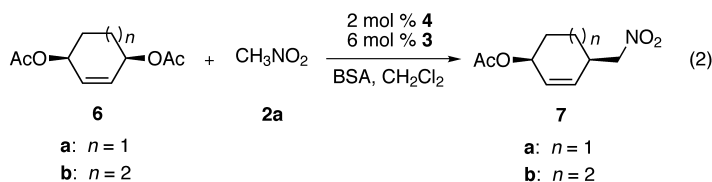
[**] We thank the National Science Foundation and the National Institutes of Health (NIH), General Medical Sciences, for their generous support of our programs. Rhône-Poulenc graciously provided a postdoctoral fellowship for J.-P. S. Mass spectra were provided by the Mass Spectrometry Facility of the University of California, San Francisco, supported by the NIH Division of Research Resources.



zoate **1a** would be superior for enantiodiscrimination. Surprisingly, using nitromethane (**2a**, 5 equiv) with the *S,S*-ligand **3** and a Pd⁰ complex **4** in DMSO with cesium carbonate as base led to no reaction. Similar results were obtained with the diacetate **1b** and dicarbonate **1c**. Switching the solvent to methylene chloride (but not THF nor acetonitrile) with the diacetate **1b** led to some reaction to produce **5b**^[7] (18 % yield) but, gratifyingly, with excellent enantioselectivity (99 %). Remarkably, changing the base from cesium carbonate to BSA (BSA = *O,N*-bis-trimethylsilylacetamide) in methylene chloride now led to smooth reaction producing **5b** in 75 % yield while maintaining high *ee* (99 %).^[8] Identical results were obtained with the dibenzoate **1a** to give **5a**^[7] however, the dicarbonate led to very low conversions.

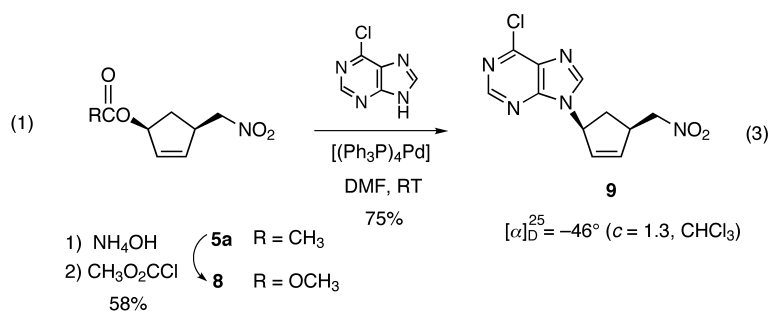
The complete reverse behavior was observed with 2-nitropropane (**2b**). Neither the dibenzoate **1a** nor the diacetate **1b** reacted to any appreciable extent in methylene chloride. On the other hand, the use of cesium carbonate in DMSO with the diacetate **1b** gave a 92 % yield of **5c**^[7] having 95 % *ee*.

The same trend was observed for nitromethane with the larger ring *meso* diesters **6a,b**. Using the optimal conditions established for **5b** [Eq. (2)], the corresponding monoalkylated products **7a**^[7] and **7b**^[7] were obtained in 82–84 % yield with near perfect enantioselectivity (99 % *ee*).

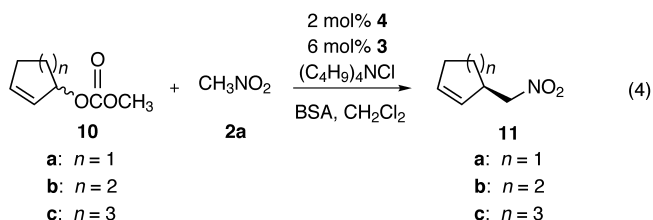


The absolute configuration is assigned by analogy.^[5, 6] Further support derives from conversion of **5b** to a known carbanucleoside intermediate **9** [Eq. (3)].^[3g] A (+) rotation is reported for the enantiomer corresponding to the “normal” enantiomeric series of the carbanucleosides, thus the (–) rotation observed for our synthetic sample indicates the absolute configuration as depicted. Since the nitromethyl side

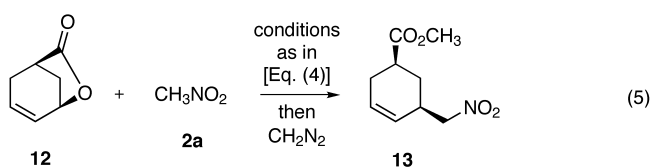
chain has been converted to a hydroxymethyl side chain in one step,^[9] this constitutes a rapid asymmetric synthesis of such carbanucleosides.



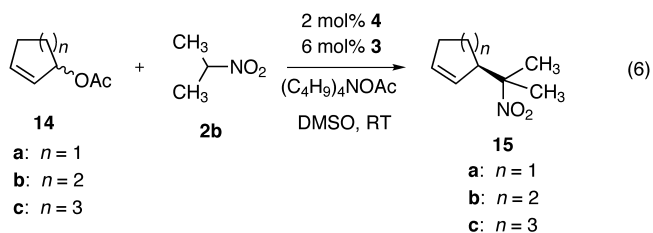
The success of the desymmetrization led to our investigation of the cycloalkenyl substrates [Eq. (4)].^[10] For the alkylations with nitromethane, the carbonates **10** proved



superior to the corresponding acetates. The conditions that proved successful in the desymmetrization with nitromethane also proved to be successful here. For the cyclopentenyl system **10a**, the alkylated product **11a**^[7] was obtained in 94 % yield (97 % *ee*). Equally satisfactory results were obtained for the six- (99 % yield, 99 % *ee* for **11b**) and seven-membered ring systems (94 % yield, 95 % *ee* for **11c**^[7]). The lactone **12** also followed the same pattern to provide, after esterification, the nitroester **13**^[7] in 74 % yield [99 % *ee*; Eq. (5)].

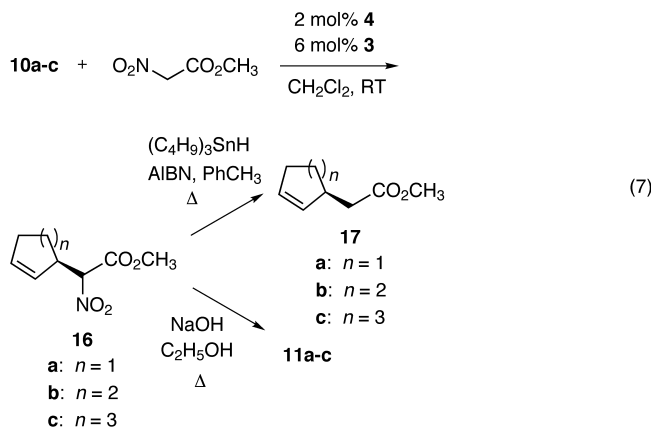


Use of 2-nitropropane allowed use of the allyl acetates as substrates [Eq. (6)]. The reactions performed in DMSO at room temperature with tetra-*n*-butylammonium acetate (for **14b**) or cesium carbonate (for **14a** and **14c**) as base gave



excellent results (**15a**^[7], 74 % yield, 97 % *ee*; **15b**^[7], 79 % yield, 94 % *ee*; **15c**^[7], 50 % yield, 96 % *ee*).

The absolute configuration was established by correlation as depicted in Equation (7). Alkylation of the allyl carbonates **10a–c** using methyl nitroacetate proceeds with no added base to give the desired products (**16a**^[7], 80 %; **16b**^[7], 93 %; **16c**^[7], 87 %). Radical denitration^[11] gave the known cycloalkenyl



acetates **17a–c**^[10] thereby establishing the absolute configuration of **16a–c**. Alternatively, hydrolysis with concomitant decarboxylation gave the nitroalkenes (**11a**, 64 % yield, 85 % *ee*; **11b**, 65 % yield, 95 % *ee*; **11c**, 72 % yield, >99 % *ee*) thereby establishing their absolute configurations as depicted.

The utility of nitroalkanes as building blocks makes a significant step forward as a result of the ability to effect AAA reactions using cyclic allyl esters. It is clear that the nitroalkane significantly influences the catalyst. The significantly different reactivity between nitromethane and 2-nitropropane highlight this fact. A possible explanation suggests that the nitronate derived from nitromethane may serve as a competitive ligand to palladium. The lack of polyalkylation of nitromethane is noteworthy especially considering that the higher nitroalkanes are better nucleophiles and the reported significance of this problem in another system.^[4] The current method provides a practical approach to these chiral nitroalkanes that enhances their utility as useful building blocks.

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Enantiomeric Self-Recognition: Cation-Templated Formation of Homochiral Isoguanosine Pentamers**

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Stereochemical information embedded within the building blocks of biopolymers is often translated into higher organization. Both the protein α -helix and the DNA duplex, structures that require homochiral chains, rely on such a hierarchy.^[1] To illustrate the impact of stereochemistry in controlling the structure of noncovalent aggregates, we describe the enantiomeric self-recognition of the racemic 5'-*tert*-butyldimethylsilyl-2'-3'-di-*O*-isopropylidene-substituted isoguanosine, isoG **1**, to give homochiral, hydrogen-bonded pentamers.

Self-recognition is a process whereby a compound selectively associates with its own kind. Self-recognition relies on: 1) reversible processes^[2] and 2) a subunit's "pre-disposition"^[3] towards self-assembly. Stereochemistry can be crucial for self-recognition. Enantiomeric self-recognition in supramolecular systems has been achieved using metal ion coordination^[4,5] or hydrogen bonds.^[6–8]

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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.

Our interest in self-recognition arose from studying isoG **1** and the guanosine derivative **G 2** (see the Supporting Information and Figure 1).^[9, 10] These nucleosides self-associate in the presence of cations,^[11] with (D)-**G 2** forming

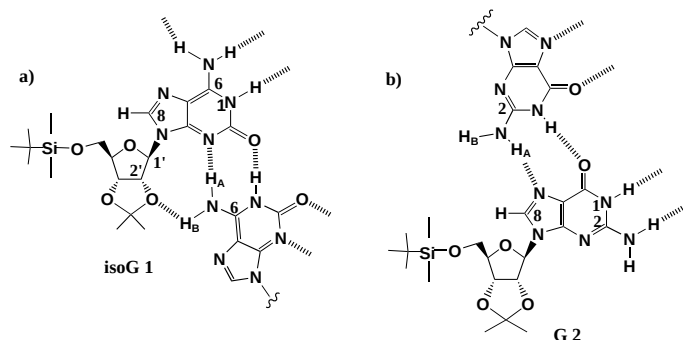


Figure 1. Hydrogen-bond geometry in a) an isoG **1**–isoG **1** basepair within an isoG pentamer and b) a **G 2**–**G 2** basepair within a G-quartet. The ribose O2' atom in the isoG–isoG basepair hydrogen bonds with the neighboring amino proton N6-H_B. A G–G pair does not show such a sugar–base interaction.

G-quartets and (D)-isoG **1** giving pentamers.^[12, 13] Crystal structure analyses^[9, 10] showed that hydrogen-bonded macrocycles sandwich the cation diastereospecifically: **G 2** forms the head-to-tail octamer (**G 2**)₈·K⁺, and isoG **1** forms the tail-to-tail decamer (isoG **1**)₁₀·Cs⁺.^[14] Nucleobase–nucleobase hydrogen bonds and cation–dipole interactions stabilize these structures. The [(D)-isoG **1**]₁₀·Cs⁺ decamer also has hydrogen bonds that link the 2'-oxygen atom of each sugar with its neighboring amine group (Figure 1).^[10] As a result of this sugar–base hydrogen bond, each monomer within an isoG pentamer interacts directly with its neighbor's chiral sugar. In contrast, sugar–base hydrogen bonds do not occur in the G-quartet. Since the isoG O2'–NH6 sugar–base hydrogen bonds are only possible in a homochiral basepair, we considered isoG **1** to be an excellent candidate for enantiomeric self-recognition. Our question was simple: will racemic (D,L)-isoG **1** form homochiral pentamers in the presence of an achiral template? Herein, we report that it does.

To better appreciate the chiral sensing properties of isoG **1**, we first describe a guanosine system that does not display enantiomeric self-recognition. (D)-**G 2** coordinates K⁺ in the solid state and in solution to give a diastereomerically pure octamer, with G-quartets stacked in a head-to-tail arrangement.^[15] Figures 2a and 2b show the isochronous ¹H NMR spectra for [(D)-**G 2**]₈·K⁺ and [(L)-**G 2**]₈·K⁺.^[16] The enantiomeric (**G 2**)₈·K⁺ octamers both have two sets of resonances, one set for each unique G-quartet within the octamer. When equivalent amounts of the enantiomers were mixed in CD₃CN the resulting spectrum showed more than ten separate signals for each proton, which indicated a diastereomeric mixture (Figure 2c). Clearly, **G 2** does not undergo enantiomeric self-recognition to give homochiral G-quartets, at least with a K⁺ ion as the template. However, as described below, the isomeric isoG **1** is pre-disposed for self-recognition.

Preparation of (D)-isoG **1** has been described,^[13a] and Scheme 1 outlines the synthesis of (L)-isoG **1**. The O6-

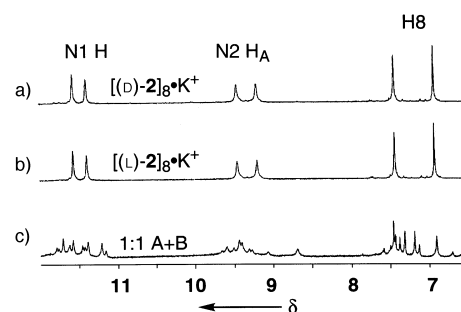
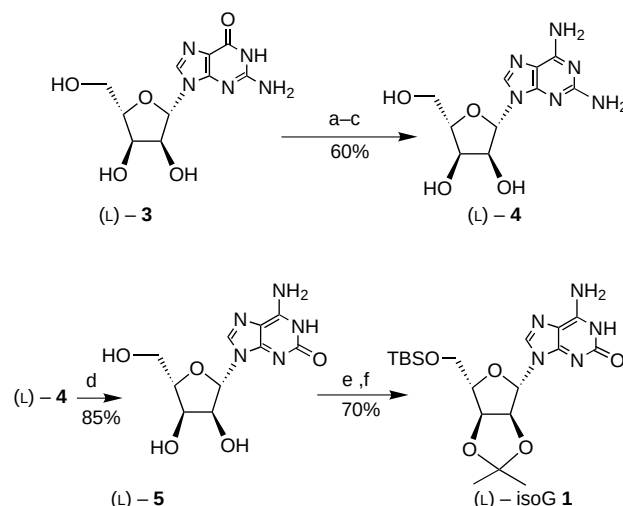


Figure 2. A region of the ¹H NMR spectra in CD₃CN at –40 °C for a) [(D)-**G 2**]₈·K⁺PF₆[–]; b) [(L)-**G 2**]₈·K⁺PF₆[–]; and c) a 1:1 mixture of [(D)-**G 2**]₈·K⁺PF₆[–] and [(L)-**G 2**]₈·K⁺PF₆[–] (the spectrum was identical 10 min and 24 h after mixing).



Scheme 1. a) Trifluoroacetic acid anhydride, pyridine; b) pentafluorophenol, pyridine; c) NH₃/H₂O, 55 °C; d) NaNO₂, HOAc, 50 °C; e) 2,2-dimethoxypropane, *para*-toluene-4-sulfonic acid; f) *tert*-butyldimethylsilyl chloride, imidazole.

activation of (L)-**G 3**,^[16] followed by aminolysis gave (L)-2,6-diaminopurine **4**.^[17] Diazotization of (L)-**4**,^[18] followed by modification of the hydroxyl groups of (L)-**5** gave the enantiomeric (L)-isoG **1**. This nucleoside and CsPh₄B crystallized from CH₃CN to give [(L)-**1**]₁₀·Cs⁺Ph₄B[–]. Optical rotations and CD spectra for the all-L decamer and the all-D decamer were of opposite sign and the same magnitude.^[19]

A ¹H NMR experiment provided the first indication for the stereoselective self-recognition of racemic isoG **1**. Figures 3a and 3b show the ¹H NMR spectra for the all-D and all-L isoG decamers in CD₃CN. Mixing equimolar solutions of these enantiomers gave a simple spectrum, with two species present in a 4:1 ratio (Figure 3c). The minor signals were identical to those observed for the all-L and all-D decamers. The major set of signals, which were different from those for the enantiomeric decamers, indicated that a new diastereomer predominates when (D,L)-isoG **1** binds a Cs⁺ ion under thermodynamic conditions.

An X-ray crystal structure analysis confirmed that (D,L)-isoG **1** undergoes enantiomeric self-recognition.^[20] The *meso* decamer [(D)-isoG **1**]₅·Cs⁺·[(L)-isoG **1**]₅Ph₄B[–] has one pentamer composed of only (D)-isoG **1** and the other

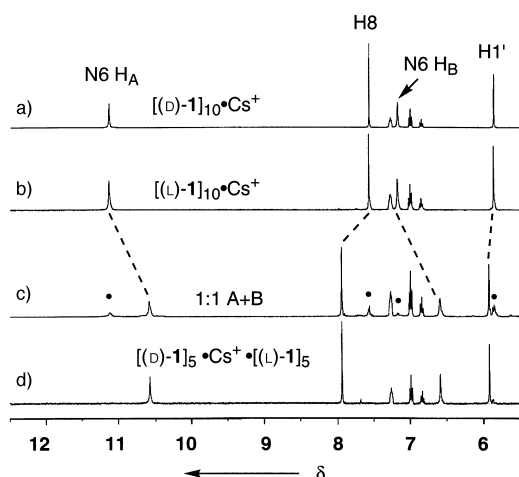


Figure 3. A region of the ^1H NMR spectra for a) $[(\text{D})\text{-isoG } \mathbf{1}]_{10} \cdot \text{Cs}^+ \text{Ph}_4\text{B}^-$; b) $[(\text{L})\text{-isoG } \mathbf{1}]_{10} \cdot \text{Cs}^+ \text{Ph}_4\text{B}^-$; c) a 1:1 mixture of $[(\text{D})\text{-isoG } \mathbf{1}]_{10} \cdot \text{Cs}^+ \text{Ph}_4\text{B}^-$ and $[(\text{D})\text{-isoG } \mathbf{1}]_{10} \cdot \text{Cs}^+ \text{Ph}_4\text{B}^-$ (after 24 h); d) *meso* decamer, $[(\text{D})\text{-isoG } \mathbf{1}]_5 \cdot \text{Cs}^+ \cdot [(\text{L})\text{-isoG } \mathbf{1}]_5 \text{Ph}_4\text{B}^-$ (immediately after dissolving crystals). Spectra were recorded in CD_3CN at 25°C after dissolving crystallized complexes.

pentamer made up of (L)-isoG **1** (Figure 4). Both homochiral pentamers have the key $\text{O}2' \cdots \text{NH}6$ sugar–base hydrogen bonds between neighbors (mean $d_{\text{N}6\text{HB} \cdots \text{O}2'} = 2.83 \text{ \AA}$, mean $\theta_{\text{N}6\text{HB} \cdots \text{O}2'} = 136^\circ$). Apparently, the five sugar–base hydrogen bonds within an (isoG **1**)₅ pentamer help overcome the entropic demands associated with enantiomeric self-sorting.

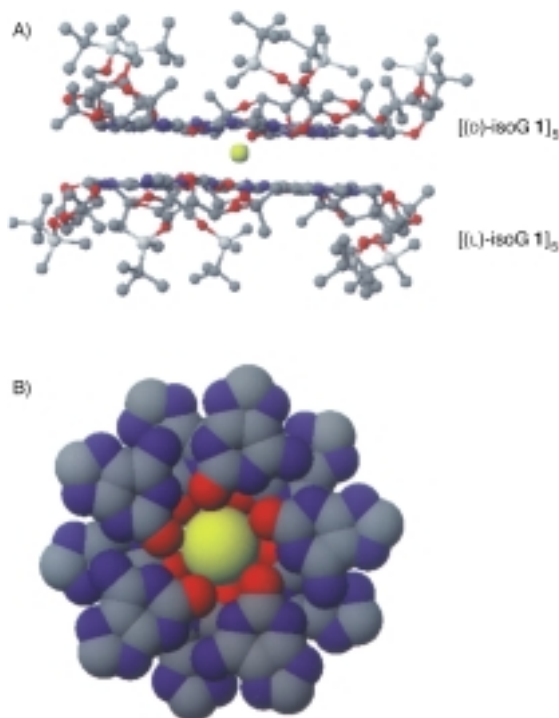


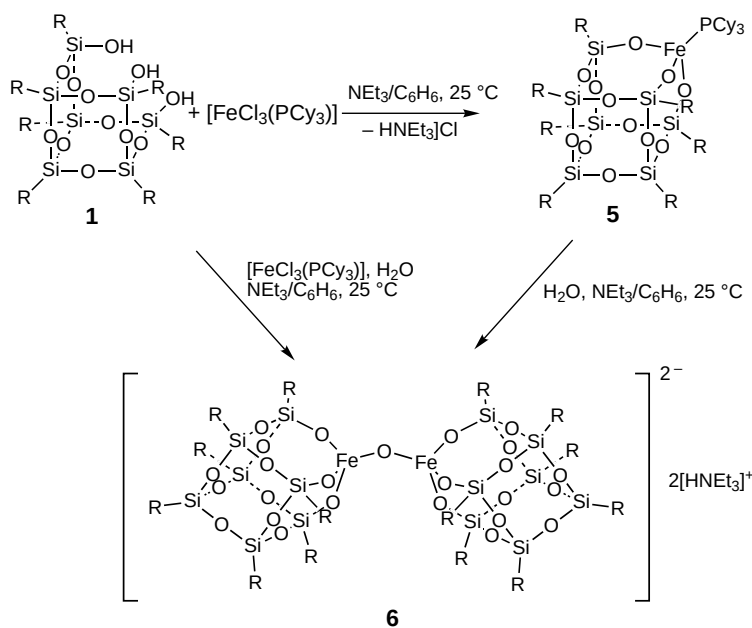
Figure 4. A) A side view of the X-ray crystal structure of the *meso* decamer $[(\text{D})\text{-isoG } \mathbf{1}]_5 \cdot \text{Cs}^+ \cdot [(\text{L})\text{-isoG } \mathbf{1}]_5 \text{Ph}_4\text{B}^-$. The Ph_4B^- ion, located above $[(\text{D})\text{-}\mathbf{1}]_5$, is removed for clarity. This view shows the stacking of the two homochiral isoG pentamers ($d = 3.26 \text{ \AA}$ between planes) around the Cs^+ ion. B) A top view with the sugar molecules and H atoms removed for clarity; Cs^+ : yellow; oxygen: red; nitrogen: blue; and carbon: gray. The encapsulated Cs^+ ion is bound to 10 oxygen atoms, with a mean $d_{\text{Cs} \cdots \text{O}} = 3.41 \text{ \AA}$.

The decacoordinate Cs^+ ion ($d_{\text{Cs} \cdots \text{O}} = 3.29\text{--}3.67 \text{ \AA}$) is sandwiched by the two homochiral pentamers. As expected, dissolving crystals of the *meso* decamer $[(\text{D})\text{-isoG } \mathbf{1}]_5 \cdot \text{Cs}^+ \cdot [(\text{L})\text{-isoG } \mathbf{1}]_5 \text{Ph}_4\text{B}^-$ in CD_3CN gave ^1H NMR chemical shifts identical to those for the major diastereomer observed in the mixing experiment (Figure 3d). The system achieved thermodynamic equilibrium within 30 minutes in CD_3CN to give a spectrum similar to that in Figure 3c.

IsoG **1**, upon coordinating a Cs^+ ion, uses sugar–base hydrogen bonds to transmit stereochemical information from one nucleoside to its neighbor. The result is a homochiral pentamer (isoG **1**)₅. Decamer formation, achieved by stacking two homochiral pentamers around a Cs^+ ion, is also diastereoselective; a *meso* isomer exists in the solid state and predominates in solution. Importantly, it is the achiral Cs^+ ion that enables enantiomeric self-recognition of isoG **1**. Experiments with other alkali cations, Li^+ , Na^+ , K^+ , or Rb^+ , have not provided convincing evidence for enantiomeric self-recognition. In a broader context, this work illustrates that the appropriate ligand and template (isoG **1** and Cs^+) may provide homochiral aggregates, while related ligand and template combinations (G **2** and K^+) yield diastereomeric libraries.^[23] In addition, nucleosides such as isoG **1** may be ideal models for exploring the origins of biomolecular homochirality.^[24]

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- [14] The pentamer's diastereotopic faces are defined such that the “head” has a clockwise rotation of the $\text{N-H} \cdots \text{O}=\text{C}$ hydrogen bonds, while the “tail” has a counter-clockwise rotation of the $\text{N-H} \cdots \text{O}=\text{C}$ hydrogen bonds.



Scheme 2. Synthesis of compounds **5** and **6**, R = *c*-C₅H₉.

The IR spectra of these complexes indicate strong C–H stretching and bending vibrations displayed at 2950, 2870, and 1450 cm^{−1} for the CH₂ groups in the cyclopentane rings as well as a strong Si–O stretching band centered at 1120 cm^{−1}. The features in the region 1000–800 cm^{−1} correspond to the Si–C and Si–O stretching, and the C–C–H bending vibrations, which are similar to those observed in a tin–silasesquioxane complex^[8a] and in zeolites.^[8b] An O–H stretching vibration is observed at 3150 cm^{−1} in the IR spectrum of **3**. The structures of **3–5** were determined by single-crystal X-ray diffraction. Similar bond lengths and angles were observed in the crystal structures of **3** and **4** (Figure 1).^[9a, 9b] The iron(II) centers in the complexes have a distorted tetrahedral coordination geometry similar to that found in the [FeCl₂(dcppe)] precursor. The Si–OH group in **3** is clearly bent towards one bridging oxo oxygen atom (O(1)) and forms an intramolecular hydrogen bond. The H(4)⋯O(1) (1.947 Å), O(1)–O(4) (2.743 Å) distances, and the O(4)–H(4)⋯O(1) (172.7°) angle are very similar to those observed for hydrogen bonds in alcohols.^[10] Further evidence for hydrogen bonding comes from the IR spectrum (see above). A similar intramolecular hydrogen bond has been reported by Duchateau et al. in an aluminum–silasesquioxane complex.^[11] The Fe(1)⋯O(4)(H) distance in **3** is 3.66 Å and the Fe(1)⋯H(4)(O) distance was determined to be around 3 Å (based on the idealized hydrogen atom position). The Fe(1)⋯O(SiMe₃) distance in **4** (6.39 Å) is much longer than the Fe(1)⋯O(4)(H) distance in **3**. This longer distance is probably caused by steric interactions between the bulky OSiMe₃ group and the Fe^{II} center. Recently Abbenhuis and co-workers reported the synthesis of Pt analogues of **3** and **4** by the reaction of [PtCl₂(dppe)] (dppe = bis(diphenylphosphanyl)ethane) with **1** and **2**.^[12] The single-crystal X-ray structure shows that [(*c*-C₅H₉)₇Si₇O₁₁–(OSiMe₃)]Pt(dppe)] has a similar structure to that of **4** except that the coordination geometry around the platinum center is distorted square planar.

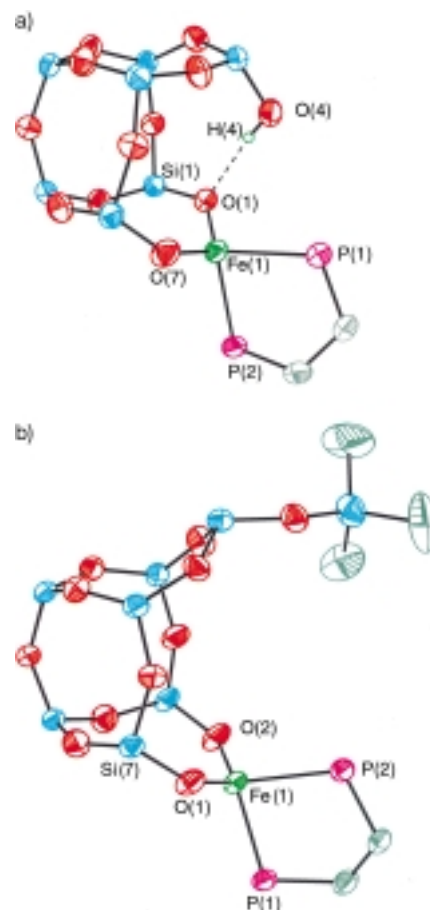


Figure 1. Molecular structures of **3** and **4**. a) **3**·0.5C₆H₆, selected bond lengths [Å] and angles [°]: Fe(1)–O(1) 1.907(3), Fe(1)–O(7) 1.869(3), Fe(1)–P(1) 2.486(1), Fe(1)–P(2) 2.469(1); O(1)–Fe(1)–O(7) 121.2(1), P(1)–Fe(1)–P(2) 83.74(4), Fe(1)–O(1)–Si(1) 130.6(2). b) **4**·1.5C₆H₆, selected bond lengths [Å] and angles [°]: Fe(1)–O(1) 1.873(3), Fe(1)–O(2) 1.866(2), Fe(1)–P(1) 2.460(1), Fe(1)–P(2) 2.434(1); O(1)–Fe(1)–O(2) 122.3(1), P(1)–Fe(1)–P(2) 83.48(3), Fe(1)–O(1)–Si(7) 137.48(15). The cycloalkyl groups, benzene molecules, and hydrogen atoms have been omitted for clarity. Si: turquoise, oxygen: red, iron: green, carbon: gray, and phosphorus: magenta.

The X-ray crystallographic characterization of **5** (Figure 2) indicates that the Fe^{III} center possesses a tetrahedral geometry which comprises of the three oxygen atoms from the silasesquioxane molecule and the phosphorus atom.^[9c] The polyhedron defined by the Fe and Si atoms is distorted, presumably because of the larger ionic radius of Fe^{III} ions (0.64 Å) compared to Si^{IV} ions (0.41 Å).^[13] The Fe–P bond (2.51(1) Å) is 0.28 Å longer than the sum of the Fe and P covalent radii (2.23 Å). This long Fe–P bond can be attributed to the low affinity of the soft phosphane ligand toward the hard metal center.

Compound **5** exhibits some interesting reaction chemistry with water (Scheme 2). The treatment of solutions of **5** in benzene with a slight stoichiometric excess of water in the presence of NEt₃ leads to the formation of the μ-oxo-bridged dianion **6**. Compound **6** could also be prepared by the reaction of **1** with [FeCl₃(PCy₃)] in the presence of H₂O and NEt₃. The single-crystal X-ray analysis of **6** (Figure 2) revealed that the bridging oxo ligand lies on a twofold rotation axis.^[9d] The distances and angles in the μ-Fe–O–Fe core are typical of

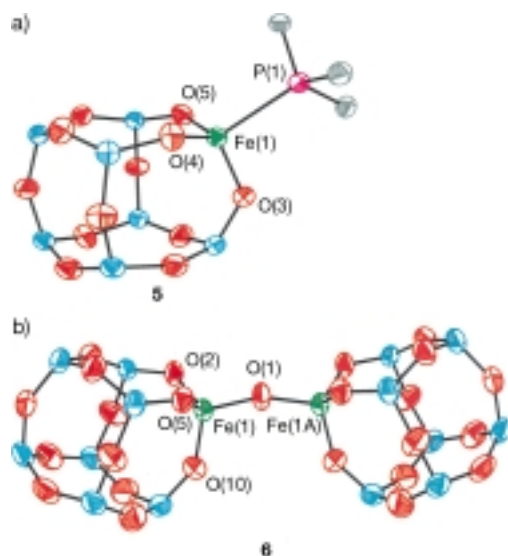


Figure 2. Molecular structures of **5** and **6**. a) **5**: C_6H_6 , selected bond lengths [Å] and angles [°]: Fe(1)–P(1) 2.5070(11), Fe(1)–O(3) 1.829(3), Fe(1)–O(4) 1.818(3), Fe(1)–O(5) 1.824(2); P(1)–Fe(1)–O(3) 105.25(8), O(3)–Fe(1)–O(4) 110.74(12). Cycloalkyl groups on Si and the benzene molecule have been omitted for clarity. Only the three *ipso*-carbon atoms of the cyclohexyl groups of the phosphane are shown. Si: turquoise, oxygen: red, iron: green, phosphorus: magenta, carbon: gray. b) anion of **6**, selected distances [Å] and angles [°]: Fe(1)–O(1) 1.790(2), Fe(1)–O(2) 1.873(5), Fe(1)–O(5) 1.882(5), Fe(1)–O(10) 1.870(5), Fe(1)–Fe(1A) 3.502(3); O(2)–Fe(1)–O(5) 109.0(2), O(1)–Fe(1)–O(2) 107.8(3), O(1)–Fe(1)–O(5) 108.3(3), O(1)–Fe(1)–O(10) 110.8(3), Fe(1)–O(1)–Fe(1A) 157.0(5). The cations, solvent molecules and cycloalkyl groups have been omitted for clarity. Si: turquoise, oxygen: red, and iron: green.

complexes with singly bridged μ -Fe–O–Fe cores.^[14] The UV/Vis spectra of **6** exhibit strong absorptions between 219 and 245 nm, similar to those found by Solomon and co-workers in their extensive studies of the Fe–O–Fe core of *met*-hemerythrin model complexes.^[15] Feher et al. have reported oxo-bridged Al– μ -O–Al and Si– μ -O–Al silasesquioxane compounds in which the X-ray analysis indicated Al/Si/O connectivity and the μ -oxo structure.^[16] Literature examples of μ -oxo-bridged metal–silasesquioxane complexes are rare, and the μ -oxo-bridged iron compound **6** is one of the few examples of such a complex.

Preliminary attempts to use complexes **3**–**6** as simple models for iron–silicate oxidation catalysts were unsuccessful. The catalytic activities of complexes **3**–**6** towards direct benzene oxidation have been tested at 30, 60, and 100 °C using N_2O (1.03 MPa) as the oxidant. No phenol or other products were produced under these conditions. The complexes decomposed overnight at 100 °C under N_2O (1.03 MPa). Whereas the homogeneous titanium–silicate systems that are excellent models of the titanium–zeolite catalysts,^[4] our results indicate that the soluble framework iron silicates do not model the catalytic chemistry of the iron–zeolite systems. This implies that the local structure of the catalytically active Fe center in these zeolite systems is different from that found in the framework iron–silasesquioxane compounds.

In conclusion, we have employed simple iron–phosphane precursors to prepare the first examples of framework iron–silasesquioxane complexes. Hydrolysis of the Fe^{III} compound yields a dimeric μ -oxo-bridged Fe^{III} compound. The Fe–

POSS complexes which should model framework metal–zeolite centers are not good models for the extra-framework centers implicated in a number of heterogeneous catalysts. We are continuing to investigate the reactivity of additional iron silicates to model extra-framework metal ion sites.

Experimental Section

$[\text{FeCl}_2(\text{dcpe})]$,^[17] $[\text{FeCl}_3(\text{PCy}_3)]$ ^[18] and **2**^[3a] were prepared by published methods. Compound **1** was purchased from Aldrich and used as received. NEt_3 and CH_3CN were purified by distillation over Na/K and CaH_2 , respectively.

3: A solution of $[\text{FeCl}_2(\text{dcpe})]$ (0.104 g, 0.19 mmol) in C_6H_6 (5 mL) was added to a solution of **1** (0.19 mmol) in $\text{C}_6\text{H}_6/\text{NEt}_3$ (10 mL; 4/1 (v/v)) and the resulting solution was stirred for 12 h at 25 °C. After filtration the solvent was removed in vacuo to give a virtually quantitative yield of crude **3** as an amorphous white foam. Recrystallization from benzene/acetonitrile afforded **3** (0.23 g, 91 % yield); elemental analysis calcd for **3**, $\text{C}_{61}\text{H}_{112}\text{O}_{12}\text{P}_2\text{Si}_2\text{Fe}$: C 54.22, H 8.30, P 4.59, Fe 4.15; found: C 54.19, H 8.09, P 3.71, Fe 3.35.

4: Same procedure as for **3** but with **2** instead of **1** as a starting material. Yield 0.24 g, 88.9%; elemental analysis calcd for **4**, $\text{C}_{64}\text{H}_{120}\text{O}_{12}\text{P}_2\text{Si}_2\text{Fe}$: C 54.01, H 8.44, P 4.36, Fe 3.94; found: C 53.62, H 8.44, P 4.36, Fe 3.21.

5: A similar procedure was adopted as for the synthesis of **3** except the solution was stirred for 3 h before filtration. Complex **5** could be obtained as colorless crystals in 91 % yield after recrystallization. Elemental analysis calcd for **5**, $\text{C}_{53}\text{H}_{96}\text{O}_{12}\text{PSi}_7\text{Fe}$: C 52.69, H 7.95, P 2.57, Fe 4.64; found: C 53.80, H 7.85, P 2.83, Fe 3.71.

6: A similar procedure was adopted as for the synthesis of **5** except that NEt_3 was used without drying. After recrystallization, **6** could be obtained as colorless crystals in 92 % yield. Elemental analysis calcd for **6**, $\text{C}_{82}\text{H}_{158}\text{O}_{25}\text{N}_2\text{Si}_4\text{Fe}_2$: C 47.44, H 7.62, N 1.35, Fe 5.40; found: C 48.51, H 7.64, N 1.31, Fe 5.57.

Crystal structure analyses: All crystals are colorless. In each case a crystal was attached to a glass fiber using a spot of silicone grease and then mounted from a matrix of mineral oil. The crystal was immediately placed on a Bruker P4/CCD/PC diffractometer ($\lambda(\text{MoK}\alpha) = 0.71073$ Å) and cooled to 203(2) K using a Bruker LT-2 temperature device. For further details see ref. [9]. 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-139882–139885. 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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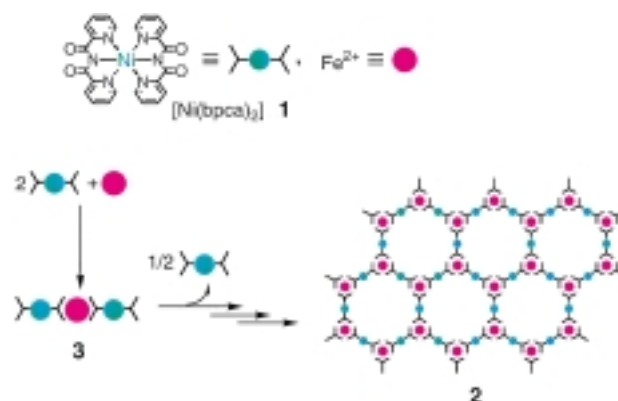
- [9] a) **3** · 0.5 C₆H₆, triclinic, space group $P\bar{1}$, $a = 15.8034(7)$, $b = 21.587(1)$, $c = 22.742(1)$ Å, $\alpha = 94.839(1)$, $\beta = 91.726(1)$, $\gamma = 103.303(1)^\circ$; $V = 7513.5(6)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.230$ g cm⁻³, $\mu = 0.409$ mm⁻¹, $1.8 < 2\theta < 53.0$. Final R factor was 0.0625 ($wR2 = 0.1514$) for 26531 observed reflections with $I > 2\sigma(I)$. b) **4** · 1.5 C₆H₆, triclinic, space group $P\bar{1}$, $a = 14.3308(6)$, $b = 14.8489(9)$, $c = 23.540(1)$ Å, $\alpha = 72.963(1)$, $\beta = 78.816(1)$, $\gamma = 61.248(1)^\circ$; $V = 4190.5(4)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.221$ g cm⁻³, $\mu = 0.387$ mm⁻¹, $3.22 < 2\theta < 52.90$. Final R factor was 0.0585 ($wR2 = 0.1413$) for 4066 observed reflections with $I > 2\sigma(I)$. c) **5** · C₆H₆, triclinic, space group $P\bar{1}$, $a = 11.0417(5)$, $b = 14.3909(7)$, $c = 22.445(1)$ Å, $\alpha = 92.955(1)$, $\beta = 98.253(1)$, $\gamma = 107.244(1)^\circ$; $V = 3354.1(3)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.274$ g cm⁻³, $\mu = 0.429$ mm⁻¹, $1.8 < 2\theta < 46.6$. Final R factor was 0.0524 ($wR2 = 0.1379$) for 9003 observed reflections with $I > 2\sigma(I)$. d) **6** · CH₃CN · 2 C₆H₆, orthorhombic, space group $Pnma$, $a = 21.838(1)$, $b = 31.824(2)$, $c = 17.1990(8)$ Å; $V = 11953(1)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.264$ g cm⁻³, $\mu = 0.449$ mm⁻¹, $2.6 < 2\theta < 53.0$. Final R factor was 0.1249 ($wR2 = 0.3333$) for 12087 observed reflections with $I > 2\sigma(I)$.
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A Graphite-Like Complex with Large Cavities Constructed with the Complex Ligand [Ni^{II}(bpca)₂]**

Asako Kamiyama, Tomoko Noguchi, Takashi Kajiware, and Tasuku Ito*

Recently metal complex assemblages have attracted much attention and several examples have been reported, including multiple helicates,^[1] grids,^[2] cages,^[3] two-dimensional sheets,^[4–6a, b] diamondoid networks,^[7] and zeolite mimics.^[8] In

such compounds cooperative interactions between the metal ions often induce properties such as magnetism,^[4–6b–h] conductivity,^[9] and photoactivity.^[6f–h] It has been shown that the use of a “complex ligand” as a building component is effective not only in the construction of the desired structures but also to design the spatial arrangement of metal ions, and to tune the metal–metal interactions. We have developed a strategy for constructing trinuclear and chain complexes by using [M(bpca)₂]^{*n*+} as bis-bidentate complex ligands (where $n = 1$ for M = Fe^{III}; $n = 0$ for M = Mn^{II} and Fe^{II}; Hbpca = bis(2-pyridylcarbonyl)amine).^[10] Herein, we report the synthesis and structure of a two-dimensional coordination polymer constructed by the reaction of [Ni(bpca)₂] (**1**) with iron(II) perchlorate (Scheme 1).



Scheme 1. Schematic representation for the formation of the honeycomb complex **2** by the reaction of **1** with iron(II) ion.

The reaction of **1** with iron(II) perchlorate hexahydrate in a 2:1 molar ratio afforded dark purple hexagonal prismatic crystals of [Fe^{II}(**1**)_{1.5}](ClO₄)₂ (**2**), a compound with a two-dimensional structure. Addition of one drop of water to the same reaction mixture afforded different deep violet crystals of the trinuclear species [Fe^{II}(**1**)₂(H₂O)₂](ClO₄)₂ (**3**) which is considered to be a precursor of **2**.

Figure 1 shows the two-dimensional honeycomb structure of **2**. The structure is that of a (6,3) net^[11] in which the triply chelated iron(II) centers act as three-connected nodes (Figure 1a). Each ring in a layer consists of six iron(II) ions at the corners and six units of **1** as the edges. The diagonal separations are Fe1#2...Fe1#3 = 16.463(3) Å and Ni1#1...Ni1#1 = 21.582(1) Å. The cavity size estimated from the space-filling model (Figure 1b) is 12 Å along the Fe1#2...Fe1#3 axis and 13 Å along the Ni1...Ni1#1 axis. The whole structure of **2** is made up, as in graphite, of layers stacked upon each other along the *c* axis and related by crystallographical mirror planes at $c = 0$ and 0.5, hence the complete channel is formed along the *c* axis. The iron(II) centers are in a chiral environment formed by three chelating complex ligands, and a single layer consists of only one of the two optical isomers (*Λ* or *Δ*). It is to be noted that **2** has noninterpenetrating networks regardless of the presence of the large cavities.^[4, 11] Interpenetration is prevented by the steric repulsion of the pyridine rings of the complex ligands. The perchlorate ions are located between the layers, half of them form two alternate

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[**] This work was supported by a Grant-in Aid for Scientific Research on Priority Area “Metal-assembled Complexes” (No. 10149102) and by Encouragement of Young Scientists (No. 11740366) from the Ministry of Education, Science, Sports, and Culture, Japan. Hbpca = bis(2-pyridylcarbonyl)amine.

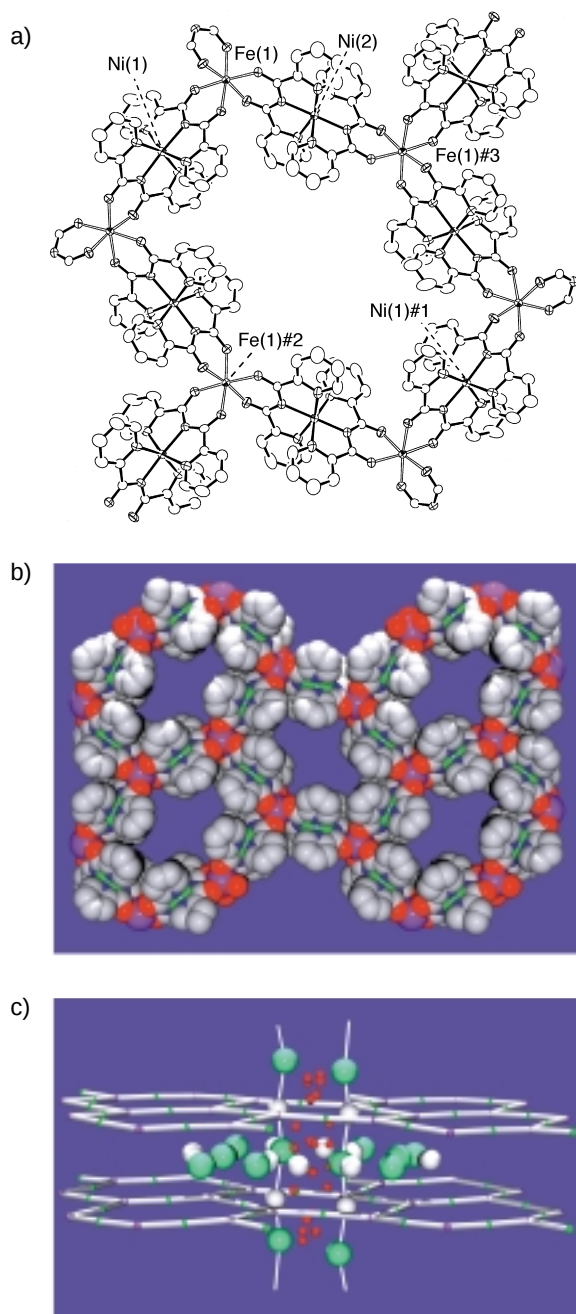


Figure 1. a) ORTEP drawing of **2** with the thermal ellipsoids set at 50% probability. b) Space-filling drawing of **2**. The hydrogen atoms are omitted. c) Schematic drawing of the counter ions and solvent molecules in **2**. Green: ClO_4^- , white: CHCl_3 , and red: H_2O , relevant distances [Å]: Ni(1)–N(pyridine) 2.087(5)–2.114(5), Ni(1)–N(amide) 2.013(5)–2.020(5), Ni(1)–Fe(1) 5.4238(11), Ni(2)–Fe(1) 5.3859(8), Fe(1)–O 2.072(4)–2.109(4); symmetry codes: (#1) $-x+1, -y+1, z$; (#2) $x, y-1, z$; (#3) $-x+1, -y+2, z$.

one-dimensional arrays with chloroform molecules along the *c* axis which penetrate the channel (Figure 1 c). More than ten water molecules were found within each ring. Compound **2** undergoes anion exchange and also exchange of the solvent molecules of crystallization in the solid state without the disintegration of the honeycomb structure.^[12]

Figure 2 shows an ORTEP plot of the complex ion in the trinuclear complex **3**. This compound consists of two $[\text{Ni}^{\text{II}}(\text{bpca})_2]$ units (shown with filled-in bonds) and a central $[\text{Fe}^{\text{II}}(\text{H}_2\text{O})_2]^{2+}$ ion (shown with open bonds), the former act as terminal ligands to the central iron(II) ion. The overall

structure of the cationic part of **3** is similar to that of the mixed valent iron trinuclear complex $[\text{Fe}^{\text{II}}\{\text{Fe}^{\text{III}}(\text{bpca})_2\}_2(\text{H}_2\text{O})_2]^{4+}$ we reported previously.^[10b] The hexacoordination of the iron(II) ion in **3** is by four carbonyl oxygen atoms from **1** and by two additional oxygen atoms from the water molecules bound *trans* to each other.

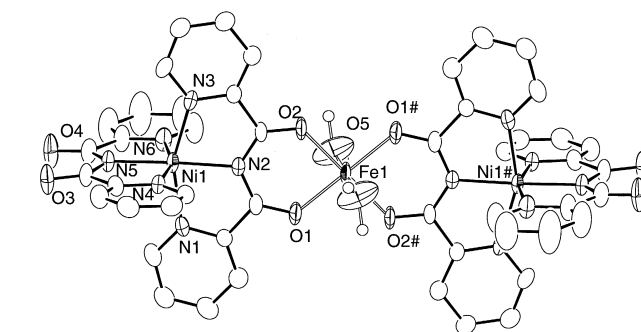
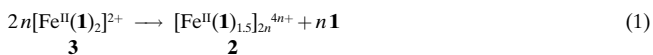


Figure 2. ORTEP plot of the cation of **3** with the thermal ellipsoids set at 50% probability. Selected distances [Å]: Ni1–N5 2.006(3), Ni1–N2 2.037(3), Ni1–N4 2.081(4), Ni1–N6 2.087(4), Ni1–N3 2.120(4), Ni1–N1 2.122(4), Ni1–Fe1 5.3681(5), Fe1–O1 2.020(3), Fe1–O2 2.065(3), Fe1–O5 2.104(6); symmetry codes: (#1) $-x, -y, -z$.

We found that the best conditions for the synthesis of the honeycomb complex **2** are the reaction of **1** and iron(II) perchlorate in a 2:1 ratio under the water free conditions. The reaction in a 3:2 ratio gave a mixture of **2** and a one-dimensional chain complex **4**^[13] even though the reaction ratio is consistent with the component ratio in **2**. To investigate the formation mechanism of **2**, we carried out an ESI-mass spectrometric study of the reaction solution of **1** and iron(II) perchlorate. The main product in a 2:1 solution was a trinuclear complex with a “doubly chelated” iron center formulated as $[\text{Fe}(\text{1})_2(\text{ClO}_4)]^+$ (*m/z*: 1176.98), and a tetranuclear complex with a “triply chelated” iron center, formulated as $[\text{Fe}(\text{1})_3(\text{ClO}_4)]^+$ (*m/z*: 1687.08), was found as a trace product. A similar result was obtained for a 3:1 solution, a triply chelated species in which three units of **1** are coordinated to the central iron(II) ion was a minor product even under these conditions. These results indicate that the precursor for the honeycomb complex **2** is the trinuclear complex **3** with a doubly chelated iron center and not $[\text{Fe}(\text{1})_3]^{2+}$ with a triply chelated iron center. Complex **3** has four coordination-free carbonyl sites (O3 and O4 in Figure 2) and thereby acts as a trinuclear complex ligand. The total reaction for the formation of the honeycomb complex **2** is expressed in [Eq. (1)].

Complex **3** has four coordination-free carbonyl sites (O3 and O4 in Figure 2) and thereby acts as a trinuclear complex ligand. The total reaction for the formation of the honeycomb complex **2** is expressed in [Eq. (1)].



The fact that the 3:2 reaction gave a mixture of **2** and **4** may be understood in the following way. Under these conditions, the reaction in [Eq. (1)] gives **2**; but since free iron(II) ions are present in larger amounts than in the 2:1 reaction, the reaction in [Eq. (2)] occurs at the same time to produce the chain



complex **4**, which, because of its low solubility is formed as a solid product. The reaction in [Eq. (1)] does not proceed when the reaction medium is wet (see above). The presence of an excess of water molecules appears to stabilize **3** and prevent the formation of **2**.

In conclusion, we have prepared the graphite-like compound **2**, which has large cavities and no interpenetration, the related discrete trinuclear and one-dimensional complexes were also prepared. Formation of these complexes depends on the reaction stoichiometry and the presence or the absence of water.

Experimental Section

Mass spectra (ESI) were recorded on a JASCO API LC-TOF/MS instrument.

2·4.5CHCl₃·3MeOH·10H₂O: Chloroform was distilled before use to remove ethanol. A solution of [Fe(H₂O)₆](ClO₄)₂ in methanol (0.025 M; 0.01 mmol, 0.4 mL) was added to a solution of [Ni(bpc)₂] (10 mg, 0.02 mmol) in chloroform/methanol (1/1 (v/v), 2 mL). Slow evaporation of the solvent at room temperature gave black hexagonal prisms of **2**·4.5CHCl₃·3MeOH·10H₂O which were filtered and dried in vacuo (11 mg).

3·4H₂O: A solution of [Fe(H₂O)₆](ClO₄)₂ in methanol (0.02 M; 0.01 mmol, 0.5 mL) was added to a solution of [Ni(bpc)₂] (10 mg, 0.02 mmol) in chloroform/methanol (1/1 (v/v), 2 mL). A drop of water was added to the resulting dark purple solution. Slow evaporation of the solvent at room temperature gave black plates of **3**·4H₂O (almost quantitatively (13 mg)).

Crystal-structure determinations: Bruker SMART-1000 CCD-based diffractometer, 200 K, MoK_α radiation ($\lambda = 0.71073$ Å). **2**·4.5CHCl₃·3MeOH·10H₂O: Orthorhombic, space group *Pnnm*, *a* = 32.999(2), *b* = 17.1473(13), *c* = 20.9802(13) Å, *V* = 11871.4(13) Å³, ρ_{calc} = 1.598 g cm⁻³, $\mu(\text{MoK}_{\alpha}) = 1.186$ mm⁻¹; ω -scans, 76878 reflections measured ($2\theta_{\text{max}} = 55^\circ$), of which 14045 were independent and 7052 were observed [$I > 2\sigma(I)$], 792 refined parameters, *R* = 0.0873, *wR*2 = 0.2532, residual electron density max./min. 1.675/−0.943 e Å⁻³. Ten peaks were found after D-Fourier peak search, and were assigned as water molecules. **3**·4H₂O: Triclinic, space group *P* $\bar{1}$, *a* = 8.9267(6), *b* = 12.2683(8), *c* = 13.3292(9) Å, $\alpha = 90.215(2)$, $\beta = 101.813(2)$, $\gamma = 97.288(2)^\circ$, *V* = 1416.63(16) Å³, ρ_{calc} = 1.624 g cm⁻³, $\mu(\text{MoK}_{\alpha}) = 1.095$ mm⁻¹; ω scans, 10168 reflections measured ($2\theta_{\text{max}} = 55^\circ$), of which 6288 were independent and 3635 were observed [$I > 2\sigma(I)$], 394 refined parameters, *R* = 0.0598, *wR*2 = 0.1323, residual electron density max./min. 0.807/−0.589 e Å⁻³. The structures were solved by the direct method with SHELXS-86^[14] or the Patterson method with DIRDIF94 (Patty)^[15] and refined by the least squares method on *F*², SHELXL-93.^[16] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were calculated by the geometrical method and refined as a riding model. 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-141630 and CCDC-141631. 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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- [12] In **2**, ClO₄⁻ ions can be exchanged with PF₆⁻ ions in the solid state, and CHCl₃ molecules can be exchanged with CHBr₃ molecules. These facts are confirmed by IR spectra and by the specific gravity.
- [13] Compound **4** is a one-dimensional alternate chain complex which consists of the bridging **1** and [Fe(EtOH)₂]²⁺ units. The iron(II) ion in this complex is coordinated by two complex ligands and two EtOH molecules. The latter comes from the stabilizer in CHCl₃. Crystal data of **4**: Monoclinic, space group *C2/c*, *a* = 10.4651(5), *b* = 18.6142(10), *c* = 20.3093(11) Å, $\beta = 102.8680(10)^\circ$, *V* = 3856.9(3) Å³, *Z* = 4, *R* = 0.0435, *wR*2 = 0.1239 [$I > 2\sigma(I)$].
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A Parallel Library of all Seven $A_2 + B_2 + C_2$ T_h Regioisomeric Hexakisadducts of Fullerene C_{60} : Inspiration from Werner's Octahedral Stereoisomerism**

Wenyuan Qian and Yves Rubin*

Compact multivalent molecules can be viewed as ideal cores for the synthesis of libraries with very high diversity.^[1–3] Cubane and trimesic acid-based scaffolds,^[2] among others,^[3] have been proposed for this purpose. Extending functional valency of these cores to octahedral or higher symmetry objects, while maintaining control of the spatial directivity of branches derived from their anchor points, would give access to “spherical” diversity suitable for probing cell surface receptors and signaling pathways. Based on these premises, octahedral stereochemistry,^[4, 5] originally proposed by Werner to explain the configuration of transition metal complexes, inspired us to develop approaches to novel scaffolds with potential applications in combinatorial chemistry.

With its six coordination sites, the octahedron has a much more complex stereochemistry than its tetrahedron counterpart, the latter possessing only configurational isomerism.^[6] In fact, if six *different* groups are placed at the O_h sites, up to fifteen diastereomers can be generated.^[5b] In a simpler case, the spatial arrangement of three pairs ($A_2 + B_2 + C_2$) of monodentate ligands at the O_h sites in transition metal complexes leads to the five diastereomers **1a–e** (Figure 1). In practice, this analysis is complicated by the lability of the ligands, hence bridging bi- or multidentate coordinating groups are required to gain control over molecular configuration.^[5, 7] This aspect restricts the number of isomers that can be prepared and does not lead to the easy elaboration of libraries with different groups at all six O_h positions.

The high symmetry of C_{60} provides an opportunity to expand the diversity of molecular libraries by using a relatively compact (10-Å van der Waals radius “nanobead”) and easily functionalizable spherical framework of thirty C=C bonds (Figure 2).^[8, 9] Although this question has been addressed in part,^[8–12] regio- and stereocontrolled additions to fullerenes remain a real challenge, and the functionalization of any of their double bonds, at will, is unthinkable at the present time.^[8a] To simplify the problem by keeping the local symmetry to a maximum, one can make a useful analogy between C_{60} and Werner's octahedral framework (Figure 2, left).^[9, 11] There are six symmetrically disposed double bonds situated at the vertices of an octahedron, which if functionalized selectively, give access to a variety of well-defined

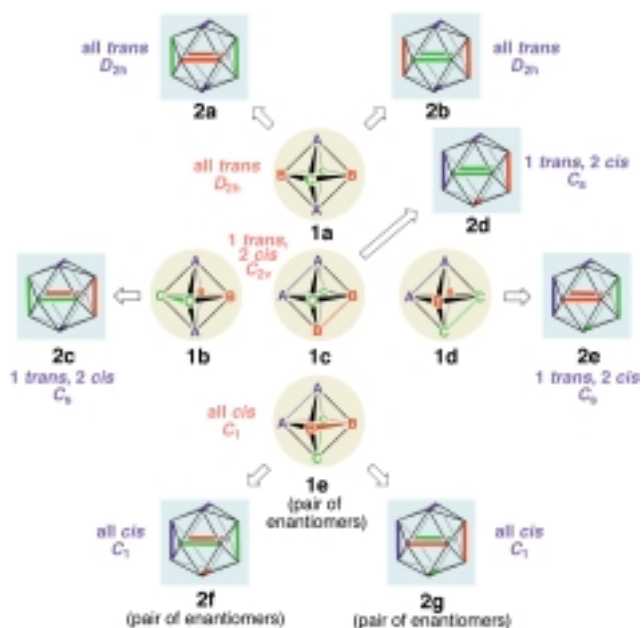


Figure 1. Stereoisomerism of $A_2 + B_2 + C_2$ addends within octahedral (**1a–e**) and pseudo-octahedral (T_h) topologies (**2a–g**).

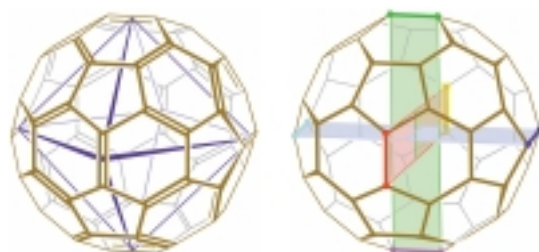


Figure 2. Octahedral and T_h topologies within C_{60} .

three-dimensional systems.^[9, 13] Contrary to the freely rotating ligand-to-metal bonds in the octahedron, these bonds are fixed in space, being included in the three planes of symmetry that are implicitly part of the T_h point group (Figure 2, right).^[6, 9a, 11] Consequently, the number of isomers that can arise from $A_2 + B_2 + C_2$ functionalization at these bonds increases to seven (Figure 1, **2a–g**), and goes up to thirty if all six adding groups are different (for symmetrical addends).

The methodology outlined here provides access to a small library of hexakisadducts (Figures 3 and 4) which have three pairs of adding groups ($A_2 + B_2 + C_2$ pattern) with topologies similar to those of the octahedral transition metal complexes, but with the added complexity of the T_h framework (Figure 1). In the octahedral model, there is only one possible diastereomer for each of the all-*trans* (**1a**) and all-*cis* (**1e**) config-

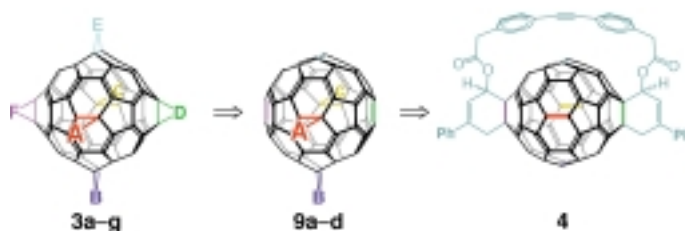


Figure 3. The “mer-3+3” retrosynthetic strategy to hexakisadducts **3a–g**.

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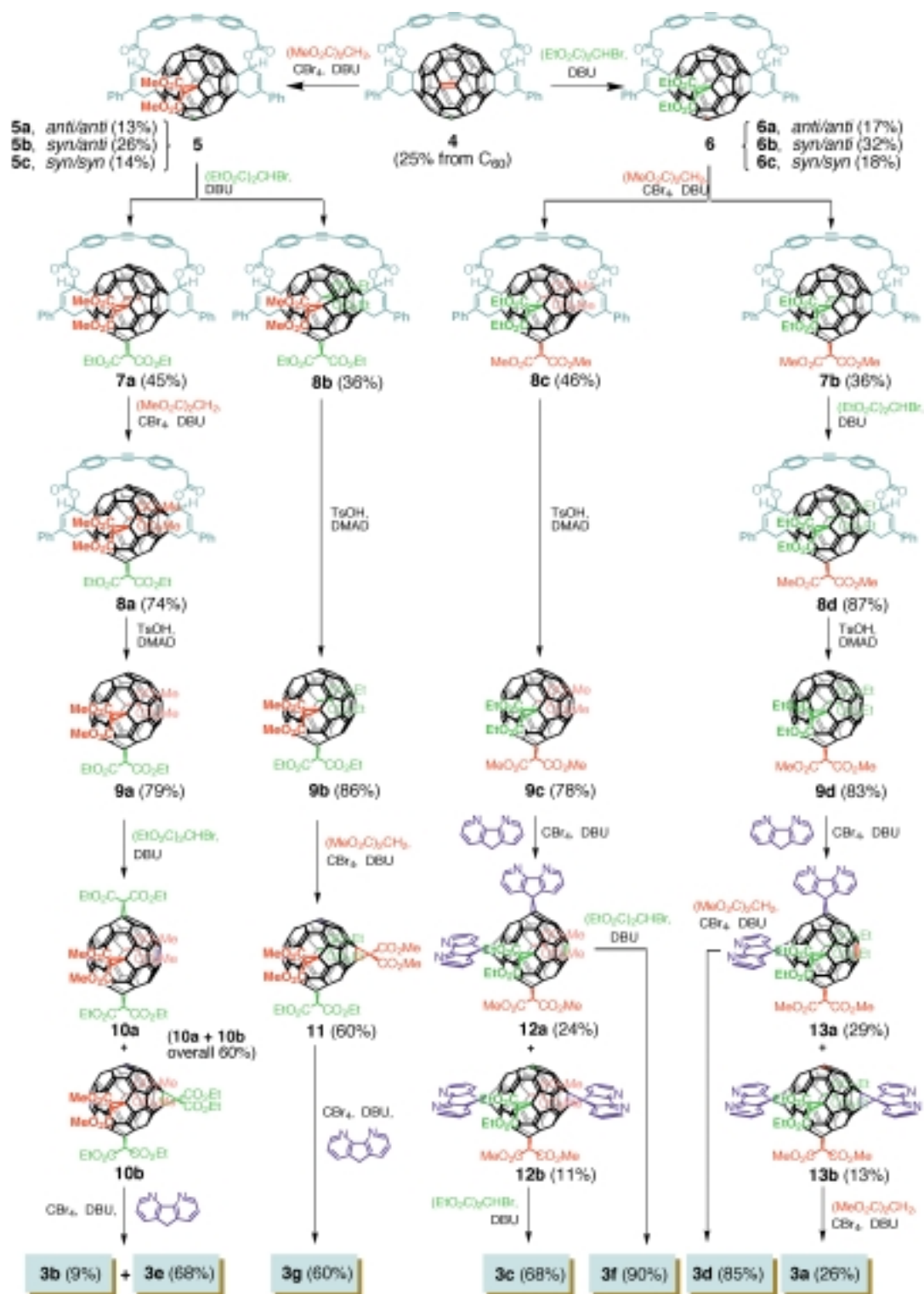
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urations, while in the pseudo-octahedral (T_h) model, each of the all-*trans* and all-*cis* relationships give rise to a pair of compounds that are *regioisomers* or “permutational isomers”^[14] (**1a**→**2a** and **2b**, **1e**→**2f** and **2g**).

The “*mer*-3+3” regiocontrol strategy^[15] employed in the present synthesis is derived from the finding that the bridged *trans*-1 bisadduct **4** displays exquisite selectivity towards the sequential addition of three groups to its equatorial positions

(Figure 3).^[13] Within the scope of this work, four differentiated “*mer*”-trisadducts (**9a–d**) were needed as key intermediates (Scheme 1). It was also anticipated that the three *e*-face, *e*-edge, and *trans*-1 arranged addends of **9a–d** would direct further additions toward the remaining three *e*-positions.^[13] However, the exact degree of regioselectivity achievable among the last three unoccupied C=C bonds at the pseudo-octahedral positions was not clear.



Scheme 1. Regioselective, parallel synthesis of the regioisomers **3a–g**.

An economical execution of this strategy was needed to give rapid access to all seven diastereomers **3a–g** (Scheme 1). Thus parallel synthesis, which has proven to be a very efficient method to obtain large numbers of structurally related compounds, was applied here.^[16] The three pairs of labeling addends, dimethyl malonate, diethyl malonate and 4,5-diazafluorene, were chosen for several reasons: they can all add to C_{60} following the Bingel–Hirsch regioselectivity, which allows them to be addressed especially well to equatorial positions.^[11] In the ^1H NMR spectra, each of the three addends appears in a characteristic region, well separated from the signals of other groups. The large magnetic anisotropy from the 4,5-diazafluorenyl moiety has a significant influence on the chemical shifts of neighboring addends, and this property plays a crucial role in the confirmation of the relative stereochemistry in each isomer. In addition, the introduction of the very polar 4,5-diazafluorenyl groups helps overcome the separation problems caused by potentially unsatisfactory regioselectivity^[13] in the late stages of the overall synthesis.

Monoaddition of a diethyl bromomalonate unit to the *trans*-1 bis-Diels–Alder adduct **4** (*syn/anti* mixture) gave one regioisomer **6** (Scheme 1).^[13, 17] The two stereocenters at the cyclohexenyl rings imply that **6** consists of three possible diastereomers **6a** (*anti/anti*), **6b** (*syn/anti*) and **6c** (*syn/syn*), which were easily separated by column chromatography (CH_2Cl_2 , SiO_2). To aid unambiguous characterization of the products in the next additions, the C_s -symmetrical compound **6a** was used in the following steps (**6b** and **6c** gave the same trimers **9c** and **9d** after the same addition and deprotection sequence). In earlier work,^[13] we found that the second Bingel addition on **6** was also highly regioselective and gave the addition pattern that corresponds to **7b**. In the present case, we used 1.5 equivalents of dimethyl malonate to afford nearly equal amounts of the easily separable tetrakisadduct **7b** and pentakisadduct **8c** in high yield (82%). Only a trace ($\leq 5\%$) of an uncharacterizable brown fraction was obtained, again confirming the high selectivity of this reaction towards the “*mer*” triple-*e* position. The tetrakisadduct **7b** was further derivatized with diethyl bromomalonate to give exclusively the pentakisadduct **8d**. One-pot elimination/Diels–Alder/retro-Diels–Alder reaction^[13, 18] removed the tethers on **8c** and **8d** to give the two trisadducts **9c** and **9d** in high deprotection yields (78% for **9c** and 83% for **9d**). Similarly, the other two heterotrisadducts **9a** and **9b** were obtained by simply switching the addition sequence on the *trans*-1 bisadduct **4**. It should be noted that these four trisadducts are only subtly different from each other, and that their rapid preparation is not possible by any other regiocontrol methodology.^[9–12]

The four trisadducts **9a–d** served as the indispensable intermediates in the overall synthesis of all seven pseudo-octahedral diastereomers **3a–g**. The relative regioselectivity among the three remaining equatorial double bonds was studied in the final synthetic steps: earlier results^[13] indicated that the fourth addition on these “*mer*”-trisadducts should greatly favor the triple-*e* position(s) (double *e*-edge, plus single *e*-face), which correspond to the “poles” of the sphere with respect to the original bis-Diels–Alder addition, rather

than the double *e*-face site on the equator. Indeed, treatment of **9b** with one equivalent of dimethyl malonate under the Hirsch conditions^[19] gave exclusively the expected triple-*e* adduct **11**. The last two *e*-double bonds were then “masked” by 4,5-diazafluorenyl groups to give the first isomer **3g** in this $A_2 + B_2 + C_2$ hexakisadduct series. Interestingly, when the C_{2v} -trisadduct **9a** was treated with one equivalent of diethyl bromomalonate under similar conditions, two tetrakisadducts **10a** and **10b** were obtained, which result from additions at both the double-*e*-face position and the triple-*e* positions, respectively. The ratio of these two inseparable isomers was 8:1 as determined by ^1H NMR spectroscopy. The reason for the reduced selectivity in this case is not clear, but one possible cause is the larger steric bulk of the diethyl bromomalonate anion compared to that of the dimethyl analogue. In any case, both isomers could be incorporated in the overall synthesis. Two 4,5-diazafluorenyl moieties were introduced in the final step to afford the desired products **3b** and **3e**. Although the tetrakisadduct isomers **10a** and **10b** were not separable, their resulting hexakisadducts **3b** and **3e** were easily separated by preparative TLC (SiO_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100/3.5) as a consequence of the different *cis* and *trans*-display of the two R_f -determining 4,5-diazafluorenyl groups.

In the final synthesis of the other four hexakisadducts **3a**, **3c**, **3d**, and **3f**, both *cis* and *trans* relationships of the two 4,5-diazafluorenyl groups on the pseudo-octahedral scaffold were required. To average out the relative amounts of the *trans* (**3c**) and *cis* (**3f**) isomers, we first treated the trisadduct **9c** with 1.4 equivalents of 4,5-diazafluorene under the Hirsch conditions. This approach afforded only the two possible pentaadducts **12a** (*cis*, 24%) and **12b** (*trans*, 11%), together with some tetrakisadduct (30%), recovered starting material **9c** (30%), and a trace of a higher adduct. These products were easily separated by column chromatography; the tetrakisadduct and starting material were recovered to prepare the pentaadducts **12a**, **b** again. The last unoccupied octahedral position in **12a** (*cis*) was easily blocked with an excess of diethyl bromomalonate to give the hexakisadduct **3f**. The fraction of the “*trans*”-adduct **12b** was contaminated with about 10–20% of a compound that could not be characterized or removed by column chromatography. The “abnormal” addition pattern of this impurity is probably due to the much more reactive nature of the 4,5-diazafluorenyl anion compared to that of the malonate anions. After treatment of this mixed fraction with excess diethyl bromomalonate under standard conditions, the resulting bright yellow all-*e* hexakisadduct **3c** was easily isolated from the orange non-octahedral byproduct by preparative TLC (SiO_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100/4). Compounds **3a** and **3d** were prepared from the trisadduct **9d** using a similar reaction sequence. Starting from C_{60} , it took only 21 steps overall (on average three reactions per isomer) to obtain all seven diastereomers. No HPLC separation was necessary in this highly divergent synthesis.

The stereochemistry of all seven isomers can be unequivocally confirmed from their NMR spectra (Figure 4), and also from their synthetic history. In accord with the initial molecular design, the diamagnetic ring current from the large π surface of the 4,5-diazafluorenyl addend has a substantial

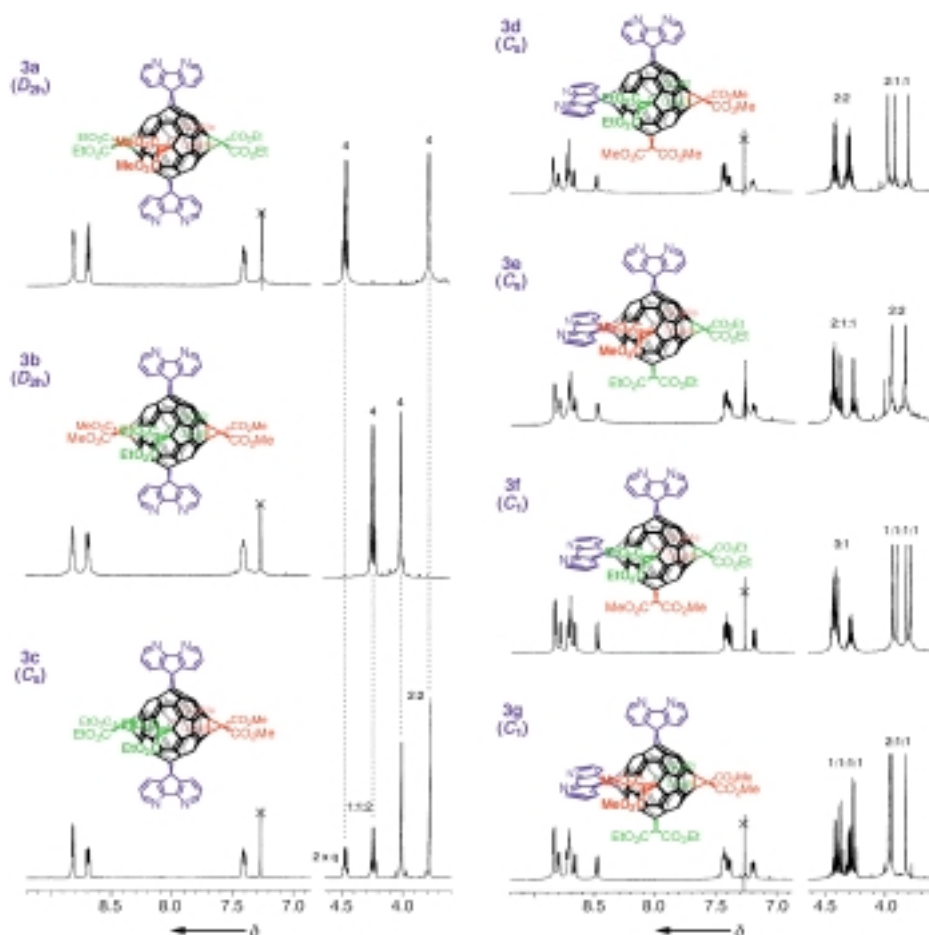


Figure 4. ^1H NMR spectra of the seven stereoisomers **3a–g** and their respective symmetry.

shielding effect on the chemical shifts of the protons of groups facing it. On the other hand, the ethyl and methyl ester groups both have a similar influence on their neighboring addends. Hence, the chemical shifts of the methylene protons (COOEt groups) or the methyl protons (COOMe groups) are particularly diagnostic in the determination of the relative position of each addend. This is especially important in distinguishing the two very similar C_1 -symmetric all-*cis* compounds **3f** and **3g**. In **3f**, there are two methoxy groups facing the π surfaces of the 4,5-diazafluorenyl moieties, thus their proton signals are shifted to higher field relative to those of the other two methoxy groups in this molecule. On the other hand, there is only one ethoxy group facing the π surface of the 4,5-diazafluorenyl addend, its protons are shielded and thus appear at higher field than those of the other three ethoxy groups in **3f**. An exactly opposite effect is observed for the isomer **3g**.

Similarly, the all-*trans* geometric pair of compounds **3a** and **3b** cannot be distinguished by the number of signals in their relatively simple ^1H or ^{13}C NMR spectra because of their common D_{2h} symmetry. However, there is a dramatic difference in the 3.6–4.6 ppm region of their ^1H NMR spectra, which depends on whether or not the methoxy or ethoxy groups are facing the π surfaces of the 4,5-diazafluorenyl addends in these two isomers. Interestingly, compared with **3a**, the upfield shift ($\Delta\delta = 0.23$ ppm) of the ethoxy methylene

protons in **3b** has exactly the same magnitude as the downfield shift of the methoxy protons. For the same reasons, the ^1H NMR spectrum of **3c** is almost the exact superposition of those of **3a** and **3b**.

The efficient synthesis of hexakisadducts **3a–g** demonstrates the utility of the stepwise “*mer*-3+3” regiocontrol strategy on C_{60} . In a broader sense, this work also provides an entry into another dimension of organic stereochemistry.^[6, 20] Furthermore, access to a diverse and relatively complex display of functional groups on the spherical framework of C_{60} can be expected to afford new classes of compounds with biological properties.^[8, 21]

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A Chip-Based Biosensor for the Functional Analysis of Single Ion Channels**

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The functional analysis of single ion channel proteins presents a serious bottleneck in the process of finding new pharmacologically active compounds. Single channel recording methods currently available (patch clamp,^[1] black lipid membrane (BLM)^[2]) are not suited for automation and miniaturization. However, new techniques such as combinatorial chemistry^[3] and combinatorial genetics,^[4] which pro-

duce large numbers of potential drugs and mutant proteins, respectively, demand efficient and reliable high-throughput screening (HTS) as well as low sample consumption.^[5] In this context, monitoring the current of single ionotropic receptors with automated biosensor devices may result in new classes of highly sensitive screening tools.

Functional studies of single ion channel proteins require the reduction of electrical background noise to 10^{-13} – 10^{-12} A and consequently a high electrical insulation of the surrounding membrane patch ($R > 10^9 \Omega$, “giga-seal”) under conditions of low dielectric loss and low electrical capacitance.^[1] Classically, tight seals are obtained manually by suction of small membrane patches (from cells or proteoliposomes) into glass micropipettes^[6] or by “painting” of lipid bilayers across holes in teflon septa.^[2, 7]

Single-channel recording systems suited for biosensor and HTS applications require a new approach. The self-positioning of unilamellar lipid vesicles on planar insulating diaphragms presents one solution. Since the surface of most native membrane vesicles and cells bears electrical charges,^[8] properly directed electrical fields can provide precise electrophoretic positioning. However, efficient electrophoretic attraction of vesicles can only be realized for electrokinetic velocities v_{ek} (vesicle) $\gg 1 \mu\text{m s}^{-1}$, which requires electric fields of several hundred volts per meter.^[9] In a homogeneous field, such field strengths are only obtained under unfavorable conditions such as high voltages or very short electrode distances. Consequently, strongly inhomogeneous fields were used for positioning, which resulted in a focused movement of vesicles towards the point with the highest voltage gradient.

An inhomogeneous electrical field (Figure 1 a) was created around a small aperture located in a thin insulating diaphragm (Figure 1 b) separating two fluid compartments. By accessing both compartments with low impedance redox electrodes (Figure 1 c) the main voltage drop after application of a potential V_C occurred within and near the aperture (Figure 1 a). The resulting radially symmetrical field E directed the electrophoretic movement of charged objects to the spatially fixed aperture. The geometry of the aperture and the limitation of the applied voltage V_C to physiological potentials (< 200 mV, to avoid artifacts such as electroporation after seal formation) define the strength of the electric field. Optimal conditions for positioning were obtained by reducing the diaphragm thickness h to the minimum size that would provide sufficient stability and insulation ($h \approx 100$ nm) and by adaptation of the aperture diameter to the vesicle size ($d_A = 0.6$ – $7 \mu\text{m}$). Figure 1 a illustrates that under optimal conditions the “electrophoretic trap” extends less than $20 \mu\text{m}$ into the buffer volume. Despite this short attractive range vesicles frequently enter the electrophoretic trap by convection during vesicle addition or by sedimentation within the small volume of the compartment (1 – $10 \mu\text{L}$, Figure 2 a).

The feasibility of electrophoretic focusing was demonstrated using negatively charged giant unilamellar vesicles (Figure 2 b) and bare silicon nitride diaphragms. Within 1 – 10 s after the addition to the *cis* compartment (Figure 1 c, upper side), vesicles were trapped and moved through the aperture. The resulting current modulations (Figure 2 a) resembled Coulter-counter events ($V_C = 80$ mV).^[10] Even vesicles larger

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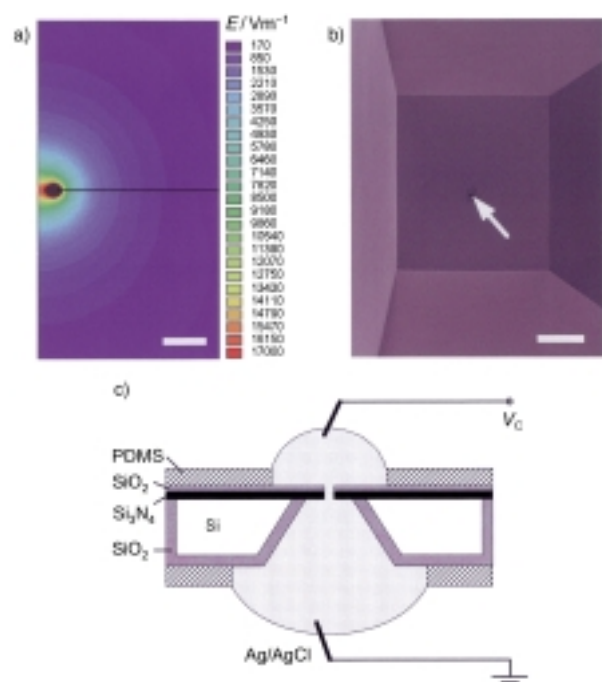


Figure 1. Characteristics of the planar recording setup. a) The electric field E near the aperture of the diaphragm mediates the electrophoretic movement of charged objects within a distance of several micrometers. The field outside the aperture along its center axis was approximated by:

$$E(L, d_A, h) = \frac{V_C \cdot d_A^3}{(h + d_A)(d_A^2 + 4L^2)^{3/2}},$$

assuming E is constant inside the aperture and $L \rightarrow \infty$ for the position of the electrodes (L = distance from the center of the aperture surface).^[24] A finite element simulation of the spatial-field distribution around a 4- μm aperture (axis of rotation to the left) for plane-parallel electrodes located 1 mm opposite the diaphragm is shown ($V_C = -100$ mV, diaphragm thickness $h = 100$ nm, scale bar: 4 μm). Near the rim E exceeds 17000 V m^{-1} and is shown in gray. b) Scanning electron micrograph showing the Si_3N_4 diaphragm and the aperture (see arrow) at the bottom of a pyramidal well. The low dielectric loss factor of $D \leq 10^{-4}$ and the high electric resistivity of $\rho > 10^{12}$ Ωm for Si_3N_4 allowed the diaphragm thickness to be reduced to $h = 100$ – 150 nm (scale bar: 25 μm , tilt = 30°). c) Schematic diagram of the recording setup. A fluid compartment was etched into a 400- μm thick silicon chip. The Si_3N_4 diaphragm contained an aperture with a diameter between 0.6 and 7 μm . The chip (cross section) was sandwiched between two polydimethylsiloxane (PDMS) elastomer pads, which provided access to the diaphragm and confined the free-standing recording compartments. The compartment volume was usually about 10 μL but can be reduced to 1 μL . Ag/AgCl redox electrodes were used for voltage application during electrophoretic positioning and for subsequent voltage clamp recordings. The distance and placement of the electrodes was not important, since the main voltage drop occurred across the aperture. An additional SiO_2 layer (thickness ca. 1 μm) was grown on the silicon bulk to reduce the capacitance ($C < 35$ pF) and consequently the capacitive noise to $\sigma < 200$ fA rms. The series resistance was between 80 to 280 k Ω in 85 mM KCl.

than the aperture passed through the unmodified Si_3N_4 diaphragm as indicated by the dwell-time distribution.

Tight and permanent binding of vesicles to the diaphragm is required for the measurement of single ion channel currents. A sufficiently close contact between the diaphragm surface and lipid membrane can be mediated by electrostatic forces.^[11] In order to exploit electrostatic attraction 20 nm of SiO_2 was deposited on the Si_3N_4 diaphragm, which was either chemically modified with 4-aminobutyldimethylmethoxysi-

lane or covered by physisorbed poly-L-lysine.^[12] The resulting positive charges on the surface within the aqueous recording environment caused a spontaneous and stable reduction in the current after the positioning of a negatively charged giant vesicle. This indicates vesicle fusion to the highly attractive diaphragm surface (Figures 2 c and d). After formation of the

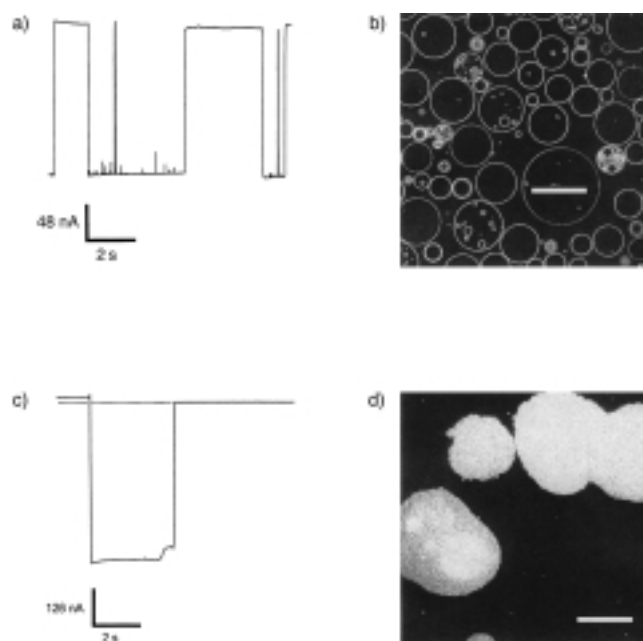


Figure 2. Voltages of $V_C < |150$ mV were sufficient to trap giant unilamellar vesicles. a) At negative potentials, vesicles added to the *cis* side of a bare Si_3N_4 diaphragm were electrophoretically trapped and moved to the opposite compartment ($h = 100$ – 150 nm, $d_A = 7$ μm). The current modulations $I(t) = V_C R(t)^{-1}$ are shown, which were caused by vesicles moving through the aperture and which depended on the vesicle size and concentration. Reversing the field orientation retracted previously passed vesicles into the *cis* compartment. b) The laser scanning microscopical (LSM) image of fluorescently labeled vesicles after size purification using a nylon net with a mesh size of 20 μm (scale bar: 50 μm). c) As in (a) but with a positively charged $\text{Si}_3\text{N}_4/\text{SiO}_2$ diaphragm (4-aminobutyldimethylmethoxysilane). Vesicles spread instantaneously on the highly attractive diaphragm surface forming G Ω seals without the need for manual control ($R = 83$ G Ω , dashed line: $I(t) = 0$ pA). In contrast to traditional BLM experiments,^[2] vesicle-derived planar bilayers are solvent-free. d) The LSM image of giant vesicles fused to the SiO_2 surface (scale bar: 50 μm). The formation of single-layered membranes was promoted by the strong electrostatic interaction between vesicles and diaphragm and by osmotic stress of the vesicles (vesicles filled with 200 mM sorbitol added to the *cis* compartment containing 85 mM KCl).

seal, the major voltage drop occurred across the newly built membrane patch. The corresponding field reduction outside the membrane patch automatically stopped the electrophoretic movement of additional vesicles. Seal resistances varied from $R = 1.4$ to 74 G Ω (poly-L-lysine) and $R = 9$ to > 200 G Ω (4-aminobutyldimethylmethoxysilane). In contrast to patch clamp techniques, calcium ions were not required in the buffer for formation of the seal. This result opens up the possibility of varying the concentration of Ca^{2+} ions and therefore performing detailed studies on calcium-dependent membrane processes.^[13]

The efficient formation of seal resistances of $R > 10^9$ Ω required vesicles with diameters at least three times greater

than the aperture size. The presence of significantly smaller vesicles presumably resulted in small negatively charged surface zones, which impaired the tight adhesion of hole-spanning giant vesicles. Stable membranes on the chip with seal resistances of $R > 10^9 \Omega$ were obtained in 99 out of 134 experiments after removal of small vesicles by dialysis (20 μm mesh size). The membranes were usually stable for more than 60 min in 10–500 mM KCl and 0–4 mM CaCl_2 .

Information about the structure of negatively charged vesicle membranes after adhesion to positively charged surfaces was obtained by scanning confocal fluorescence microscopy using fluorescently labeled vesicles. Complete binding of vesicles occurred instantaneously after sedimentation of the vesicles to the surface. The resulting membrane patches were extremely flat (thickness $< 0.8 \mu\text{m}$, below the z -resolution of the microscope) and correlated in size to the surface area of vesicles in solution ($3293 \pm 692; 3300 \pm 165 \mu\text{m}^2$, $n = 34$), which suggests the formation of planar unilamellar bilayers (in contrast to an expected ratio of 1:2 for two adjacently layered membranes of intact but flattened vesicles). The observed formation of single bilayers upon spreading giant, osmotically stressed vesicles on highly attractive surfaces is in agreement with previously described work.^[11]

Planar unilamellar membranes on low capacitance silicon chips ($C < 35 \text{ pF}$) are ideally suited for low noise electrical recording of transmembrane ion currents. The general functionality of this new concept was demonstrated with alamethicin, a peptide known to self-integrate into bilayers and to form voltage-gated ion channels of defined conductance by oligomerization.^[2, 14] The voltage-dependent activation of single alamethicin channels with up to seven conductance states was observed (Figures 3a and b) 10–150 s after adding the peptide (final concentration, 2.5 ng mL^{-1}) to the *cis* side of a vesicle-derived planar bilayer.^[15] The dwell-time distribution and the nearly symmetrical current–voltage relationship derived from these events are typical for the activation of alamethicin in asolectin membranes.^[2, 16] Changing the lipid composition to include 45 % 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 25 % cholesterol restricted the channel activation to positive potentials, as previously reported.^[16]

In this work we have shown that stable gigaohm seals over micrometer-sized holes can be obtained in a time-frame of seconds by the electrophoretic self-positioning of charged lipid membranes. For the first time, single-channel events were recorded on silicon microstructures. Self-integrating channel-forming peptides, such as alamethicin and gramicidin,^[17] or pore-forming proteins, such as α -haemolysin^[18] and OmpF,^[19] are expected to have applications in screening assays based on single-channel recordings, including high-speed DNA sequencing^[20] and metabolite detection. In the future, this method could be extended by either functional reconstitution of ion channels into giant vesicles^[21] or their fusion (small proteoliposomes) into preformed bilayers.^[7, 22] The possibility of combining this type of planar set-up with high numerical optics may also enable the simultaneous observation of electrical and optical signals, which would help to elucidate the coupling between ligand-binding and channel events of ionotropic receptors.^[23]

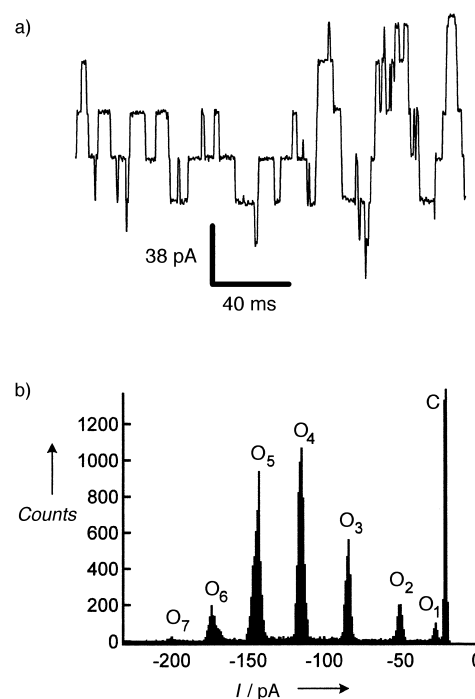


Figure 3. Voltage-dependent activation of single alamethicin channels. a) The I versus t diagram recorded at $V_c = -130 \text{ mV}$ in 0.2 M KCl and 8 mM HEPES, at pH 7.4 shows the typical activation pattern comprising up to seven conductance states. b) Current histogram derived from alamethicin traces recorded under conditions given in (a). The conductance states O_1 to O_7 are shown. C reflects the current baseline with the channel closed.

Recording chips can be produced in large numbers with defined geometry and material properties by standard silicon technology. Multiple recording sites can be integrated on one single chip because of the small lateral size of the diaphragm (less than 0.1 mm^2). The combination of silicon technology with the demonstrated principles of fast and reliable self-formation of low noise recording membranes in small sample volumes of 1–10 μL offers a unique approach for the realization of multiarray biosensors based on natural or designed^[18] channel-forming peptides or ion channel proteins, as well as for large-scale functional screening in fundamental research and drug discovery.

Experimental Section

Chips ($3 \times 3 \times 0.5 \text{ mm}^3$, Si wafer, (100) orientation, covered by a $0.1\text{--}1\text{-}\mu\text{m}$ thick plasma enhanced chemical vapor deposition (PECVD) Si_3N_4 layer) were structured by a combination of anisotropic KOH silicon etching and reactive-ion etching of the aperture. The anisotropic etching process was stopped at the Si_3N_4 layer, and resulted in a free-standing Si_3N_4 diaphragm of $10^{-9}\text{--}10^{-8} \text{ m}^2$, into which the hole was etched. For chemical and physical modifications of the diaphragm, a 20-nm SiO_2 PECVD layer was deposited onto the Si_3N_4 surface. To reduce the capacitance an additional insulating SiO_2 layer (thickness $\geq 1 \mu\text{m}$) was grown by thermal oxidation onto the silicon. Positively charged diaphragm surfaces were prepared by incubating the chips either for $\geq 5 \text{ min}$ in 0.1 % poly-L-lysine (relative molecular mass $M_r = 70\text{--}150 \text{ K}$) solution or overnight in dry toluene containing 1 % of 4-aminobutyldimethylmethoxysilane.

Giant unilamellar vesicles were prepared by overnight swelling of vacuum-dried lipid films (1.25 mg total lipid) of either 70 % asolectin, 25 % 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (POPG), 5 % cholesterol or 45 % POPE, 25 % cholesterol, 22.5 % 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 7.5 % POPG in deionized water

(10 mL) containing sorbitol (200–1000 mM). Lipid bilayers were labeled with *N*-(6-tetramethylrhodaminethiocarbonyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (TRITC-DHPE, 0.07 %). The vesicles in solution and fused to surfaces were observed with a laser scanning microscope (Zeiss, LSM 510, Zeiss 60x/1.25 apochromat water immersion objective, pinhole size 100 μ m) on either poly-L-lysine or 4-aminobutyldimethylmethoxysilane-modified glass coverslips coated with a 50 nm PECVD-SiO₂ layer.

Standard experiments were performed in 85 mM KCl, 2 mM *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), at pH 7.4 using vesicles filled with 200 mM of sorbitol.

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A Room-Temperature Discotic Nematic Liquid Crystal**

Sandeep Kumar* and Sanjay Kumar Varshney

Liquid crystal displays (LCDs) have played a vital role in information technology. The twisted nematic and supertwisted nematic display devices have dominated commercial displays since their invention. The liquid crystal layer in these devices is made up exclusively of calamitic liquid crystals (composed of rod-shaped molecules). A number of calamitic nematic liquid crystals having room-temperature mesophases—such as, cyanobiphenyls, phenylpyrimidines, phenylcyclohexanes, and Schiff bases—have been synthesized and used in practical displays.^[1, 2]

The major disadvantage with these types of devices is the narrow and nonuniform viewing cone, which is considered to be an unacceptable aspect of their performance. Several methods have been put forward to improve the viewing angle characteristics, for example the multidomain technique,^[3] the introduction of an optical compensator to reduce the amount of light leakage in the dark state,^[4] the application of an electric field parallel to the plane of the substrates,^[5] and the “amorphous” twisted nematic liquid crystal displays (TNLCD).^[6] We recently disclosed a novel approach to overcome this problem by utilizing discotic nematic liquid crystals instead of calamitic nematic liquid crystals.^[7] Compared to the large number of calamitic molecules showing a nematic phase, there are only few disk-shaped molecules showing a discotic nematic phase N_D. Several derivatives of triphenylene,^[8, 9] truxene,^[10] thiotruxene,^[11] and naphthalene^[12] show stable discotic nematic phases, but these phases are high-temperature and narrow. For any application as a liquid crystal device, stability of the mesophase well below and above room temperature is one of the most important criteria.

Hitherto, no room-temperature discotic nematic liquid crystal was known. The common procedure for extending the mesophase range of calamitic liquid crystals is by mixing different components and preparing an eutectic mixture, but such a procedure has not been applied to discotic nematic till now. Therefore, it is of great practical interest to prepare room-temperature discotic nematic liquid crystals. Here we report on the synthesis of the first example of a room-temperature discotic nematic liquid crystal.

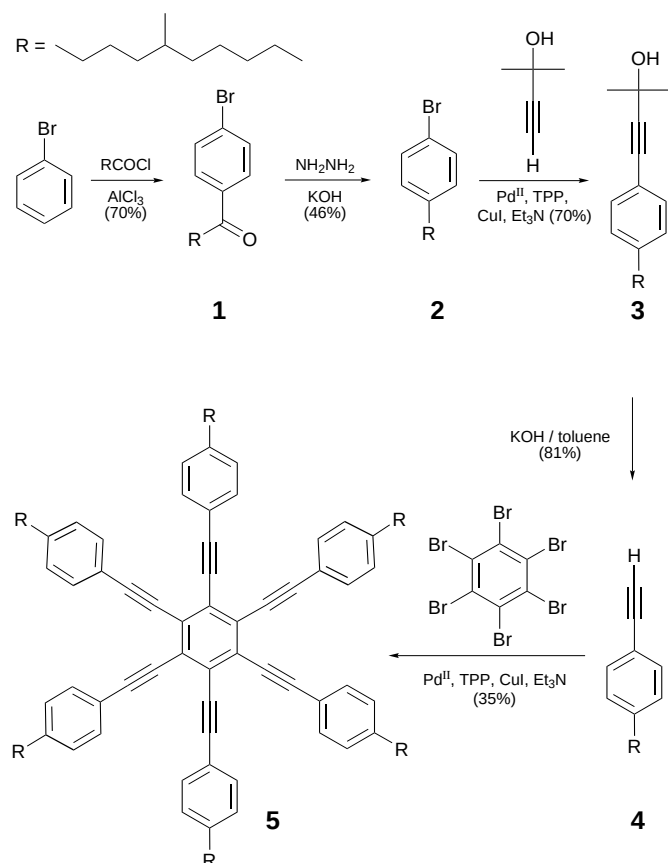
Hexa- and pentaalkynylbenzene derivatives having N_D phases have recently received a great deal of attention as their clearing temperatures are not very high and they show a chiral nematic-discotic phase on doping with suitable chiral molecules. Several hexa- and pentaalkynylbenzenes and their dimers were prepared and studied extensively.^[9, 13]

When the peripheral alkyl chains are attached to the phenyl ring in the hexaalkynylbenzene derivatives through a hetero-

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atom such as oxygen, the melting and clearing temperatures are higher than when the alkyl chains are directly attached to the ring.^[14] Furthermore when the peripheral aliphatic side chains of various cores are branched, the mesophase is widened, but the type of the mesophase formed is not affected by the introduction of branching in many cases.^[15] We anticipated that by replacing the normal alkyl chains by branched alkyl chains connected directly to the phenyl ring, the melting points of the alkynylbenzene derivatives would reduce and thus stabilize the mesophase. The synthesis of the new branched chain hexalkynylbenzene is outlined in Scheme 1.



Scheme 1. Synthesis of **5**. TPP = triphenylphosphane.

4-Methylnonanoic acid was converted into the acid chloride with oxalyl chloride at room temperature. Friedel–Crafts acylation of bromobenzene with this acid chloride gave 1-bromo-4-(4-methylnonanoyl)benzene (**1**). Wolff–Kishner reduction of this ketone furnished 1-bromo-4-(4-methylnonyl)benzene (**2**). Palladium-catalyzed alkynylation of **2** with 2-methyl-3-butyn-2-ol afforded the protected phenylacetylene **3**, which was deprotected with KOH in refluxing toluene to yield phenylacetylene **4**. Palladium-catalyzed coupling^[16] of this branched, alkyl-chain-substituted species with hexabromobenzene provided the desired hexakis[4-(4-methylnonyl)phenylethynyl]benzene (**5**). The product, purified by repeated column chromatography, was homogenous according to thin-layer chromatography (TLC), and the HPLC purity was found to be 99.8% (silica, hexane; flow

1.2 mL min⁻¹, retention time 39.25 min.). The spectral data were in accordance with the structure.^[17] Both the starting material (4-methylnonanoic acid) and the product **5** were optically inactive.

The thermal behavior of **5** was checked by polarizing microscopy and by differential scanning calorimetry (DSC). The compound exhibits textural features of a nematic phase at room temperature (Figure 1). On heating it transforms to the



Figure 1. Optical textures of **5**. Photomicroscopic images of the mesophase were obtained with a polarizing microscope (Leitz DMRXP equipped with a Mettler FP82HT hot stage) on cooling from the isotropic liquid at 25 °C (crossed polarizer, magnification $\times 100$).

isotropic phase at about 70 °C. The nematic phase reappears with very limited supercooling and crystallizes at –12 °C. These crystals melt at about 6 °C on subsequent heating. The DSC traces obtained on the heating and cooling runs are shown in Figure 2.

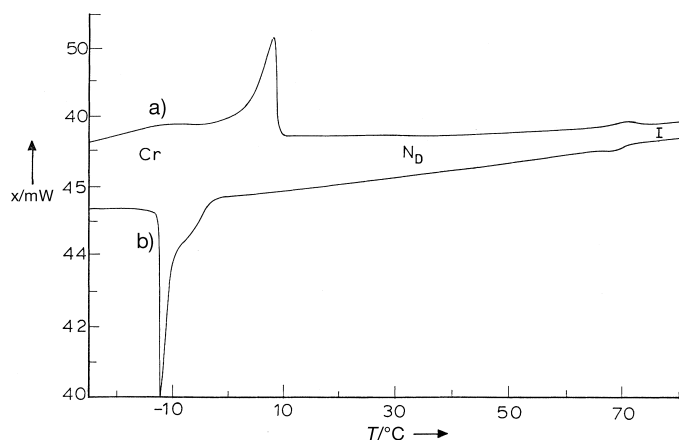


Figure 2. DSC traces for compound **5** on a) heating and b) cooling (scan rate 5 °C min⁻¹). x = heat flow, Cr = crystal phase, Nd = nematic phase, I = isotropic phase.

Compound **5** shows a broad melting transition at 6.1 °C (peak temperature, enthalpy 18.1 kJ mol⁻¹) and a transition from nematic discotic to isotropic at 69.3 °C (enthalpy 0.3 kJ mol⁻¹). The cooling run shows the isotropic to nematic transition at 68.1 °C (enthalpy 0.2 kJ mol⁻¹) and a broad transition from the nematic phase to the crystal phase at –12.2 °C (enthalpy 18.2 kJ mol⁻¹). However, we realized that the thermal behavior of **5** obtained in different batches is not

consistent, though the spectral data are identical. This could be due to the stereoheterogeneity of the product (with one chiral center in each of the six side chains, the product derived from racemic starting material consists of a mixture of stereoisomers). A mixture of stereoisomers could be the cause of the varying melting ($0-10^{\circ}\text{C}$) and clearing points ($40-80^{\circ}\text{C}$) in different batches.

Efforts to crystallize the material in various solvents at low temperature met with failure, but **5** can be fractionalized by triturating with acetone at about -10°C . Two such treatments give a product with higher melting and clearing points. Fractionation of **5** is also possible by column chromatography over alumina and elution with hexane/dichloromethane in small fractions (1 mL). The first few collections have a relatively lower clearing point ($40-50^{\circ}\text{C}$) than the latter fractions ($70-80^{\circ}\text{C}$). This indicates that the product **5** is a statistical mixture of stereoisomers. The different solubilities of the isomers account for the variations in the composition of the different batches. The preparation of pure stereoisomers of 4-methylnonanoic acid and other derivatives is currently under investigation.

The nematic phase shown by **5** is stable well below and above ambient temperature. Previously, experimental studies have been presented to demonstrate the wide and symmetrical viewing angle of devices having similar but high-temperature nematic discotic material.^[7] Similar studies with the present room-temperature nematic discotic material will be presented in due course.

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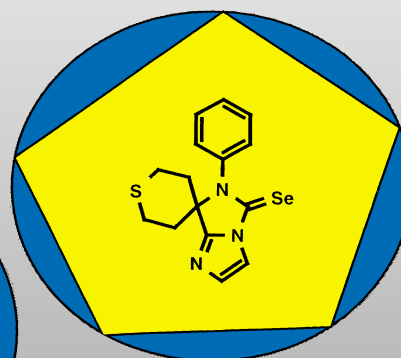
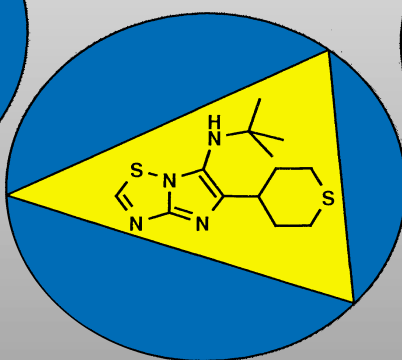
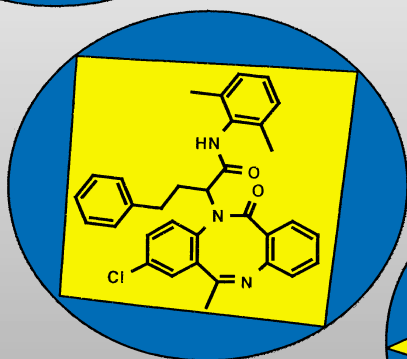
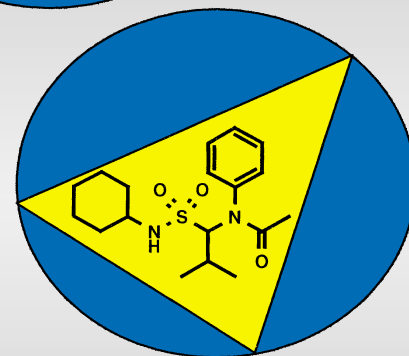
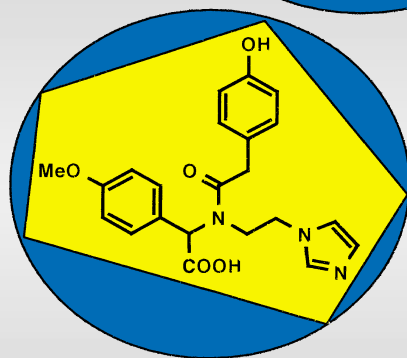
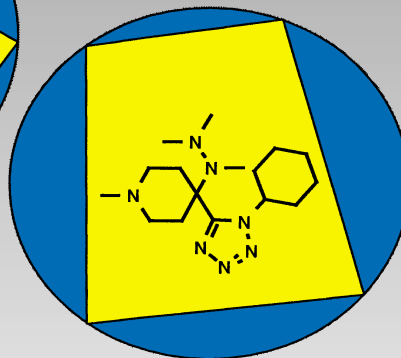
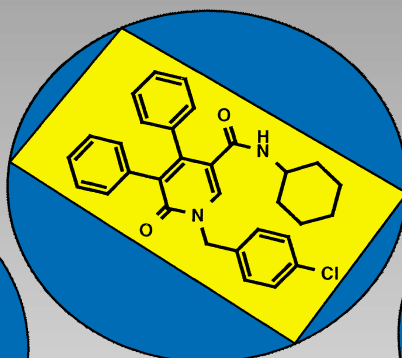
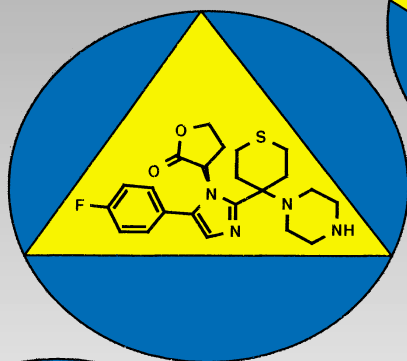
Cover Picture

Ivan S. Neretin, Konstantin A. Lyssenko, Mikhail Yu. Antipin, Yuri L. Slovokhotov, Olga V. Boltalina, Pavel A. Troshin, Andrei Yu. Lukonin, Lev. N. Sidorov, and Roger Taylor

The cover picture shows the single-crystal X-ray structure for $C_{60}F_{18}$, the first fully characterized aromatic fullerene, possessing a flat and fully delocalized hexagonal benzenoid ring embedded at the center of the fluorinated crown. The bond lengths in this hexagon are all of equal length (1.372 Å). The un-addended part of the molecule possesses the normal C_{60} fullerene structure so that the overall appearance is that of a tortoise shell. $C_{60}F_{18}$ is potentially an important building block for donor–acceptor derivatives with photovoltaic/photonic applications, combining enhanced cage electron withdrawal, with a vacant area for location of donors. The unique geometry also makes the molecule a hexasubstituted benzene, which may be able to participate in η^6 coordination to metallic species. Details on this tortoise molecule are described by Slovokhotov, Boltalina, and Taylor et al. on p. 3273 ff.



MCR



Multicomponent Reactions with Isocyanides

Alexander Dömling* and Ivar Ugi

Multicomponent reactions (MCRs) are fundamentally different from two-component reactions in several aspects. Among the MCRs, those with isocyanides have developed into popular organic-chemical reactions in the pharmaceutical industry for the preparation of compound libraries of low-molecular druglike compounds. With a small set of starting materials, very

large libraries can be built up within a short time, which can then be used for research on medicinal substances. Due to the intensive research of the last few years, many new backbone types have become accessible. MCRs are also increasingly being employed in the total synthesis of natural products. MCRs and especially MCRs with isocyanides offer many opportunities to

attain new reactions and basic structures. However, this requires that the chemist learns the “language” of MCRs, something that this review wishes to stimulate.

Keywords: combinatorial chemistry • isocyanides • multicomponent reactions • synthetic methods

1. Introduction

Organic-chemical syntheses have reached a high degree of skilfulness. There is hardly a complicated natural product that escapes a multistep total synthesis, in which the target molecules are often synthesized divergently rather than convergently, or sequentially, in many steps according to their complexity. The progress made in the last decades in the total synthesis of natural products becomes visible when Woodward's strychnine synthesis is compared with newer syntheses.^[1] It is founded in part on the optimization of synthetic operations and reactions. For example, organometallic variations of classic reactions often are more diastereo-, enantio-, regio-, and/or chemoselective and often afford the products in higher yields. Improved analytical and separation methods contribute to more efficient synthetic steps. Whereas Woodward obtained strychnine from his 28-step synthesis in $6 \times 10^{-5}\%$ overall yield, the groups of Magnus, Overman, Kuehn, and Rawal were able to produce this complex compound in less synthetic steps and with 10^3 to 10^4 times higher total yield.

The “ideal synthesis” (Figure 1) should lead to the desired product in as few steps as possible, in good overall yield and



Figure 1. The ideal chemical synthesis (adapted from Wender et al.).^[2] Although virtually no natural product, however complex it may be, escapes a chemical total synthesis, the organic synthesis is far from the elegant, effective and economical biosynthesis.

by using environmentally compatible reagents.^[2] The synthetic variables that have to be optimized are time, costs, overall yield, simplicity of performance, safety, and environmental acceptability. In multistep syntheses the temporal and preparative complexity increases in proportion to the number of steps in a first approximation. It is reflected in many isolation and purification operations, such as crystallization, extraction, distillation, or chromatography.

Besides the multistep, sequential synthesis of a target molecule, the desired product can also be obtained in one-pot reactions of three or more starting compounds, the multicomponent reactions (MCRs), in many cases. Here the starting materials do not react simultaneously in one step, but rather in a sequence of elementary steps according to a program. Reactions with an irreversible step, which drives the

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preceding equilibrium to the product side, are especially favorable. In contrast to two-component reactions, MCRs are especially variable. Very many products can be synthesized from only a few starting substances. Many common MCRs fall back on easily accessible, archetypical functional groups, which are available in a great variety, that is an MCR is economical with resources. As MCRs, being one-pot reactions, are practically single-step conversions, they are easier to carry out than multistep syntheses. Thus, the MCRs already come quite close to the idea of an “ideal synthesis”. If a product can be synthesized by means of an MCR, this procedure offers many advantages.

A large and important class of MCRs are the isocyanide multicomponent reactions (IMCR). The chemistry of isocyanides began in 1838. Apart from short active periods, isocyanide chemistry has slumbered for more than a century (Figure 2).

In the last 150 years, the classical MCR chemistry was developed, which is summarized in 1960 in the book “ α -Aminoalkylierung” by Hellmann and Opitz.^[3] Many of these classical MCRs are name reactions, among them well-known classics, such as the Mannich reaction (a three-component reaction, M-3CR) or its intramolecular variant, the Pictet–Spengler reaction (a two-component reaction, PS-2CR).

A short time after the Ugi four-component reaction (U-4CR; January 1959) was discovered, it was found that xylocain

4—an important local anaesthetic then as well as now—could be synthesized in only one step from 2,6-dimethylphenyl isocyanide (**1**), formaldehyde (**2**), and diethylamine (**3**) with good yield (Scheme 1). Ownership of the patent which was immediately applied for^[4] changed over to the company A.B. Astra. At that time, Ivar Ugi was scientific consultant for the Swedish company. He recommended to the synthetic chemists and research heads to produce whole collections^[5] of different α -amino amides, based on the four-component reaction which is highly variable and easy to carry out in one pot, and to test them for their anaesthetic effect. Later, more than a dozen local anaesthetics based on the xylocain structure were developed and marketed by various pharmaceutical companies.

In the last decade a change of thought has taken place in the pharmaceutical industry which has led to a renaissance of the MCRs. Characteristic for this paradigm change in pharmaceutical research are the fields of combinatorial chemistry, high-throughput screening, and genome and proteome analysis.^[6] Whereas only a few years ago the substance productivity of a medicinal chemist was relatively low, today thousands of substances can be produced and characterized every day by means of automated methods.

Although the suitability of the MCRs for building up large compound libraries was published as early as 1961,^[7] these reactions were of little interest in the following decades.

Alexander Dömling, born in 1964, studied biology and chemistry at the Technische Universität München and completed his doctorate in chemistry under the supervision of Prof. Ivar Ugi in 1994. As holder of a Humboldt fellowship he spent one year at the Scripps Research Institute in the group of Barry Sharpless. Then he began his habilitation at the TU München. In 1996 he founded the company Morphochem AG with Wolfgang Richter. In the same year he was awarded the Friedrich-Weygand prize by the Max Bergmann circle. His research interests are the discovery of new multicomponent reactions and their application in total syntheses of natural compounds, ideally in one step (one-pot syntheses). Further interests are in “other forms of life” and complex artificial enzymes



A. Dömling

I. Ugi

Ivar Ugi, born in 1930 in Estonia, studied chemistry at the Universität Tübingen from 1949 to 1952. In 1954 he obtained his doctorate at the Universität München. His habilitation on aryl pentazoles and isocyanides followed in 1959. From 1962 to 1968, he worked in the Central Research Laboratories of the Bayer AG in Leverkusen, where he was the chairman of the commission for basic research and head of research for the last three years. In 1964 he was awarded the research prize of the scientific academy in Göttingen. From 1968 to 1971 he was a professor at the chemical institute of the University of South California, Los Angeles (USA), and then became a professor for organic chemistry at the Technische Universität München in Garching (Germany), holding the Hans Fischer chair, where he remained until 1999. He is a member of the Royal Swedish Academy of Science in Uppsala (since 1987), the Estonian Academy of Science (since 1991), and the US Academy of Science in New York (since 1994). In 1988 he received the Challenge Future Prize from the Philip Morris Foundation, in 1992 the Emil Fischer Medal from the Gesellschaft Deutscher Chemiker for his discovery of the four-component condensation and the development of mathematical models for the logical structures of chemistry, in 1995 the Ugi-Dugundji-Medal, awarded for the first time, to honor his achievement in applying mathematics and information technology to chemistry, and in 1999 he was awarded the Max Bergmann Medal.

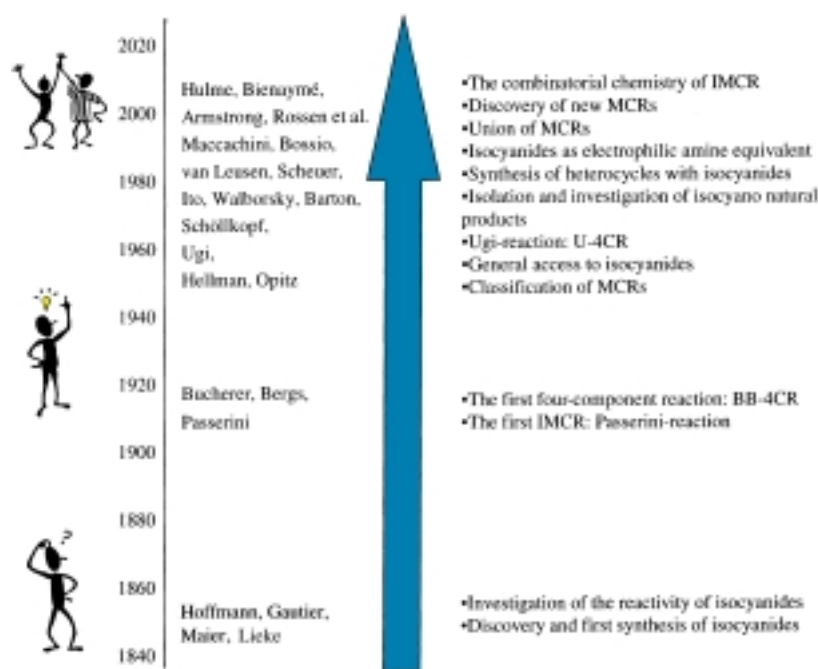
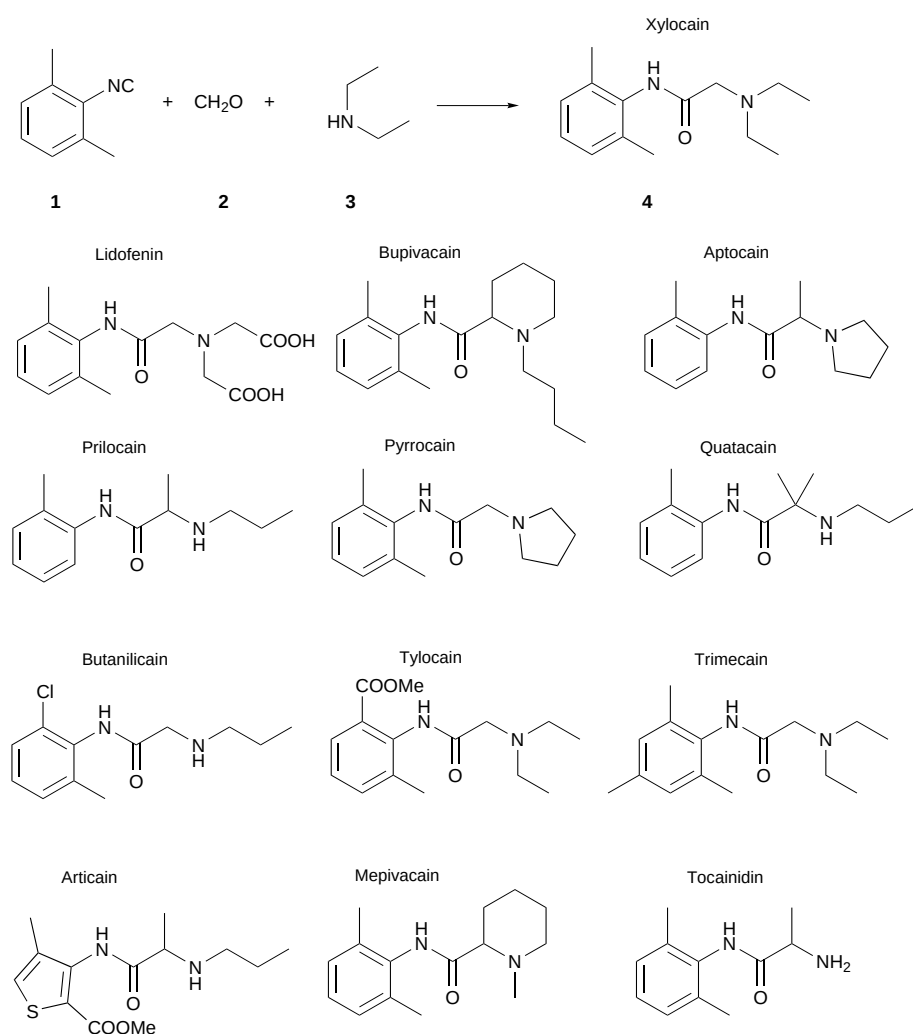


Figure 2. The history of the isocyanides and that of their MCRs took place in three large, parallel waves. The first wave was the constituting one, in which the isocyanides were discovered and their structure and identity recognized, some properties were examined and limited preparative access was found. In the following, research on this area was sporadic with only a few publications until the Italian Passerini discovered the self-named, first IMCR and investigated it for many years (the second wave). It grew silent again for many decades until a general and good approach to the class of the isocyanides was found. In this third wave, the largest so far, which may not even have reached its climax yet, the Ugi reaction and many new IMCRs, many biologically active natural compounds, new heterocycle syntheses with isocyanides and other important synthetic applications of isocyanides were described. Finally, the immense advantages of the IMCRs in drug research were recognized (the figures at the edge of the diagram were taken from Microsoft ClipArt).



Scheme 1. One-pot synthesis of xylocain (4) and a selection of commercial analogues based on the α -aminoamide structure.

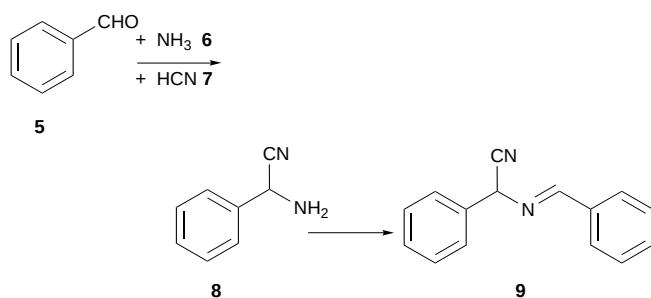
Especially due to their efficiency, the ease of their automation, and the enormous number of possible products, they have moved into the focus of contemporary endeavors to find new active substances in shorter periods of time. Great efforts have been and still are being made to find and develop new MCRs.^[8b] With the concept of unions of MCRs described in Section 7, new reactions of even more components have been and will in future be described.

In the following the history and the historical development of the MCRs in the last one and a half centuries are presented. The overview pays special attention to new aspects and applications of the MCRs of isocyanides;^[8] other MCRs are only mentioned in passing. The new IMCR literature is summarized and discussed with special emphasis on combinatorial applications, and new concepts of the MCRs are introduced.

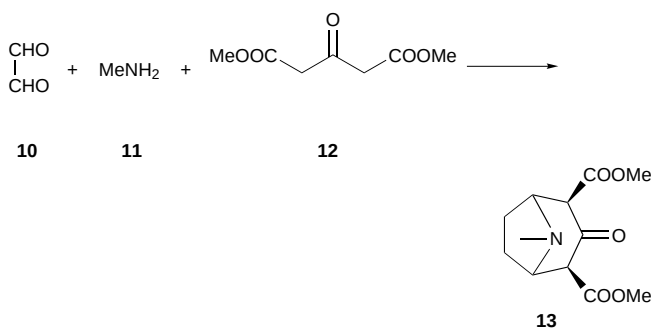
1.1. History of the MCRs

The general ordering principle of MCRs is followed by the α -amino alkylation, in which an oxo compound and a primary or secondary

amine undergo electrophilic addition to an electron-rich position of a molecule.^[3, 9, 10] The Strecker synthesis of α -amino acids via α -amino cyanides was first published in 1850 and is generally considered to be the first MCR.^[11] However, in the reaction of bitter almond oil and ammonia 12 years earlier, Laurent and Gerhardt isolated a poorly soluble product that had evolved in an MCR.^[12] In this reaction, the crude product, hydrocyanic acid-containing benzaldehyde (**5**/**7**), reacts in a Strecker reaction with ammonia (**6**), giving aminobenzyl cyanide (**8**), whose Schiff base with benzaldehyde was called “benzoyl azotide” **9**.

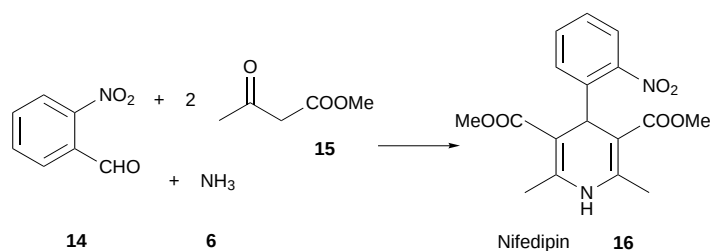


Robinson's synthesis of the alkaloid tropinone (**13**) from succinic dialdehyde (**10**), methylamine (**11**), and dimethyl acetonedicarboxylate (**12**), carried out in 1917, is the first important application of MCRs in natural product synthesis.^[13] Two decades later, Schöpf was able to carry out analogous syntheses under physiological conditions.^[14] Mannich-type reactions (M-3CR) proved in the following to be extremely valuable for the total synthesis of natural products.^[10]



Many important heterocycle syntheses are MCRs. 1,4-Dihydropyridines such as **16** were first synthesized over one hundred years ago in a four-component reaction by Hantzsch (H-4CR) from ammonia (**6**), aldehyde **14** and acetoacetic ester **15**.^[15] As this preparation is particularly simple to carry out, it was listed in the popular laboratory manual by Gatterman for half a century.^[16] Finally, at the Bayer AG company, a very successful dihydropyridine preparation for the therapy of cardiovascular disease named Nifedipin was developed, based on the Hantzsch synthesis.^[17]

The first MCR with isocyanides was described in 1921 by the Italian Passerini (P-3CR).^[18] In many publications, he was able to show the preparative possibilities of this reaction.^[19a]



A further important MCR is the Bucherer–Bergs reaction (BB-4CR).^[20] It can be understood as an extension of the S-3CR by one component (CO_2). Whereas the Strecker three-component reaction (S-3CR) is an equilibrium reaction and often delivers the products in unsatisfactory yields, the BB-4CR is practically irreversible upon addition of CO_2 . It still is an important method for the synthesis of unnatural α -amino acids.

Some more important MCRs leading to heterocycles are listed in Table 1.

1.2. Isocyanides

Isocyanides, formerly known as isonitriles, are compounds with an extraordinary functional group. Its unusual valence structure and reactivity were discussed for over one and a half centuries. Isocyanides are the only class of stable organic compounds with a formally divalent carbon.^[27] In exothermic reactions C^{II} is oxidized to C^{IV} . This was already noted in 1892 by Nef.^[28] Owing to its reactivity the isocyanide group differs fundamentally from other functional groups.

Hundreds of isocyanide group containing natural products were isolated, above all from marine species. The name Scheurer is synonymous for the investigation of natural isocyanides from marine sources.^[29] Many natural isocyanide show a strong antibiotic, fungicidal, or antineoplastic effect. The potential of isocyanides as possible agents for crop protection, discovered as early as the 1960s, also found its way into the patent literature,^[30] in which the antibiotic, acaricidal, fungicidal, or insecticidal activity with simultaneous low toxicity for warm-blooded animals is described. Di- and triisocyanides stand out because of their extraordinary antibiotic activity and often show no signs of resistance even after hundreds of generations.^[31] Antifouling properties similar to those of copper sulfate were recently described for marine, terpenoid isocyanides.^[32]

Many natural products are isolated as *N*-formamides. As these can be regarded either as precursors or as products of the hydrolysis of isocyanides, presumably many more isocyanides occur naturally than is generally assumed. A small selection of bioactive natural products with isocyanide functionality, **17**–**28**, is shown in Scheme 2.

Isocyanides were first synthesized in 1859 by Lieke, who did not recognize them as such and first believed them to be nitriles.^[33] He tried to transform the putative nitriles into the corresponding carboxylic acids by means of hydrolysis, but obtained formamides instead. At the time, isocyanides were

Table 1. Many of the historically significant MCRs are based on the reactivity of carbonyl or imine groups.

Name of the reaction	Year of discovery	Example ^[a]
Strecker synthesis ^[10]	(1838) 1850	
Hantzsch dihydropyridine synthesis ^[15]	1882	
Radziszewski imidazole synthesis ^[21]	1882	
Hantzsch pyrrole synthesis ^[22]	1890	
Biginelli reaction ^[23, 24]	1891	
Mannich reaction ^[25]	1912	
Bucherer–Bergs hydantoin synthesis ^[20, 26]	1941	

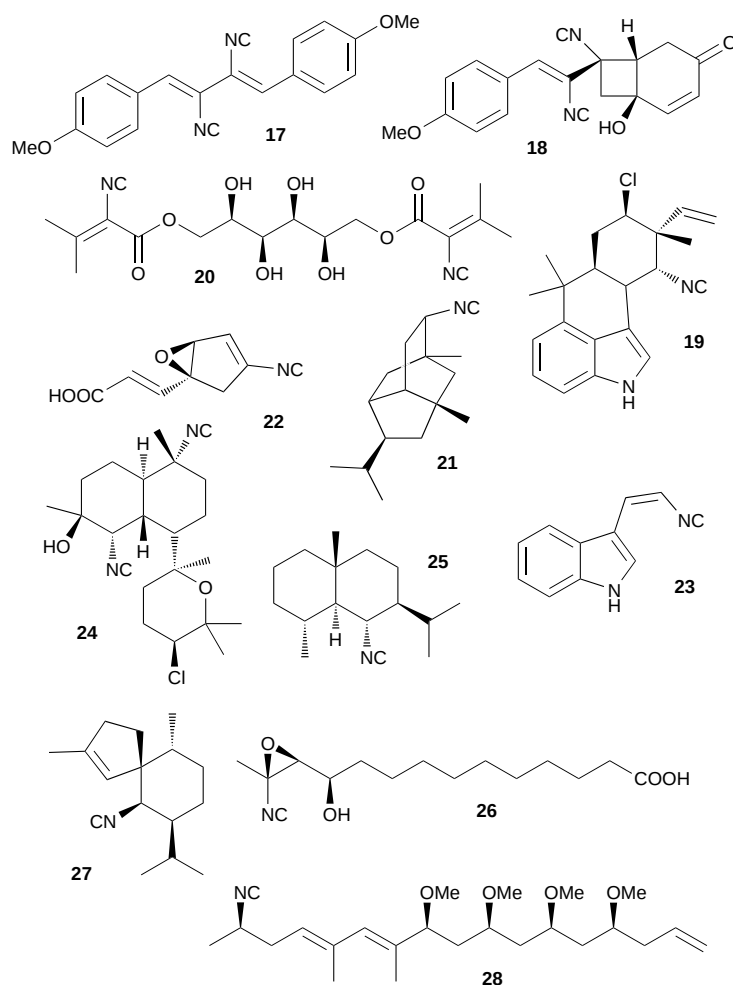
[a] T = thymine.

produced in substitution reactions of reactive alkyl halides with silver cyanide. Owing to the poor scope of substrates for this reaction, only few isocyanides were available. The isocyanides stood out because of their strange odour which forced the chemists to work outdoors. Almost all commercially available isocyanides are volatile and carry this repulsive odor “which is reminiscent of artichokes and phosphorus at the same time.”^[34] People who have inhaled volatile isocyanides such as allyl, benzyl, methyl, or *tert*-butyl isocyanide over a longer period of time report the sensory perception of the smell of hay.^[35] The more long-term inhalation of isocyanides is also said to increase the intensity of dreams at night. Other liquid isocyanides, such as the position isomeric picolyl isocyanides, on the other hand, do not smell. The isocyanide derived from L-phenylglycine methyl ester smells of rhubarb. In fact most isocyanides are solid and odorless. Gautier already investigated the toxic properties of isocyanides by dribbling them into the eyes of dogs.^[34] However, according to toxicological examinations of hundreds of isocyanides carried out in the 1960s by the Bayer AG company, this class of compounds is only slightly toxic, apart from few exceptions.^[36]

Once again, it was Gautier who first discovered the isomeric nature of relationship between the isocyanides and the nitriles.^[37] At the same time, Hoffmann found a new approach to isocyanides with the reaction of primary amines with potash and chloroform. All methods for the preparation of isocyanides known at the time were, however, complicated, poorly generalizable, and delivered the products in low yields. Often the isocyanides could not be separated from the accompanying nitriles. The preparative availability and their bad odor may have been the reasons why the chemistry of isocyanides was investigated only sporadically and not very intensely for 100 years. Whereas only twelve isocyanides were known in the first 100 years, 325 isocyanides were described by 1971.

1.2.1. Methods for the Preparation of Isocyanides

Even though dozens of methods for the preparation of isocyanides have been described,^[38] the reaction of the *N*-formamides with phosgene or phosgene surrogates such as di- and triphosgene or other inorganic dehydratants and matching bases is the method of choice regarding cost, yield, and



Scheme 2. Examples of naturally occurring isocyanides: the antibiotic xanthocillin (**17**) from *Penicillium chrysogenum*, leptocillin (**18**), hapalindole A (**19**), the antibiotic, antimycotic, and antihypertensive compound A-32390 (**20**) from the fungus *Pyrenochaeta spaeropsidales*, isocyanopupukean (**21**) from *Phyllidia varia*, 4-((*E*)-2-carboxyvinyl)-3,4-epoxy-1-cyclopentenyl isocyanide (**22**), B 371 (**23**) from *Pseudomonas* sp., the antimalarial kalihinol A (**24**) from *Chromobacterium spec.*, acenthelin-1 (**25**), aerocyanidine (**26**), axisonitrile-3 (**27**), and mirabilene isonitrile A (**28**).

execution in most cases.^[39] Another convenient and advantageous method of preparation is the dehydration with POCl_3 .^[40] Racemization-sensitive α -amino acid isocyanides can be synthesized with oxomethylenebis(3*H*-imidazolium)-bismethanesulfonate.^[41]

In the last century Gautier already attempted to obtain isocyanides from *N*-formamides.^[42] As he omitted to add appropriate equivalents of base to the reaction mixture, the isocyanide formed in situ soon was converted back to the *N*-formamide.^[43] The use of various organic bases such as triethylamine, pyridine, quinoline, 1,4-diazabicyclo[2.2.2]octane (DABCO), or diisopropylamine has been described. Depending on which further functionalities are present, many alternative production methods can be drawn upon (Table 2). Very sensitive isocyanides, such as the isocyano analogue 3'-azido-3'-deoxythymidine (AZT), can only be synthesized under special precautions.^[44] Finally, isocyanides were linked to the solid phase very early on.^[45]

Despite the obviously easy availability of isocyanides from accordingly variable precursors, only a few are commercially

available. However, for the combinatorial application of MCRs, all classes of starting materials should ideally be present with a great scope of compounds. An interesting solution to the problem is the combinatorial formation of isocyanides.

One possibility for the diversification of the isocyanide group was described by Bienaymé with the P-3CR. In the course of this the expression "reagent explosion" came up. He describes the synthesis of various β -dialkylamino α -isocyanoacrylates.^[58] *N*-imidazomethyl diethyl acetal (**29**) was treated with methyl isocyanoacetate (**30**) and primary as well as secondary amines to give the β -substituted isocyanoacrylates **31–36**.

Bossio's group, which is very productive in the field of IMCR, described the two-step synthesis of isocyanides by means of a U-4CR with ammonium formate and subsequent dehydration (Scheme 3).^[59] This method allows the combinatorial production of isocyanides from other isocyanides and aldehydes.

Based on the work by Schöllkopf, Armstrong et al. have developed an in situ method in which isocyanides are obtained from α -alkylations and employed in MCRs without former isolation.^[60] Different benzyloisocyanides are first treated with BuLi and alkylating reagents, then with aldehydes, carboxylic acids, and amines. This way the limited number of commercially available isocyanides can be increased significantly with little preparative effort.

Finally, the problem of isocyanide diversity and availability can also be circumvented by linking the isocyanide group to a solid phase. Many such examples are discussed in the following.

1.2.2. Chemistry of Isocyanides

The chemistry of isocyanides is characterized by three properties: the α -acidity, the α -addition, and the easy formation of radicals.

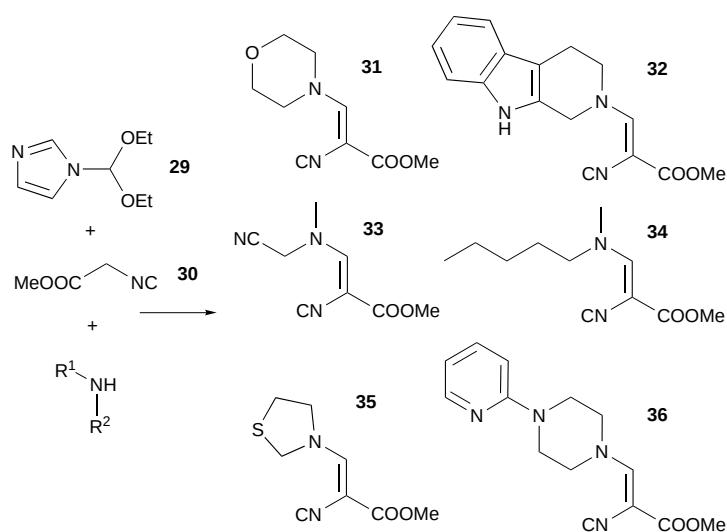
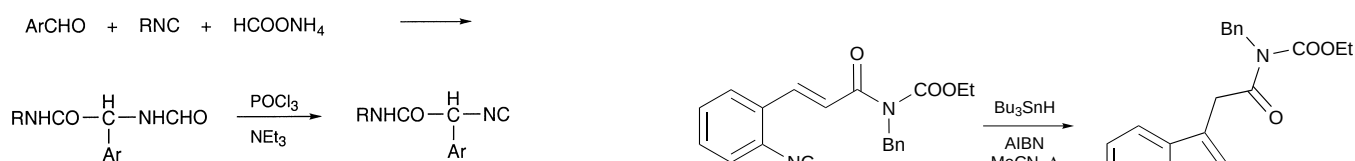


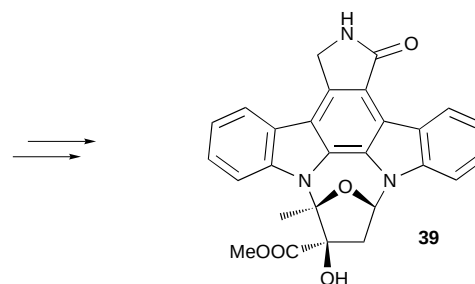
Table 2. Important preparative isocyanide syntheses with examples.

Method	Example ^[a]	Ref.
Lieke, 1859		[33]
Meyer, 1866		[46]
Gautier, 1867	the silver cyanide method, often referred to as the Gautier method, was first described by Lieke and Meyer.	[47]
Hoffmann, 1867	$\text{PhNH}_2 + 3 \text{KOH} + \text{CHCl}_3 \longrightarrow \text{PhNC} + 3 \text{KCl} + \text{H}_2\text{O}$	[48]
Hoffmann, 1870		[49]
Ugi, 1958		[50]
Ugi, Weber, Gockel, 1972	improved Hoffmann carbylamine method, in $\text{CH}_2\text{Cl}_2/\text{CHCl}_3/\text{H}_2\text{O}$ with phase transfer catalyst	[51]
Appel, 1972		[52]
Schöllkopf, 1971		[53]
Barton, 1988		[54]
Gassman, 1982		[55]
Baldwin, 1990		[56]
Kitano, 1998		[57]

[a] DABCO = 1,4-diazabicyclo[2.2.2]octane, TMS = Me_3Si , Tol = tolyl.

Scheme 3. Two-step isocyanide synthesis by means of a U-4CR and subsequent dehydration. This method allows the combinatorial preparation of isocyanides from other isocyanides and aldehydes.

Isocyanides and especially phenyl isocyanides are substrates for radical-induced cyclizations. Complex indole-containing natural products like the kinase inhibitor (+)-K252a (**39**) can be synthesized starting from *o*-isocyanocinnamic acid amides such as **37** (via **38**) by means of radical cyclizations (AIBN = α,α' -azoisobutyronitrile).^[61] A further famous example is the synthesis of the ABCD ring moiety of the



topoisomerase II inhibitor camptothecin and numerous derivatives by Curran et al.^[62]

The α -acidity as a striking feature of the isocyanides is increased by further electron-withdrawing substituents in the α -position such as carboxylic esters, nitriles, phosphonic esters, or sulfonyl groups. α -Metalated isocyanides are versatile starting materials for the synthesis of α,β -unsaturated isocyanides, heterocycles or amino acids.^[63] Schöllkopf found that α -metalated isocyanides are easy-to-handle α -amino anion equivalents.

Isocyanides polymerize under Lewis acid catalysis to polyiminomethylenes. The corresponding polymers have the structure of cylindrical helices.^[64]

The synthetically most important property of isocyanides is the reaction with nucleophiles and electrophiles at the isocyanide carbon atom. The α -addition of nucleophiles and electrophiles leads to the α -adduct (Figure 3). Most other

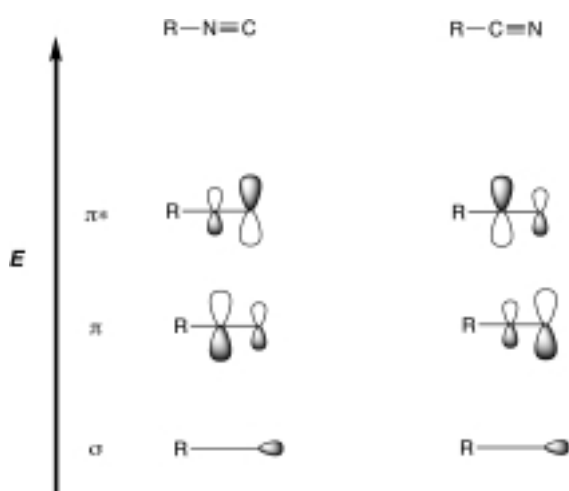


Figure 3. Qualitative comparison of the frontier orbitals of isocyanides and nitriles, showing the different reactivities of the two isomeric functional groups. Compared to the nitriles, the orbital coefficient of the isocyanides at the carbon atom in the π^* orbital is higher, leading to nucleophilic attack of the carbon atom. Electrophiles react with the σ orbital of the HOMO-1 and therefore also with the carbon atom. Nitriles, on the other hand, are attacked by nucleophiles at the carbon atom (higher π^* orbital coefficient) and by electrophiles at the nitrogen atom (higher π orbital coefficient).

functional groups in organic chemistry react with nucleophiles and electrophiles at different centers. Only carbenes and carbon monoxide share this property with the isocyanides. Thus, many parallels can be found in the chemistry of these classes of compounds.

One of the classic themes in the chemistry of isocyanides is heterocycle synthesis. The names of Schöllkopf and van Leusen stand for many pioneering developments in this field.^[65] Among others, useful imidazoline, oxazoline,^[66] thiazoline, pyrrole,^[67] imidazole,^[68] oxazole,^[69] and thiazole syntheses^[70] have been described. Enantioselective methods for the preparation of dihydrooxazoles and therefore of hydroxy-amino acids were elaborated by Ito and Togni with planar-chiral ferrocene catalysts.^[71] A comprehensive review of heterocycle syntheses with isocyanides has been published.^[72]

1.3. Definitions of MCRs

Reactions in which more than two starting compounds react to form a product in such a way that the majority of the atoms of the starting material can be found in the product are called multicomponent reactions. This rough definition is suitable to distinguish traditional two-component reactions and domino reactions, in which usually only one or two starting compounds are converted, from MCRs. A more sophisticated view is introduced in the following (Table 3).

Table 3. The basic types of MCRs.

MCR type	General reaction scheme
I	$A + B \rightleftharpoons C \rightleftharpoons \dots O \rightleftharpoons P$
II	$A + B \rightleftharpoons C \rightleftharpoons D \dots O \rightarrow P$
III	$A \rightarrow B + C \rightarrow D \rightarrow \dots O \rightarrow P$

MCRs whose starting compounds, intermediates, and products are in a mobile equilibrium are classified as type I MCRs. As different states of balance can prevail, yields between 0 and 100 % are possible. In most cases the products occur as mixtures with the intermediates and/or starting materials and are difficult to isolate. As the reaction often is incomplete, side reactions are possible which lead to further impurities.

MCRs whose elementary reactions are equilibria and irreversible partial reactions and whose last reaction step is irreversible belong to type II. Reactions of this type are preparatively advantageous as the total equilibrium is shifted to the side of the products by the last irreversible step. Such irreversible steps are, for example, the result of strongly exothermic reactions, such as the $C^{II} \rightarrow C^{IV}$ conversion of the isocyanides, a ring-closure reaction, or an aromatization.

MCRs of the type III are sequences of irreversible elementary reactions. They seldom occur in preparative chemistry. On the other hand, biochemical reactions in the living world fall into this category. Many of these reactions are de facto irreversible partial reactions, either due to the thermodynamic circumstances or due to the combination of endothermal with exothermal reactions (e.g. ATP coupling). These reactions correspond to enzymatically accelerated and mostly highly selective reactions so that significant amounts of side products are seldom formed. Should this nevertheless be the case, the side products are eliminated enzymatically and returned to the circuit.

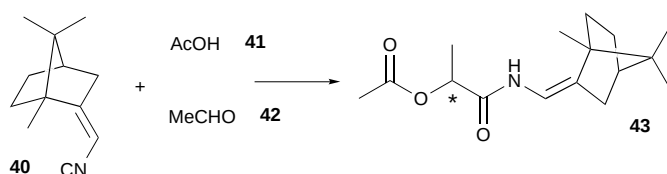
It must be considered in the above classification that these are idealizations. Many reactions can not be counted to one of the classes, rather the transitions are fluid.

In the following the Passerini reaction, the Ugi reaction, and other IMCRs as well as their new variants shall be introduced. It will be attempted to subdivide the presentation of the examples according to reaction as well as to structure features.

2. The Passerini Reaction

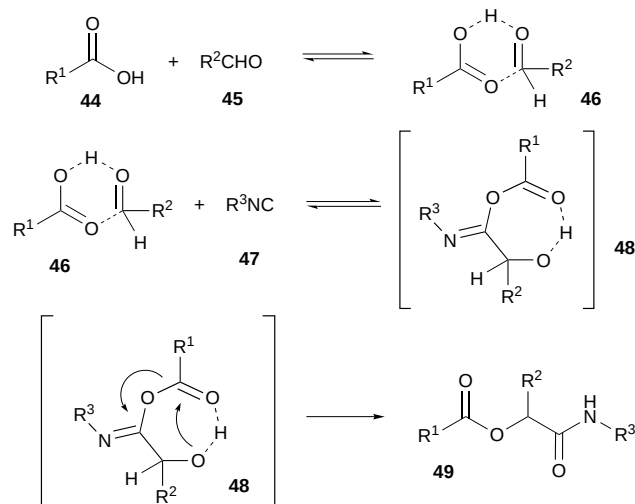
The classic reaction between carboxylic acids, oxo compounds and C-isocyanides, described by Passerini in 1921 and

later given his name, opens the access to α -acyloxy-carbox-amides in one step.^[18] The mechanism of the reaction has often been discussed.^[19] Kinetic and preparative investigations have led to different mechanistic suggestions.^[73] In contrast to the Ugi reaction, the Passerini reaction is accelerated in aprotic solvents, indicating a nonionic mechanism.^[74] The results concerning the influence of chiral isocyanides on the stereochemical course of the P-3CR seem contradictory. Mostly it was stated that chiral isocyanides have no influence on the diastereoselectivity of the P-3CR. The α,β -unsaturated isocyanide **40** derived from camphor is an exception. Thus, the P-3CR of the chiral isocyanide **40** with acetic acid **41** and acetaldehyde **42** yielded one diastereomer **43** with 93 % *de*.^[75]



Passerini himself postulated hemiacetals between the carboxylic acid and the oxo compound as intermediates. From kinetic studies and the observation of a third-degree reaction order, bipolar intermediates were assumed.^[19c] Other authors have discussed N-protonated isocyanides as reactive intermediates.^[19d] A P-MCR in the ligand sphere of organo-metallic catalysts has also been described.^[19e]

A plausible mechanism which agrees with the experimental data is the formation of a loosely hydrogen-bonded adduct **46** from a carbonyl compound **45** and a carboxylic acid **44**, followed by the α -addition of the electrophilic carbonyl carbon and the nucleophilic oxygen atom of the carboxylic acid to the isocyanide carbon of **47** (Scheme 4) under formation of a cyclic transition state with all three parent compounds. The α -adduct **48**, which cannot be isolated, rearranges in an intramolecular transacylation to the stable α -acyloxy carboxamide **49**.

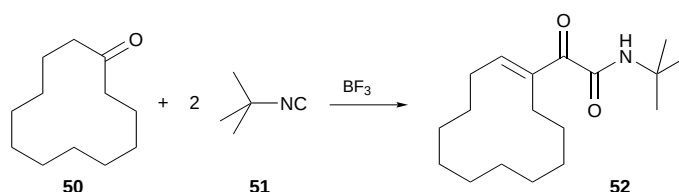


Scheme 4. Suggested mechanism of the P-3CR.

The Passerini reaction is carried out at high concentrations of the starting materials and in inert solvents at or below room temperature. There rarely are limitations concerning the oxo compound; thus, perfluorinated aldehydes and ketones also react, for example.^[76] Some sterically hindered and α,β -unsaturated ketones obviously are no substrates of the P-3CR.^[77] Bicyclic, sterically extremely hindered ketones such as camphor or bicyclo[3.2.1]octanone-2 do not react with isocyanides in a Passerini reaction, even in the presence of $\text{BF}_3 \cdot \text{OEt}_2$.^[78]

Besides normal C-isocyanides, Me_3SiCN (TMSCN), which is in equilibrium with TMSNC and is a formal derivative of the most basic isocyanide, namely isocyanic acid, also reacts with mineral acids in the presence of ZnI_2 to give 2-hydroxy-carboxylic acid amides.^[79]

If the ketone **50** is treated with two equivalents of the isocyanide **51** in the presence of $\text{BF}_3 \cdot \text{OEt}_2$ in nonpolar solvents, the β,γ -unsaturated α -oxocarboxylic amide **52** is formed.^[80] One equivalent of the isocyanide is the source of the α -C atom in this reaction.



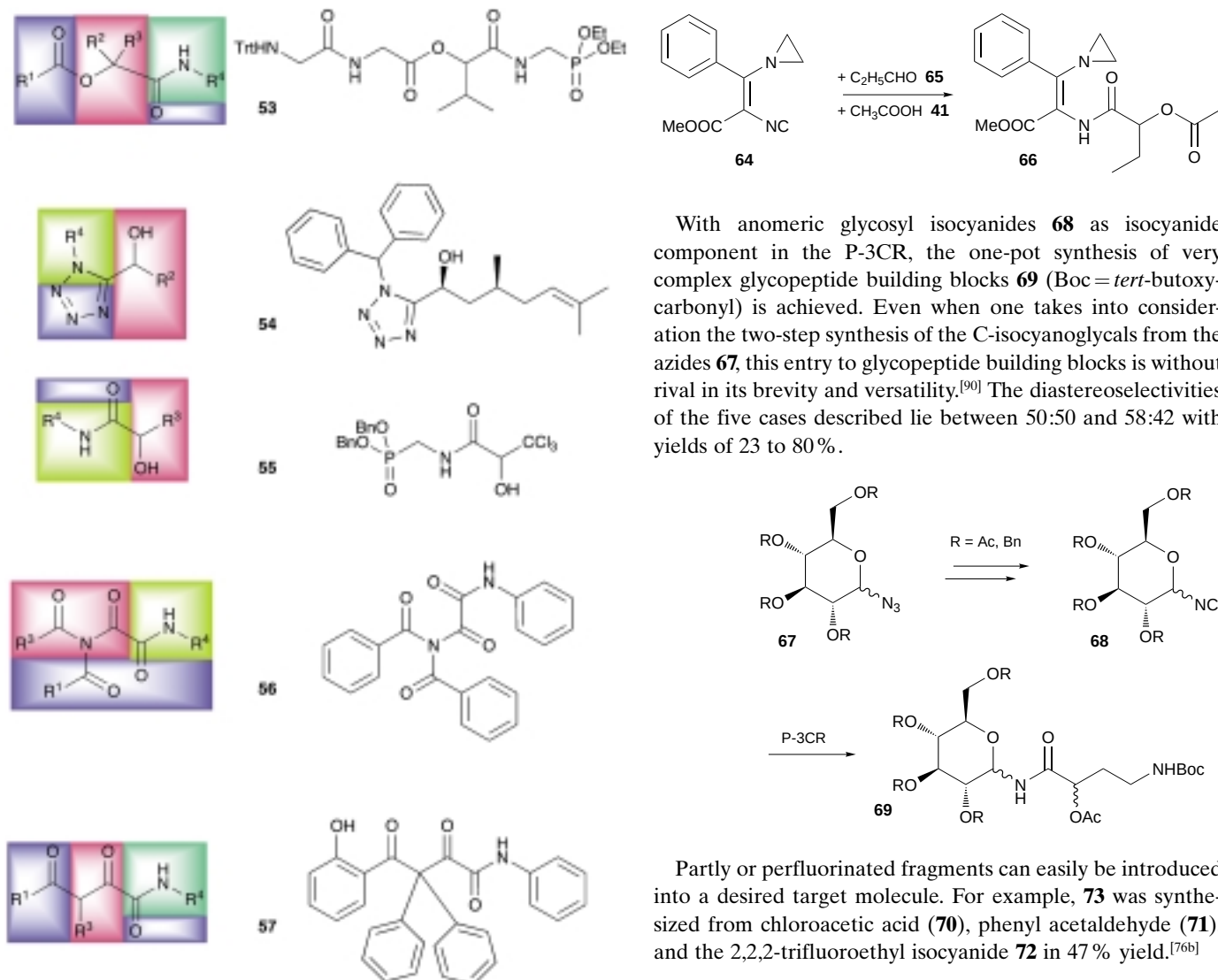
The α -acyloxy carboxamide group is a frequently recurring motif in many natural products, for example in the pharmacologically interesting depsipeptides. Thus, the P-3CR is particularly suitable for the synthesis of many compounds of this class and their derivatives. As an example, the synthesis of the complex phosphonic acid derivative **53** was achieved in one step from commercially available starting materials in 60 % yield (Scheme 5).^[81]

Lewis and Brønsted acids catalyze the formation of α -hydroxytetrazoles such as **54** from isocyanides, HN_3 , and oxo compounds.^[82, 83] Highly chlorinated oxo compounds are converted to α -hydroxycarboxamides such as **55**, even without catalysts.^[84] A little-known variant of the P-3CR is the reaction of aryl isocyanates with carboxylic acids and isocyanides under formation of *N*-alkyl or *N*-aryl *N',N'*-diacyloxamides such as **56**.^[85] The reaction of ketenes with isocyanides and carboxylic acids to α,γ -dioxocarboxylic amides such as **57** is also counted among the P-3CRs.^[86, 94]

Acetyl cyanide (**58**) as the oxo component was transformed into α -cyano- α -alkyloxamides in several examples in 75–89 % yield.^[87] Compound **61** can be isolated in 58 % yield after the reaction of formic acid **59** and isocyanide **60**.

The easily performed reduction of Passerini products opens the way to a multitude of *N*-substituted β -hydroxyamines. In this way, **63** can be isolated in a one-pot reaction without isolation of the Passerini product in quantitative yield over both steps! Many analogous examples have been described.^[88]

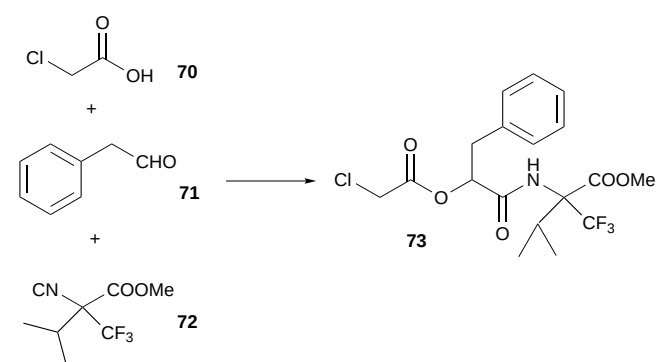
The parallel synthesis of dehydroamino acid derivatives such as **66** has been described.^[89] The alkene geometry



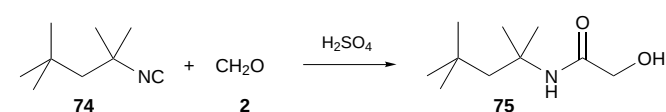
Scheme 5. Classic variations of the Passerini reaction. The components are color-coded: The acidic component is blue, the oxo component red, and the isocyanide component green or yellow. Trt = Ph₃C.

With anomeric glycosyl isocyanides **68** as isocyanide component in the P-3CR, the one-pot synthesis of very complex glycopeptide building blocks **69** (Boc = *tert*-butoxycarbonyl) is achieved. Even when one takes into consideration the two-step synthesis of the C-isocyanoglycals from the azides **67**, this entry to glycopeptide building blocks is without rival in its brevity and versatility.^[90] The diastereoselectivities of the five cases described lie between 50:50 and 58:42 with yields of 23 to 80 %.

Partly or perfluorinated fragments can easily be introduced into a desired target molecule. For example, **73** was synthesized from chloroacetic acid (**70**), phenyl acetaldehyde (**71**), and the 2,2,2-trifluoroethyl isocyanide **72** in 47 % yield.^[76b]



Finally, otherwise elusive *N-tert*-alkyl glyoxylic acid derivatives such as **75** can be obtained elegantly in one step in a P-3CR of tetramethylbutyl isocyanide **74** with formaldehyde **2** under acidic conditions.^[91]



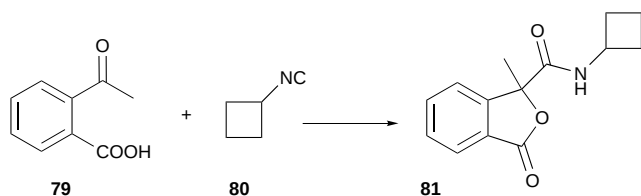
brought in by the isocyanides is retained in the target molecule. The dehydroamino acid moiety is introduced by α,β -unsaturated isocyanides like **64**. The dehydroamino acid motif occurs in many pharmacologically interesting natural products. Therefore, this reaction was also used for the synthesis of corresponding libraries.^[8b]

2.1. Cyclic Variations of the P-3CR

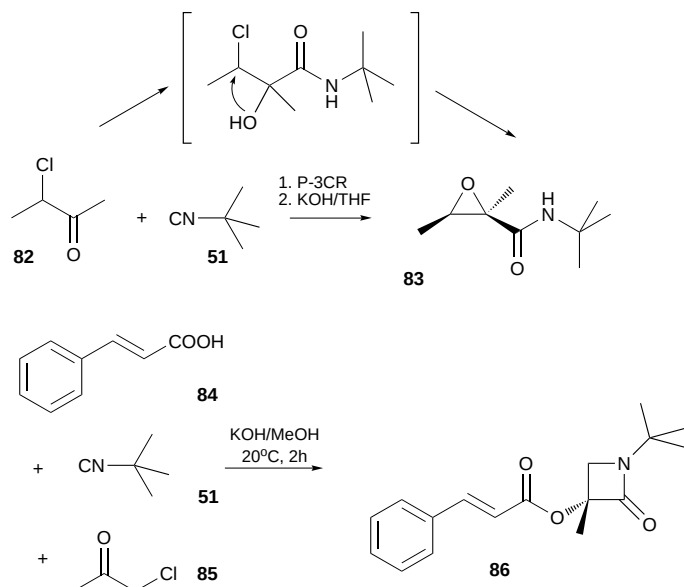
α -Hydroxytetrazoles such as **54** were described as early as 1931 as products of the reaction between hydrazoic acid, oxo compounds, and isocyanides.^[92] With less reactive oxo compounds, the well-known tetrazole formation due to the reaction of the isocyanide **76** with HN_3 **77** to **78** according to Oliveri-Mandala and Alagna comes to the fore.^[93] If $\text{Al}(\text{N}_3)_3$ is used as the azide source, unreactive oxo compounds can also be brought to react.^[73]



If bifunctional starting materials such as **79** with a ketone or aldehyde function and a carboxyl group are used in the P-3CR, lactones of various ring sizes, such as **81**, are obtained depending on the carbonyl–carboxylic acid distance.^[95]

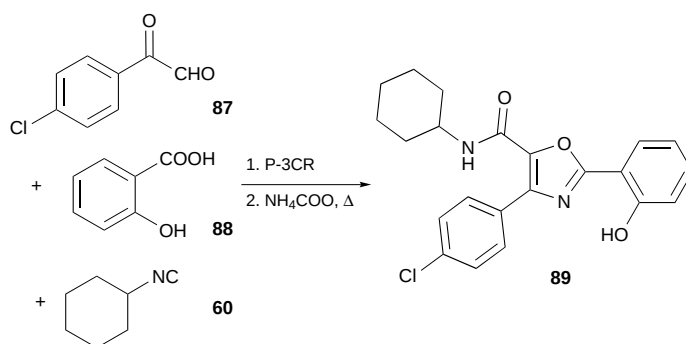


α -Epoxyamides such as **83** and 3-acyloxy-2-azetidinones like **86** can be synthesized from α -chloroketones (**82** and **85**, respectively), isocyanides (**51**) and carboxylic acids (**84**) depending on the conduction of the reaction.^[96, 97]

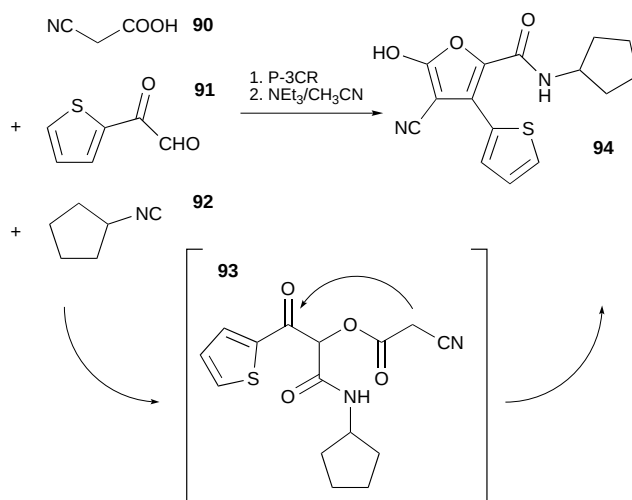


Oxazoles such as **89** can be generated very elegantly and with high diversity from α -oxoaldehydes (**87**), carboxylic acids (**88**), and isocyanides (**60**). The *N*-alkyl-2-acyloxy-3-aryl-3-

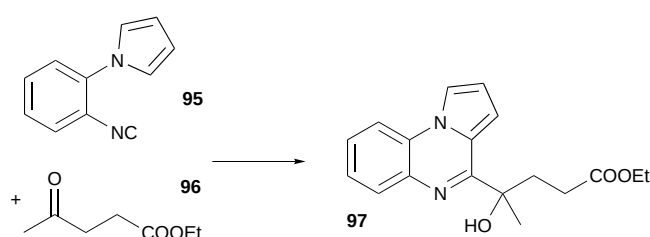
oxopropionic amides formed intermediately react smoothly and with good yields with ammonium formate in acetic acid under reflux, leading to the 2,4,5-trisubstituted oxazoles.^[98] All three substitutable positions of the oxazole can be varied independently in this two-step sequence.



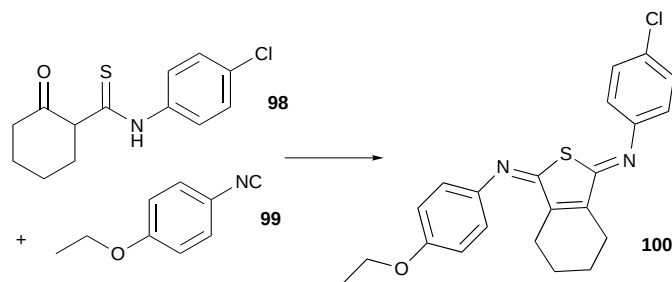
Cyanoacetic acid **90** as acid component, α -oxoaldehydes (**91**), and isocyanides (**92**) lead to the expected Passerini products (**93**), which cyclize to 2-hydroxyfurans such as **94** upon the addition of base in a Knoevenagel condensation.^[99]



A Japanese group has described an interesting intramolecular variation of the P-3CR which leads to heterocyclic CNS-active substances: The 1-(2-isocyanophenyl)pyrrole **95** obtained from 1,2-phenylenediamine reacts with oxo compounds (**96**) in the presence of $\text{BF}_3 \cdot \text{OEt}_2$, leading to 4-(1-hydroxyalkyl)pyrrolo[1,2-*a*]quinoxalines like **97**.^[100] Optimum catalytic properties were shown by $\text{BF}_3 \cdot \text{OEt}_2$ towards TiCl_4 , SnCl_4 , AlCl_3 , and ZnCl_2 . The pyrrole-CH group in 2-position reacts as a nucleophile or as the acid component.



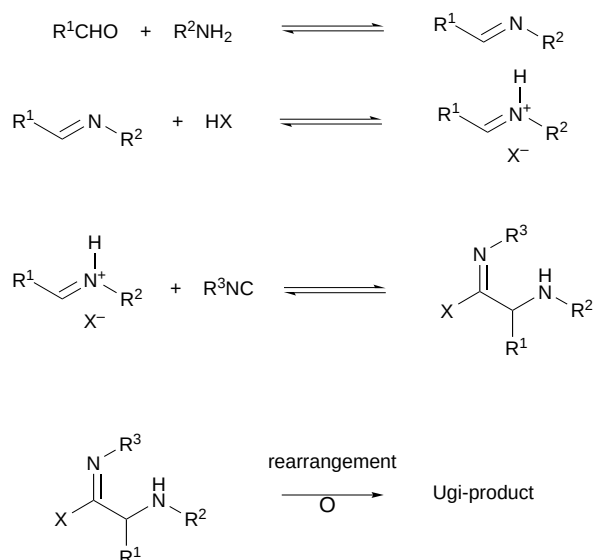
In another cyclic variation of the P-3CR, a β -oxothioamide (**98**) reacts intramolecularly as the acid and the oxo component to give benzo[*c*]thiophenes like **100**.^[101] The tautomeric pair thioamide $\rightleftharpoons$ α -sulfanylimide functions as the acid component.



The Passerini reaction has had a renaissance in the last few years. Compared with the Ugi reaction, however, the number of different suitable types of acid components is modest. The carbonyl group of the ketones and aldehydes in the P-3CR and the imine group of the imines in the U-4CR are isoelectronic. Thus, the chances of finding further new acid components that can be utilized in the P-3CR are high. Generally, many variations of the U-4CRs should also be possible as P-3CRs under accordingly modified reaction conditions. Finally, there are many as yet undiscovered ways to reach new basic structures starting from bifunctional compounds and using secondary reactions.

3. The Ugi Reaction

In 1959, Ugi et al. described the most important variants of the four-component condensation, the U-4CRs.^[102] Within a few weeks most of the condensation types known today were discovered. Carboxylic acids, hydrazoic acid, cyanates, thiocyanates,^[103] carbonic acid monoesters, salts of secondary amines, water, hydrogen sulfide as $\text{Na}_2\text{S}_2\text{O}_3$ and hydrogen selenide as the acid components in the U-4CR react with ketones or aldehydes, primary and secondary amines, hydrazines, and hydroxylamines as the amine components, and C-isocyanides.^[104] The Bayer AG company carried out investigations on the reaction mechanism and the theory of the stereoselectivity of the U-4CRs. In these investigations, extensive, analytically insoluble systems of equations were solved numerically with one of the first Zuse computers that were commercially available at the time.^[105] A strongly simplified reaction mechanism for U-4CRs with carboxylic acids as the acid component is shown in Scheme 6. In the first step the oxo component and the amine condense to the imine, the Schiff base, via a hydroxy aminal. It is also possible that the hydroxy aminal can be transformed directly in the course of the reaction without formation of a Schiff base under certain circumstances. Imines can be understood as carbonyl analogues. Like most imine reactions, the U-4CR runs better upon activation of the Schiff base. For this, the acid component protonates the nitrogen atom of the Schiff base,



Scheme 6. Simplified mechanism of the U-MCR and its main variants: The variability of the basic structures of the U-MCR depends above all on the acid component, but also on the properties of the other components.

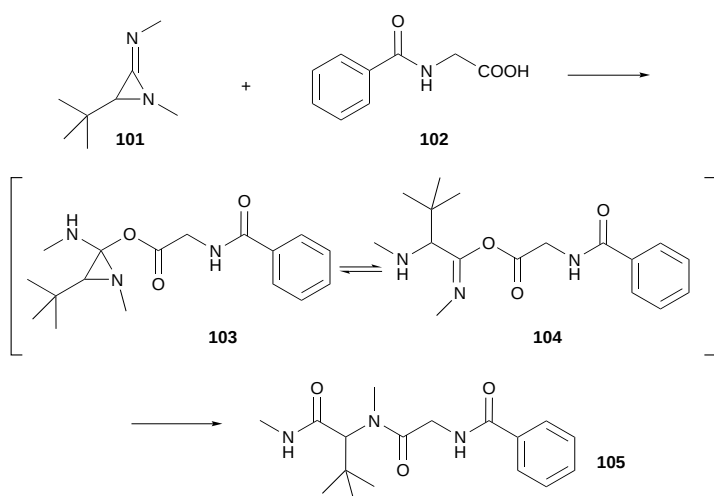
thus increasing the electrophilicity of the $\text{C}=\text{N}$ bond. Another way to increase the electrophilicity of the imines is the addition of Lewis acids such as TiCl_4 or $\text{BF}_3 \cdot \text{OEt}_2$. Depending on the solvent, the ions can be present as a salt pair or separately. The electrophilic iminium ion and the nucleophilic acid anion add to the isocyanide carbon atom. The α -adduct thus formed can be seen as a hetero analogue of an acid anhydride in which an *exo*-oxygen atom has been substituted by an NR group. Acid anhydrides are strong acylating agents, as are their heteroanalogues formed here. The closest acylable atom is the nitrogen of the former amine. After an intramolecular acylation and subsequent hydroxylimine $\rightarrow$ amide rearrangement the stable Ugi product is obtained. This type of intramolecular acylation was first described in 1910 by Mumm and was subsequently called the Mumm rearrangement.^[106] All elementary steps of this reaction sequence are equilibria; however, that of the last step, the rearrangement to the stable α -acylaminoamide, lies exclusively on the product side. In this respect this type II MCR is very different from other MCRs. The driving force of the total reaction sequence is the oxidation of the isocyanide C^{II} atom to the amide C^{IV} atom.

It is instructive to follow the changes in nucleophilicity and electrophilicity of the components during the U-4CR. In the course of the individual steps the reactive centres of the acid component and the imines change the sign of their reactivity several times. At first the $\text{C}=\text{N}$ bond of the imine behaves like a base towards the acid component. Then the protonated Schiff base functions as the electrophilic and the acid anion as the nucleophilic component of the α -addition. Due to the α -addition to the isocyanide, the amine nitrogen atom becomes the nucleophilic reaction partner of the electrophilic *O*-acylcarboxylic acid amid system in the α -adduct. In the course of the cycloaddition and the elimination, the reactive centres change their philia signs once again.

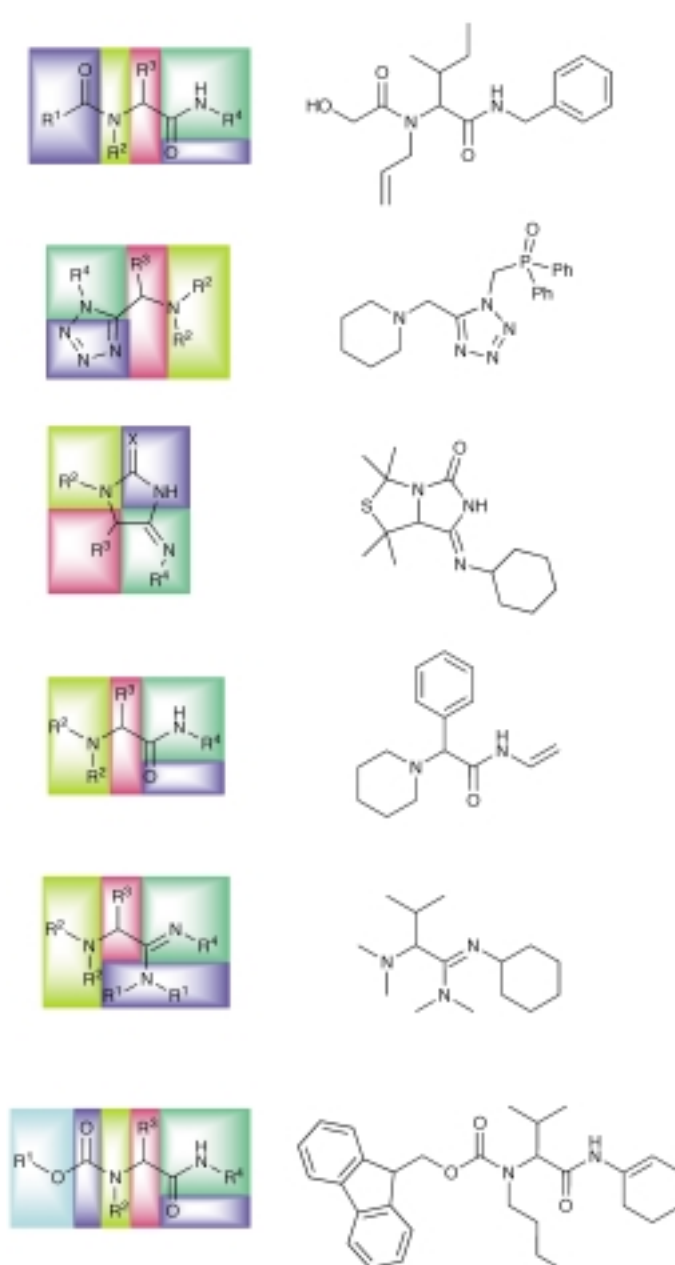
In the course of the U-4CR, one C–C bond and several heteroatom–C bonds are newly formed.

Reactivity series for isocyanides have been set up. In competitive experiments, the product distribution of different isocyanides was examined in the U- and the P-MCR as a function of the solvent and the concentration.^[107] The reactivity is mainly influenced by inductive and mesomeric and to a lesser extent by steric effects. The reactant concentration is much more important for the reactivity than the properties of the solvent! McFarland was the first to examine the product distribution of the U-4CR depending on different reaction conditions systematically.^[83]

Iminoaziridines such as **101** also react with carboxylic acids like **102** to give the Ugi products (**105**). They are known to decompose to isocyanides and imines under thermal conditions or acid catalysis. In this work, detailed spectroscopic examinations of the elusive α -adducts (**104**) were carried out for the first time.^[108]



The diversity of the basic structures of the U-4CR depicted in Scheme 7 is primarily due to the variety of the acid components and their rearrangement opportunities, but also to the structures of the amines as well as the many intramolecular variations. The classic U-4CR can be carried out in solution as well as on a solid phase. The following experiences made in over 40 years are valid for the reaction in solution: Low-molecular-weight alcohols, such as methanol, ethanol, or trifluoroethanol^[109] are used as solvents. Aprotic polar solvents like DMF, chloroform, dichloromethane, THF, or dioxane have also been described as advantageous. In addition, the U-4CR can be performed in biphasic, aqueous solvent mixtures. The exothermic U-4CR usually proceeds fast, within seconds or a few minutes at room temperature or below. Therefore, external cooling is recommended for large batches. Generally the reaction proceeds better if the reactants are present in high concentrations, that is 0.5 to 2 molar. The precondensation of amine and oxo compound usually has a positive effect on the yields. The addition of Lewis acids can be advantageous, which is understandable according to the mechanism of the U-4CR discussed above. This is especially the case for electron-rich, weakly electrophilic Schiff bases. Recently, the rate-accelerating effect of microwaves on the U-4CR was described.^[110]



Scheme 7. Diversity of the basic structures obtained in U-MCRs by varying the acid component. The components are color-coded: The acid component is blue, the oxo component red, the amine component yellow, and the isocyanide component green. N=N–N and R₂N in the second and fifth formulas, respectively, stem from the acids HN and H₂NR₂Cl, respectively; in the sixth example the acid R¹OCOOH comprises CO₂ (blue) and R¹OH (light blue).

3.1. Peptides, Peptoids, and Stereoselective U-4CRs

The classic version of the U-4CR described in many textbooks is the reaction of a primary amine, an oxo component (aldehyde or ketone), an isocyanide, and a carboxylic acid to give an α -amino acylamide. Two amide bonds are formed in the course of this reaction. The possibility of a peptide synthesis with the U-4CR was therefore recognized and discussed early on. Two strategies were followed, namely peptide segment coupling with U-4CR (4CC-SC) and the synthesis of peptide fragments by means of

stereoselective U-4CR (4CC-SSS), which were both discussed in detail in a review.^[111]

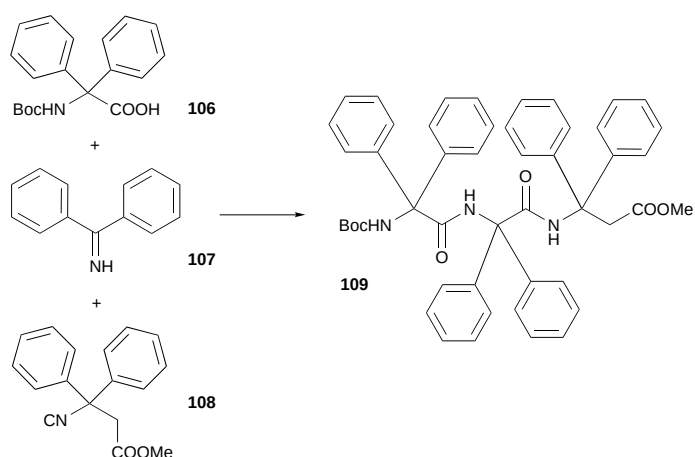
In the 4CC-SC, suitable peptide fragments with protected carboxy and amino termini are brought to react with an aldehyde and an isocyanide, in the course of which a cleavage point is inserted, for example by using *o*-nitrobenzaldehyde. After the U-4CR the coupling ballast is removed.

In the 4CC-SSS an aminoterminally protected peptide fragment is brought to react with a chirality-inducing amine, serving as a chiral ammonia equivalent, an isocyanopeptide, and an aldehyde. The directive amine residue is cleaved after the U-4CR. By repeating this sequence several times a peptide can be built up.

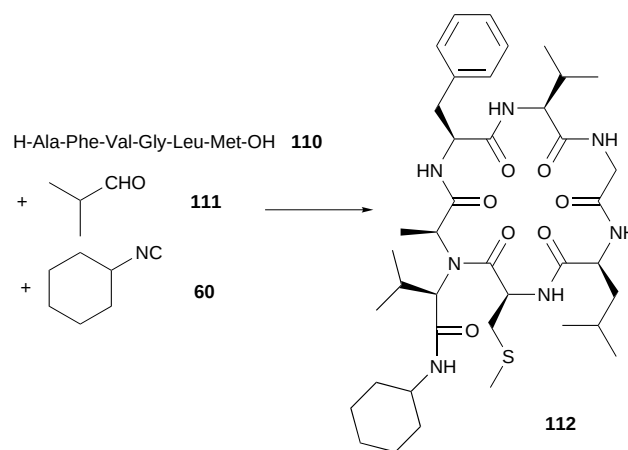
Until today the problem of control of the newly formed stereocenter has not been solved conclusively. Many paths were followed: So far, sugar amines and α -ferrocenyl alkylamines, which can be cleaved and in principle recycled after the U-4CR, have shown themselves to be the most promising reagents.^[112]

The potential of the Ugi reaction for the production of nonnatural α -amino acids was recognized very early. For example, Joullié et al. reported the synthesis of enantiopure heterocyclic α -amino acids by using chiral phenylethylamine.^[113]

The U-4CR has also proved itself in the synthesis of α,α -dialkyl or -diaryl dipeptide fragments. In this way, α,α -diisopropyl and α,α -diphenyl dipeptides were synthesized, among others.^[114] To our knowledge, this is the only method to obtain such sterically hindered peptide building blocks. The great steric strain becomes especially obvious when one attempts to build space-filling models of these compounds. A Japanese group has synthesized α,α -diphenyl dipeptides in a U-4CR under very high pressure.^[115] The same group also synthesized compounds of this type under normal pressure and at room temperature: Precondensed diphenylimine (**107**) was treated with N-protected α,α -diphenylglycine (**106**) and methyl α,α -diphenyl isocyanopropionate (**108**), and the resulting tripeptides such as **109** were obtained at room temperature after 14 h in 55 % yield following chromatography.^[116]



Linear hexapeptides such as **110** can be cyclized to give 18-membered cyclohexapeptides such as **112** by means of the reaction of isocyanides (**60**) and aldehydes (**111**) in good yields.^[117] This reaction proceeds via a 21-membered α -adduct.



If further reactions are to be carried out with the primary U-4CR product, preformed cleavage points play an important part. At these predetermined breaking points, new functional groups for follow-up reactions evolve. The development of such cleavable reagents has been worked on since the beginnings of the U-4CR. Table 4 gives an overview over the cleavable reagents described so far.

In 1996, Armstrong et al. described further applications for cyclohexenyl isocyanide (**113**) introduced by Ugi in 1961 as an isocyanide for subsequent cleavage to primary amides **114**. With this reagent, derivatizations to carboxylic acids, esters **115**, thioesters **116**, pyrroles **117**, and benzodiazepines **118** can be carried out after the U-4CR (Scheme 8).^[118, 134] A münchnone^[135] is discussed as an intermediate in this reaction. Because of the versatile transformation possibilities, Armstrong introduced the term “universal isocyanide” for **113**.^[136]

Generally, one dimension is lost with invariable (e.g. resin-bound) components, that is with a “universal isocyanide” a U-4CR in fact becomes a reaction in which only three components and therefore only three substituents are variable. The cyclohexenyl isocyanide only provides one C atom in the product molecule.

Peptidomimetic libraries of α -methylated amino acid derivatives were described from the corresponding methyl ketones in solution as well as on Rink resin in 31–71 % yield.^[137] The corresponding α -methyl- α -alkyl(di)peptides are expected to show better pharmacokinetic properties than the natural peptides.

N-protected α -amino acids react with cyclic thiazolidines, 2*H*-1,3-oxazines^[138] and 2*H*-1,3-benzoxazines of the Asinger type to give unusual peptide fragments containing nonproteinogenic α -amino acids.^[139] The reaction of precondensed Schiff bases, for example of Asinger heterocycles, with unprotected amino acids, di- or tripeptides, and isocyanides is suitable for the one-step synthesis of unprotected oligopeptides. This constitutes a protecting-group-free peptide synthesis.^[140]

A repetitive U-4CR with multifunctional N-protected α -isocyanamines such as **122**, opening a short route to Nielsen’s polyamidonucleic acid (PNA), has been described.^[141] This route is also suitable for the synthesis of highly diverse PNA

Table 4. Cleavable reagents for IMCRs described so far. One example each is listed with reaction conditions and yields.

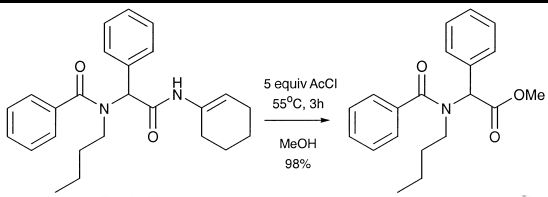
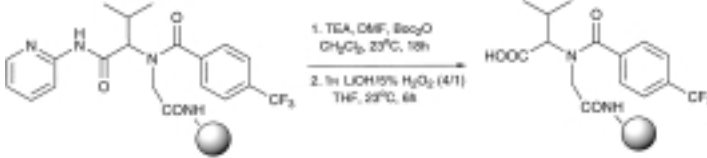
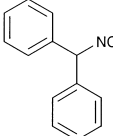
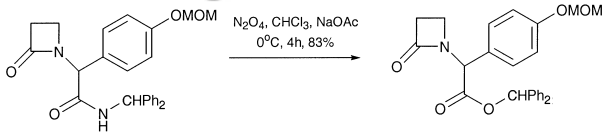
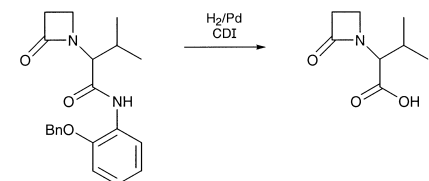
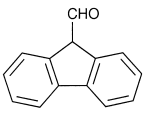
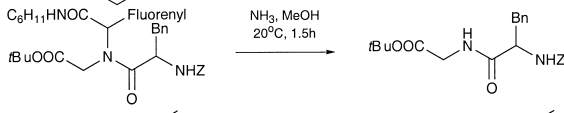
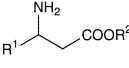
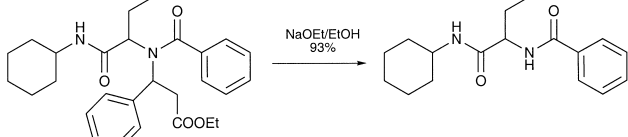
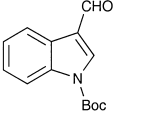
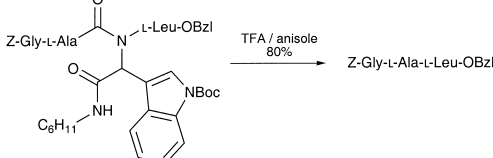
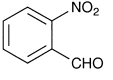
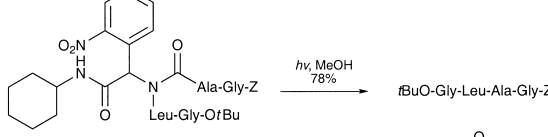
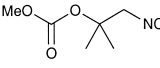
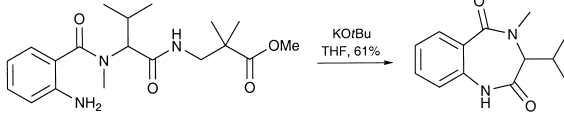
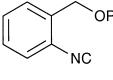
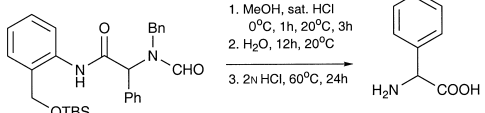
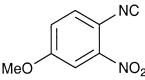
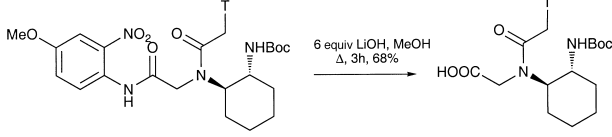
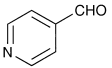
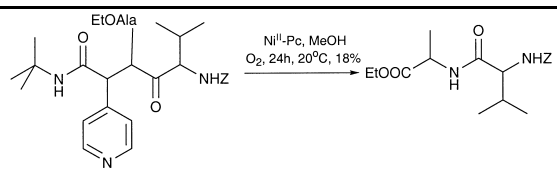
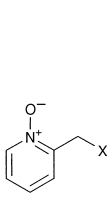
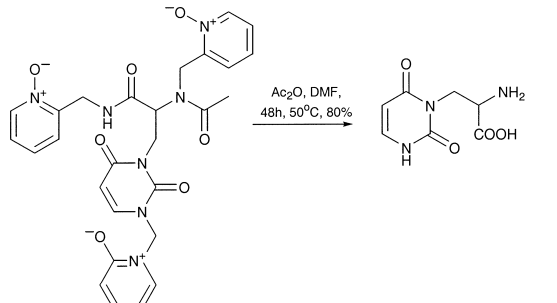
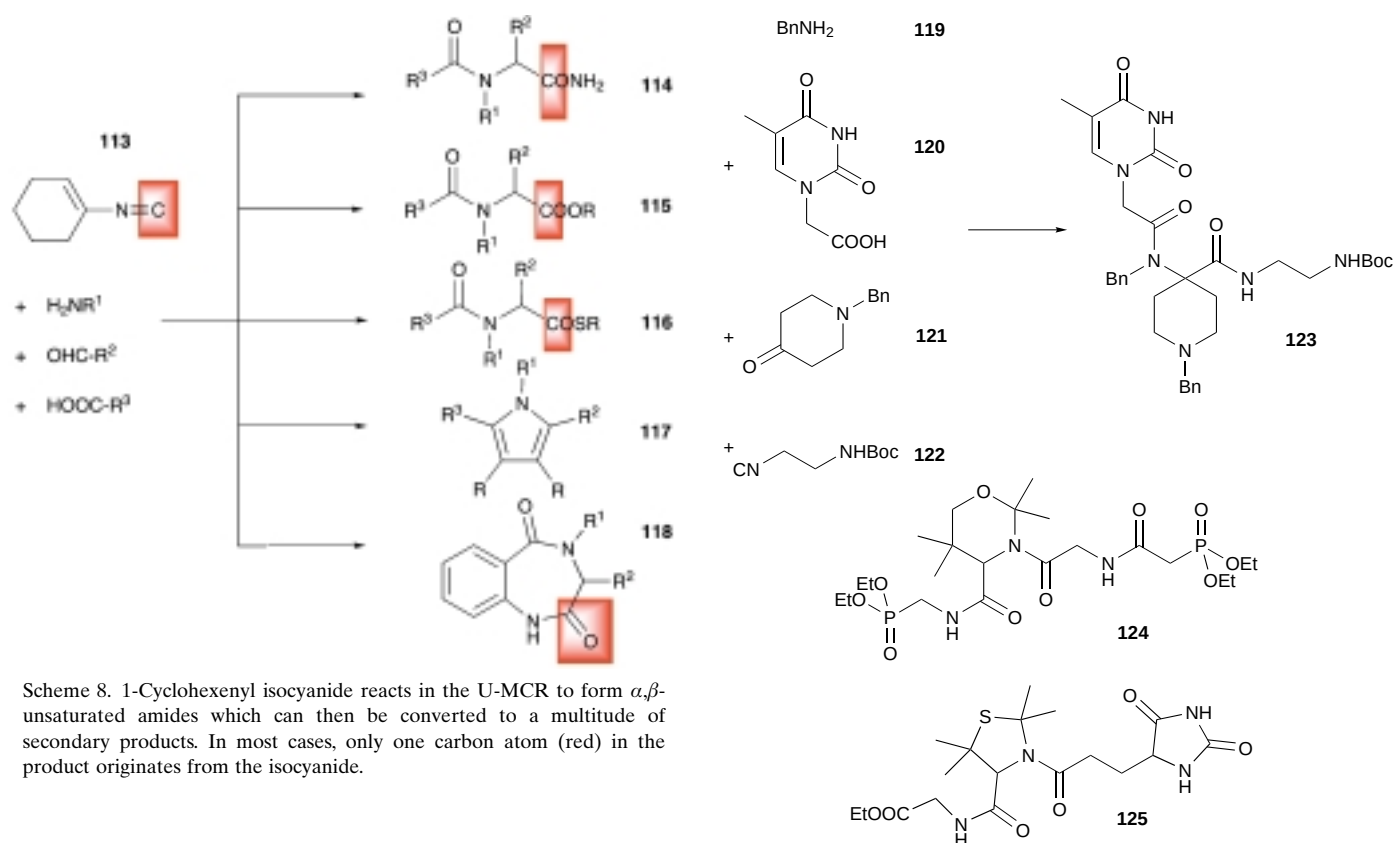
Entry	Cleavable reagent	Example ^[a]	Comment	Ref.
1			cleavable to various functional groups via münchnones (see Scheme 8); alternatively, benzyl isocyanides can be used	[118]
2			2-pyridinamides which can be hydrolyzed on solid phase and lead to pyrroles via münchnones or to carboxylic acids	[119, 120]
3			oxidative cleavage with N ₂ O ₄ to the Ph ₂ CH esters is possible; in the same way <i>p</i> -nitrophenyl(PNP) isocyanide can oxidatively be converted to PNP esters	[121, 122]
4			U-4CR, cleavage of the protecting group with H ₂ /Pd and condensing cleavage with CDI	[123]
5			cleavage of the aldehyde-isocyanide moiety with NH ₃ in MeOH at room temperature	[124]
6			mild cleavage of the amine residue, good ammonia equivalent	[125]
7			acidic removal of the aldehyde-isocyanide moiety	[126, 127]
8			photochemically cleavable	[128]
9			basic cleavage of the secondary amide bond to form esters and carboxylic acids with KOtBu	[129]
10			cleavable isocyanide which leads to a high diastereoselectivity with sugar amines	[130]
11			isocyanide cleavable to the carboxylic acid	[131]

Table 4. (Continued).

Entry	Cleavable reagent	Example ^[a]	Comment	Ref.
12			O ₂ /Ni ^{II} -phthalocyanine(Pc)	[132]
13			2-picolylamine-1-oxide (X = NH ₂) and 2-picolyl-isocyanide-1-oxide (X = CN) as components of the U-4CR with subsequent acidic cleavage to the primary amine or the acid, respectively	[133]

[a] TEA = triethylamine, MOM = CH₂OCH₃, CDI = carbonyl imidazole, Bn = benzyl, Bzl = benzoyl, Pg = protecting group, TBS = *t*BuMe₂Si, T = thymine.



Scheme 8. 1-Cyclohexenyl isocyanide reacts in the U-MCR to form α,β -unsaturated amides which can then be converted to a multitude of secondary products. In most cases, only one carbon atom (red) in the product originates from the isocyanide.

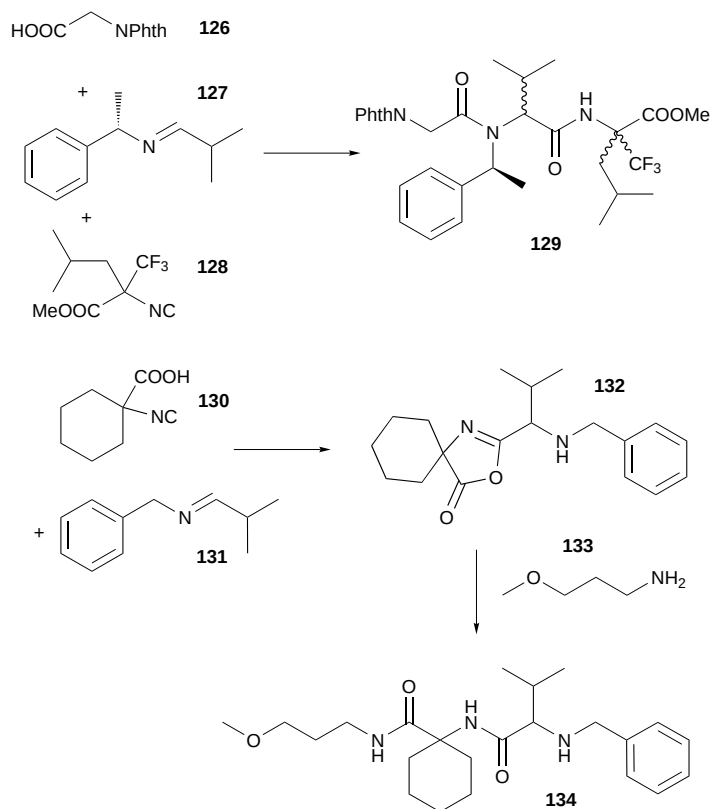
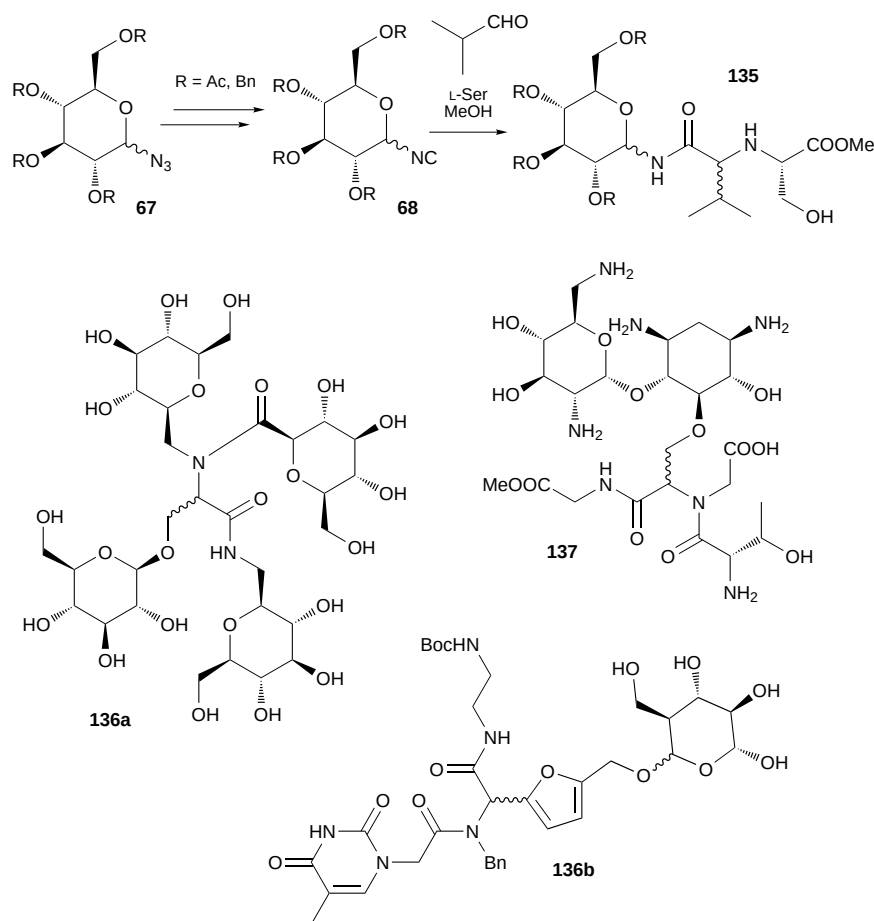
monomers such as **123**.^[142] Another group has described an alternative route to PNA monomers.^[131] In this reaction, nucleobase acetic acids are treated with amines, oxo compounds and cleavable isocyanides. After the cleavage of the amide group, PNA monomers result (Table 4, entry 11). This monomer synthesis is highly convergent, short and very variable, compared to the classic monomer synthesis.^[143] In the same paper, the synthesis of phosphonopeptides like **124** as well as glutathione derivatives such as **125** by means of U-4CR was also published.

Fluorinated α -amino acid derivatives, which are otherwise hard to synthesize but pharmacologically very interesting, can be obtained with partly or perfluorinated oxo compounds. α -Trifluoromethyl-substituted α -amino acids such as **129** (PhthN = phthalimidyl) were inserted at the C and N termini of peptides by means of U-4CR.^[76b]

When the bifunctional starting material 1-isocyanocyclohexane-1-carboxylic acid (**130**) is treated with the Schiff base

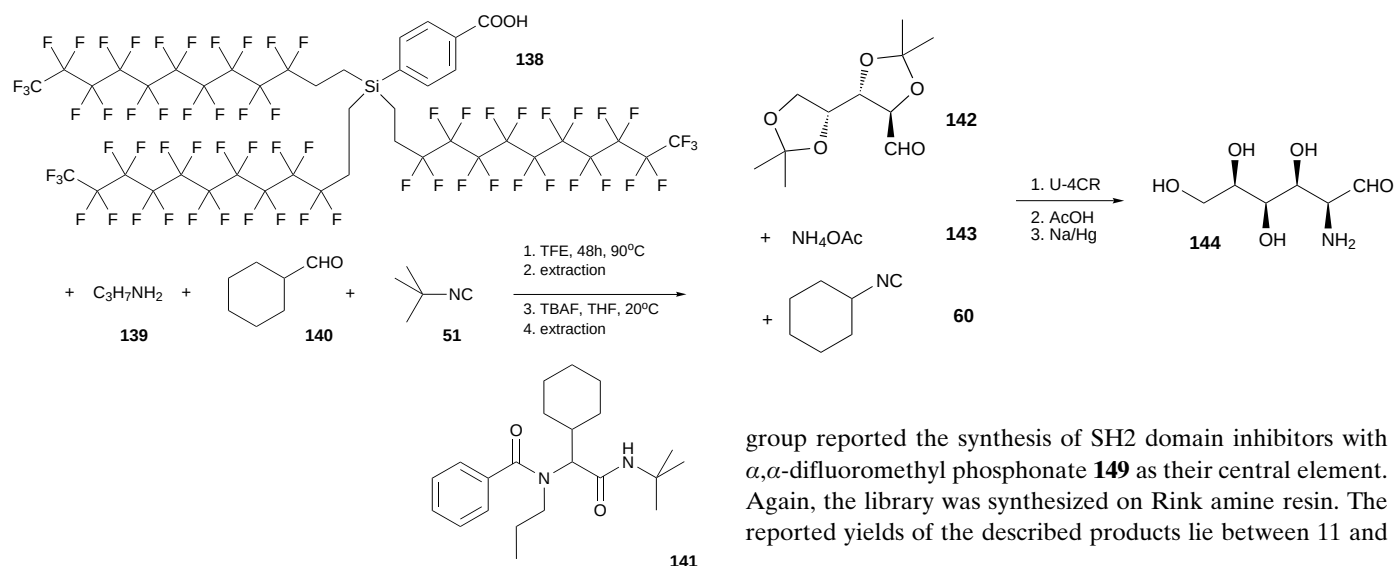
131 in the U-4CR,^[144, 145] the intermediary α -adduct **132** can be isolated and treated with a further nucleophile, for example a second amine **133**, in the same reaction vessel to yield **134**. In sum, this synthesis of **134** corresponds to a U-5CR. The intermediate **132** is the first stable α -adduct to be isolated and described.

The fast and diverse construction of glycopeptide libraries is achieved with glycosyl isocyanides as U-4CR components. As was to be expected, the diastereoselectivity is very low.^[90, 146] On the other hand, highly complex glycopeptide fragments were built up in one step and with satisfactory yields considering the complexity, whereas the time-consuming conventional synthesis would go over many steps. Thus, **135** was obtained in a U-3CR of serine methyl ester, the glycosyl isocyanide **68** and isobutyroaldehyde in 15% yield after chromatography. Another paper and a patent describe the utilization of the U-4CR for the synthesis of glycoconjugates.^[147, 148] In this way, glycosyl derivatives of all four components were synthesized and successfully converted to the expected tetrapseudoglycoside **136a**. All glycosyl derivatives used were protected. In our experience this is often unnecessary as



the reaction also takes place in the presence of free hydroxy groups with very good yields. For example, the nucleobase glycoconjugate **136b** can be synthesized from the corresponding unprotected furfural sugar, benzyl amine, thyminoacetic acid and 2-Boc-1-isocyanoethylamine in nearly quantitative yield.^[148] Park et al. synthesized Ras-Raf protein-binding Ugi libraries that were active against HIV such as **137** on polyethylene glycol (PEG) and in solution. The yields of the compounds synthesized in solution were no better than on PEG; however, the purification of the libraries was less complicated, being a simple precipitation with diethyl ether.^[149]

Curran et al. emphasize the advantages of components with long-chained perfluorinated residues, for example **138**, in the U-4CR (TFE = tetrafluoroethylene; TBAF = tetrabutylammonium fluoride).^[150] These fluororous labels make the purification of the substance libraries easier. The products of Ugi reactions ("Ugis") with fluorinated substrates (these reactions are also called "Flugis") can be liberated from excess reagent by means of liquid-liquid extraction with fluororous phases. Subsequently the fluororous tags are eliminated from the product. Analogous methods have also been described for the Biginelli reaction ("Fluginelli" reactions). So far the conventional method has not been compared to the "Flugi" method concerning purity and yield as well as synthetic complexity and reagent costs. It remains to be seen whether this technique achieves greater importance.

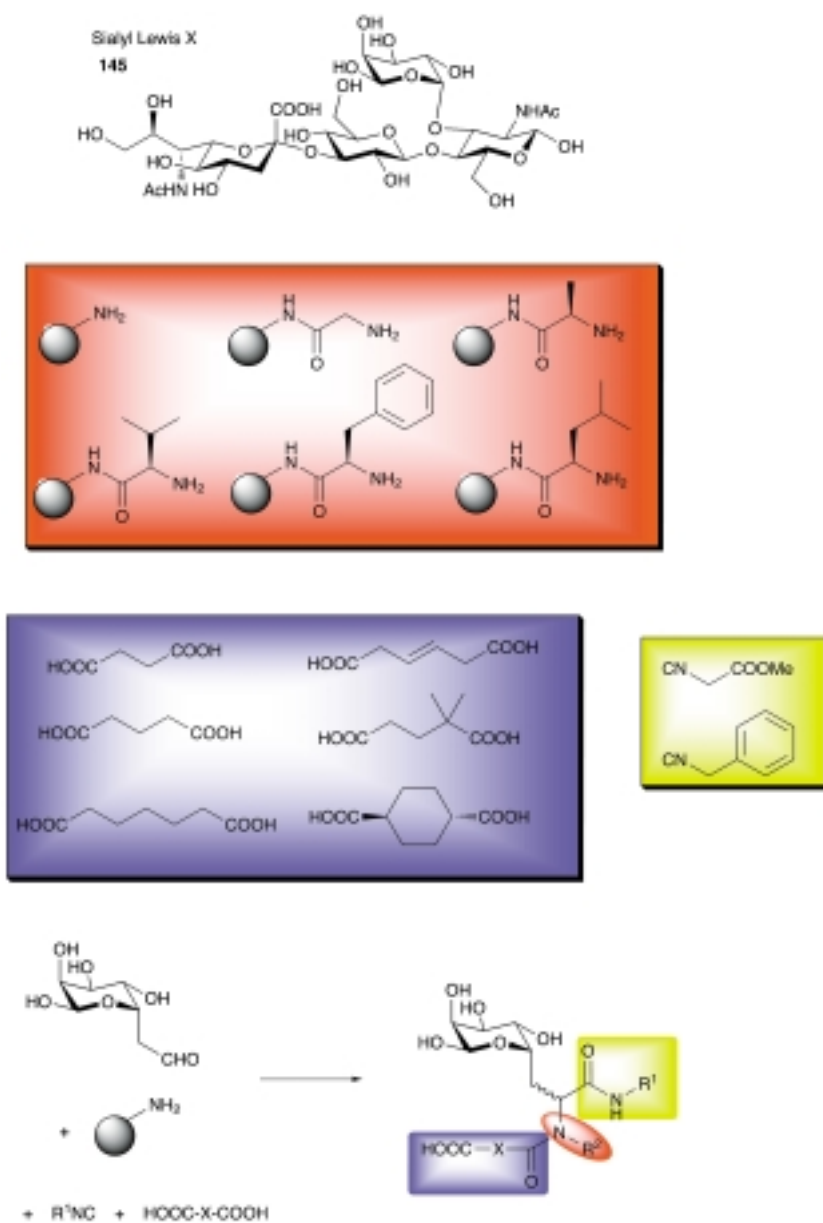


Russian authors have utilized the U-4CR in the synthesis of glucosamine **144**. Ammonium acetate reacted with the isocyanide **60** and the chiral aldehyde **142** derived from glucose to give the Ugi product. Acidic cleavage of its acetal functions and reductive cleavage of its amide functions then yielded **144**.^[151]

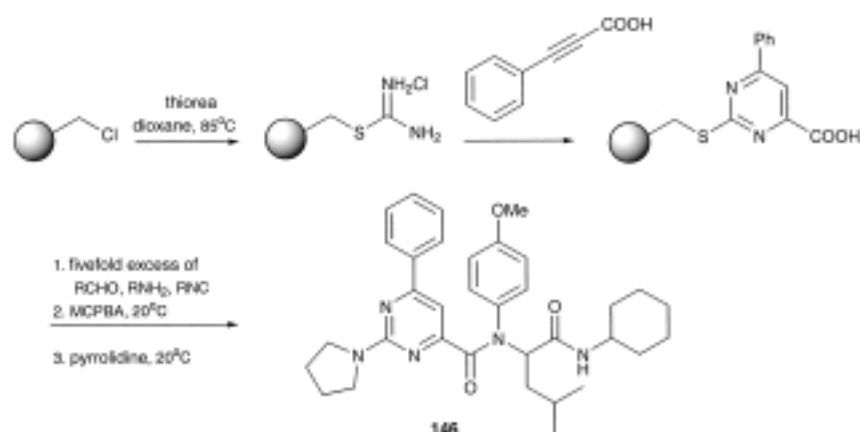
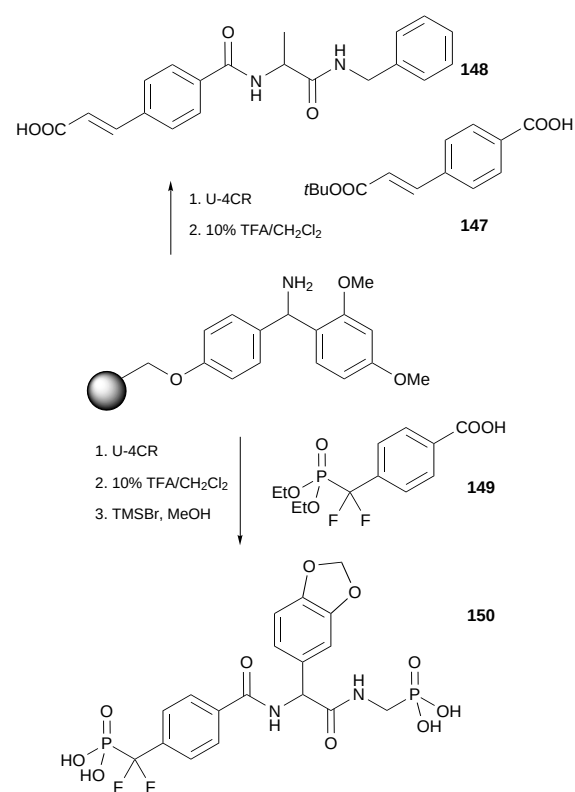
Sialyl-Lewis-X **145** plays a prominent role in cell–cell recognition. Therefore, libraries of sialyl-Lewis-X mimetics are the goal of diverse synthetic efforts. C-Glycoside-peptide ligands were prepared by means of U-4CR on Rink resin (Scheme 9).^[152] With the resin-bound amine component, remarkably, it is possible to use the unprotected dicarboxylic acids, which react only once on the solid phase due to the high degree of dilution. A corresponding direct synthesis with unprotected dicarboxylic acids is impossible in solution.

The application of resin-bound starting materials in MCRs has been described by various authors. Besides Merrifield resin, the preferred resins are Wang, Tentagel, and Argogel. The Ugi reaction for the synthesis of pyrimidines such as **146** on highly loaded Merrifield resin was described by a group at Hoffmann–LaRoche (Scheme 10).^[153]

The production of potent inhibitors of the hematopoietic (blood-forming) protein-tyrosine phosphatase by means of U-4CR was described by a group at the company Ontogen. The overexpression of the enzyme occurs in acutely leukaemic cells. A common element of the library is *tert*-butyl-4-carboxycinnamate (**147**), which appears in all compounds, for example **148**. The library was again built up on Rink resin (TFA = trifluoroacetic acid).^[154] The application of radio frequency tags was described for an analogous U-4CR library.^[155] Another research

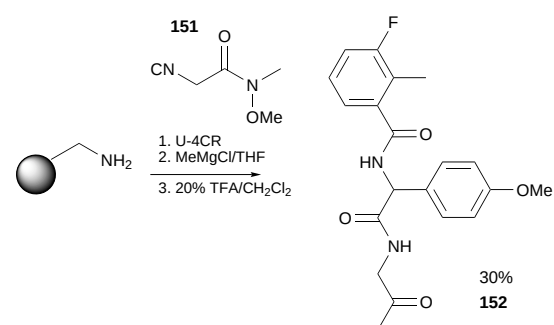


Scheme 9. Synthesis of sialyl-Lewis-X analogues by solid-phase U-4CR.

Scheme 10. Synthesis of **146**; MCPBA = *meta*-chloroperbenzoic acid.

95% with an HPLC purity of usually far beyond 80%.^[156] For example, **150** was isolated in 77% yield.

The build-up of α -oxodipeptides **152** as serine proteinase inhibitors is achieved by introduction of Weinreb's amido

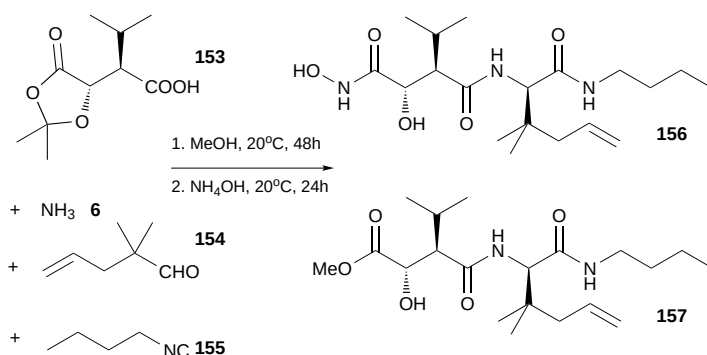


isocyanide **151** into a U-4CR library and subsequent derivatization with Grignard reagents on Rink amine resin. Compounds of this type are interesting as anticonvulsants.^[157]

3.2. Variation of the Amine Component

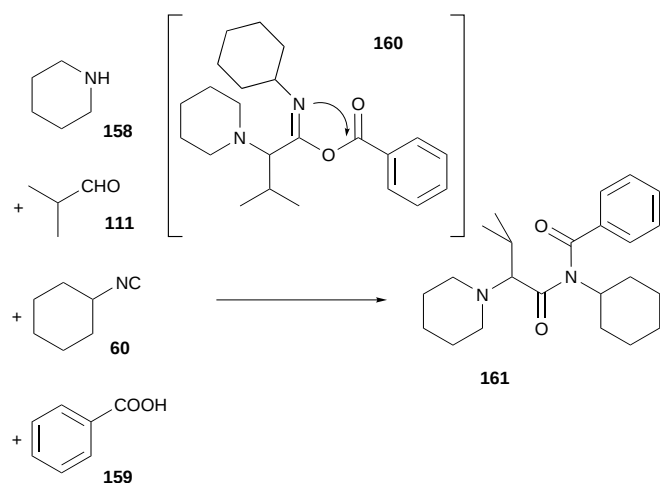
The structural diversity of the U-MCRs is brought about by the different types of acid compounds used, but also by the multitude of “amine-like” components.

Ammonia as the simplest amine reacts in the U-4CR. Libraries of analogues of the naturally occurring metalloproteinase inhibitor marimastat were described by a group at the company British Biotech. In a two-step reaction, ammonia (**6**), the carboxylic acid **153**, the aldehyde **154**, and the isocyanide **155** were converted in a U-4CR and transformed to the hydroxamic acid **156** with hydroxylamine without isolating the intermediate. The product was isolated in moderate to good yields. The side reaction is the ammonia-catalyzed formation of the methyl ester **157**, which can be suppressed by means of precondensation of the Schiff base.^[158] In some cases, however, the turnovers and yields are low with ammonia as the amine component. Alternatively, 2,4-dimethoxybenzylamine, for example, can be used as the amine component and the corresponding amide cleaved in the familiar way with acid catalysis.^[159]



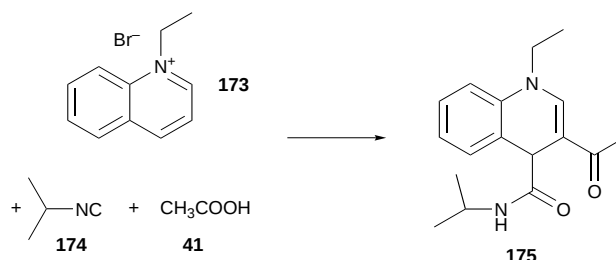
Primary amines react as described above to afford the corresponding α -amino acylamides. When secondary amines such as **158** are used in the place of primary amines, the amine nitrogen of the α -adduct (**160**) cannot be acylated anymore. Instead, the former isocyanide nitrogen atom is acylated, and α,α' -diacylimides such as **161** result. This reaction variation proceeds much faster if the enamines are precondensed.^[83, 160]

Ammonia derivatives, such as hydrazine, hydrazides, or hydroxylamine, react in an analogous way to yield the expected products, for example **163** and **166**. Hydrazine leads to the mono, bis, tris, and tetrakis products as well as mixed Ugi–Mannich products in reactions that are hard to control (UM-5CR). On the other hand, monoacylate hydrazines can be converted selectively only once to the highly substituted

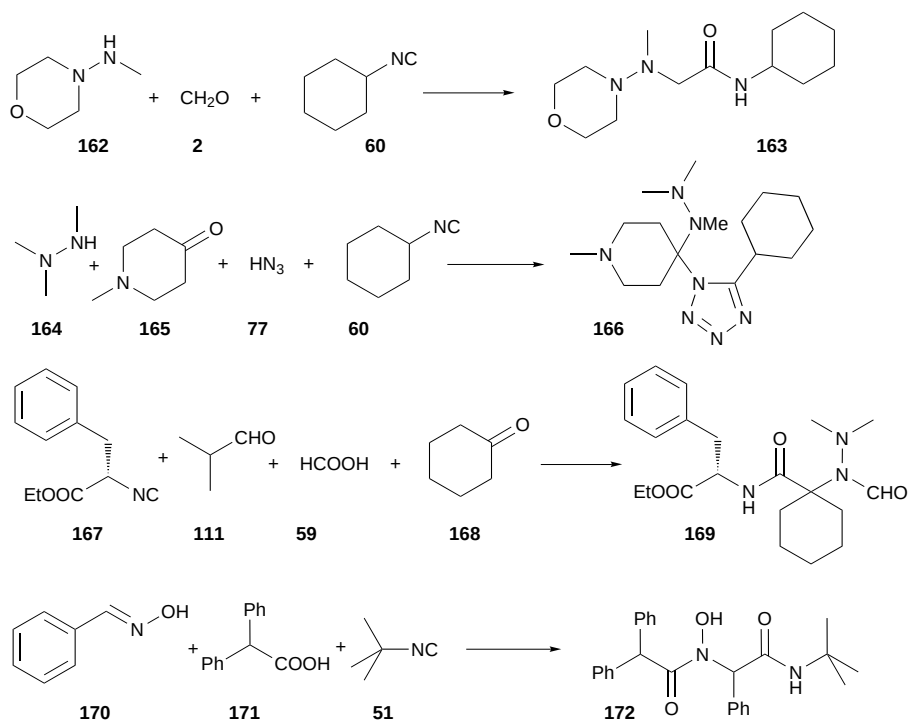
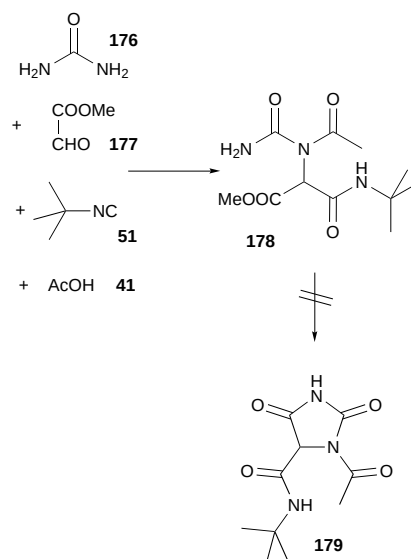


hydrazines in an Ugi reaction.^[161] Not only unnatural amino acids are easy to build into peptides, *N*-aminopeptides like **169** are also accessible in one step with the hydrazine variant of the U-4CR.^[162] Depending on the reaction conditions, hydroxylamine leads to substituted hydroxylamines, α -hydroxylamino-*N*-hydroxyamidines, 2-hydroxylaminoamides, or 2,2-iminodicarboxdiamides.^[38d] Sterically extremely hindered *N*-hydroxy peptides are accessible by means of an oxime U-4CR. The resulting *N*-hydroxy group can easily be reduced to the amide with TiCl_3 .^[163] Hydroxylamine and oximes such as **170** open routes to the pharmacologically extremely interesting class of hydroxamic acids (**172**). Hydroxamic acids are potent metalloproteinase inhibitors with possible applications in the therapy of proliferative and inflammatory diseases.^[164] The hydroxamic acids available through U-4CR are otherwise hard to obtain with this substitution pattern and in this multitude.^[104, 165]

Like conventional immonium ions, *N*-alkylquinolinium compounds such as **173** react in the U-4CR according to their electrophilic character. Theoretically, two isomeric products can arise through the reaction in the 2- or 4-position. In fact, the addition in 4-position leading to the formation of **175** takes place exclusively.^[166]



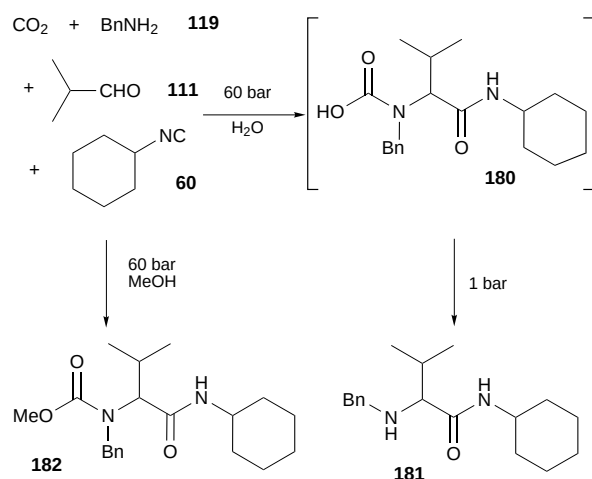
Recently it was shown for the first time that urea (**176**) is also basic enough to function as the amine component in the U-4CR. However, under the experimental conditions the desired cyclization of **178** to **179** did not take place.^[167]



3.3. Variation of the Acid Component

The great structural variety of the U-MCRs is primarily due to the variety of the acid components (see Scheme 7). Aqueous mineral acids react with amines, oxo components, and isocyanides to give α -amino alkylamines. In this reaction, water formally reacts as the acid component.^[83, 160, 168, 169]

An alternative and complementary method for the preparation of α -amino amides like **181** is the reaction of isocyanides with an amine, an oxo compound, and CO_2 under pressure. The carbamido

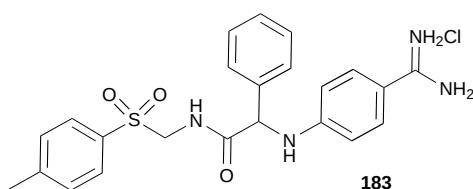


acid (**180**) formed intermediately is hydrolyzed after expansion of the pressure reactor.^[170]

Whereas hydrogen sulfide can only be converted to α -amino thioamides as the acid component in the shape of thiosulfate, hydrogen selenide reacts smoothly to the α -amino selenoamides.^[102]

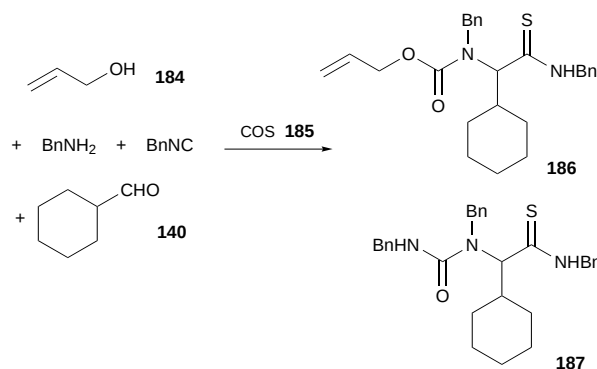
In a CO_2 atmosphere, alcohols are in equilibrium with their monoalkyl carbonates. These can function as the acid component in the U-MCR (for example, see the formation reaction of **182**). As the carbonic acid is formed in situ, this reaction corresponds to a U-5CR.^[102, 171] Whereas the yields are good with low-molecular-weight, liquid alcohols, they decrease sharply with higher molecular weight, solid alcohols. Nevertheless, 9-fluorenyl methanol can serve as the alcoholic component for the introduction of the 9-fluorenyl methoxycarbonyl(Fmoc) protecting group into amines.^[172]

Weber et al. have described the synthesis of very potent thrombin inhibitors with water as the acid component. For this, they used a novel technique to discover new active substances, the Genetic Algorithm.^[173] This way the most potent substance in a large collective of compounds can be found within a short space of time. The described virtual library comprised 160 000 compounds. Within a few synthesis and screening cycles, the inhibitor **183**, which is effective in nanomolar concentrations, was found.^[174]

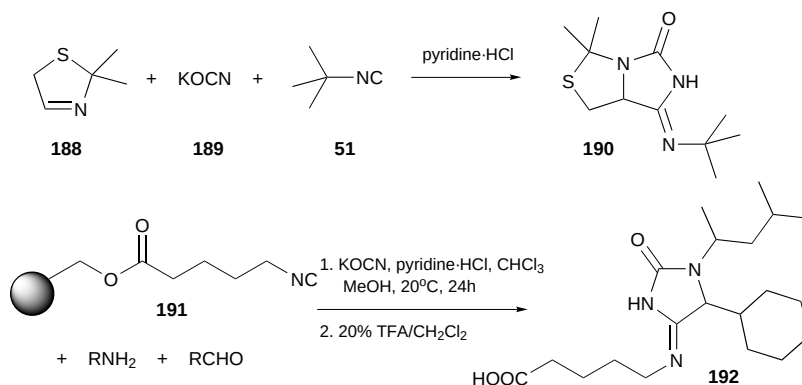


It was also researched whether dithio- and thiocarbonic acids can be used as acid components in the U-4CR instead of carbonic acids. In analogy to the formation of the carbonic acids from carbon dioxide and alcohol, CS_2 and COS were treated with alcohols. With CS_2 , mixtures of α -aminothio-

amides, thioureas and α -thioacylamide thioureas evolve. The authors discuss several mechanisms for their formation.^[172] In the case of carbon oxide sulfide **185**, the expected product **186** can be isolated besides the α -thioacylamide urea **187** and the corresponding α -aminothioamide. In the case of the urea, a second equivalent of the amine reacts with the α -adduct. The reaction seems to be useful for the synthesis of α -aminothioamides and α -amino thioacylcarbamates.



Cyanic acid and hydrogen thiocyanate react as acid components with primary amines, oxo components and isocyanides, yielding iminothiohydantins and thioiminothiohydantins, respectively.^[103] The reaction is generally carried out with amine hydrochlorides and cyanate or thiocyanate salts, respectively; alternatively the amines, the cyanate- (**189**) or thiocyanate salts, respectively, and pyridinium chloride can also be used. Often the pure products precipitate from the reaction solution. With cyclic imines such as **188**, bicyclic hydantins such as **190** are formed.



The synthesis of hydantins such as **192** from the four components isocyanide, amine, aldehyde, and cyanate has also been described on solid phase. Wang resin-bound isocyanide **191** is treated with pyridinium chloride and potassium cyanate at room temperature. After removal of the products with 20% TFA in dichloromethane, the hydantins can be isolated in 41–81% yield. The analogous thiohydantoin synthesis on Wang resin led to unsatisfactory results, compared to the synthesis in solution.^[175]

Hydrazoic acid reacts as the acid component with primary or secondary amines and oxo compounds as well as isocyanides, affording 1,5-disubstituted tetrazoles.^[103, 161a, 168, 176]

Formaldehyde and primary amines can react with each other twice and yield bistetrazolyl amines. With ammonia and small aldehydes like formaldehyde, tristetrazolyl amines are obtained. The solution of HN_3 in benzene which is often described can be replaced advantageously by TMSN_3 in methanol, which is easy to handle and less toxic.^[177]

The solid-phase (Wang resin) synthesis of tetrazoles and α -alkylamino amides is achieved with moderate yields. The corresponding syntheses in solution proceed with much better yields.^[178]

An important group of U-MCRs runs via bifunctional carboxylic acids.

In analogy to the synthesis of β -lactams from β -amino acids by means of U-4CR, it was attempted to prepare α -lactams from α -amino acids in the initial phase of the 4CR. However, iminodicarboxylic acid derivatives were isolated instead of the expected α -lactams.^[179]

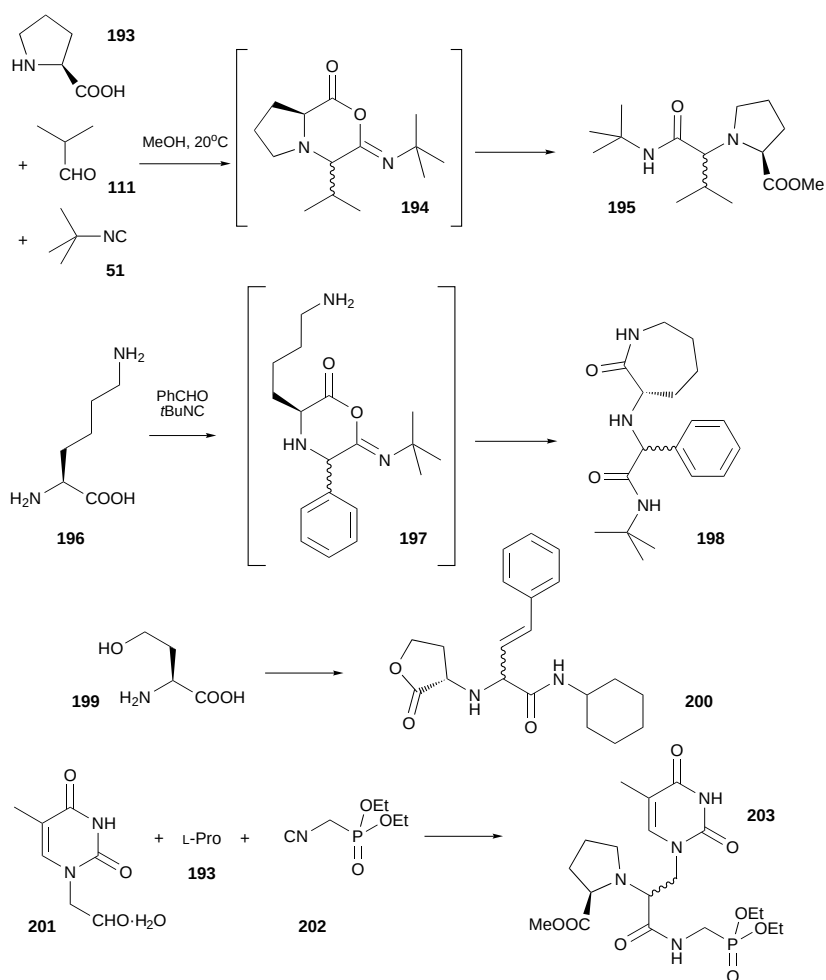
The nonisolable, six-membered intermediate **194** from the α -amino acid **193**, the aldehyde **111**, and the isocyanide **51** is transformed to the stable product **195** by the solvent alcohol in a nucleophilic reaction. The new-formed stereocenter is often generated with surprisingly high diastereoselectivity. The selectivity can be reversed by addition of metal salts.^[180] Apart from cysteine, all known natural and many unnatural α -amino acids can undergo this reaction with aldehydes, mostly with good to excellent yields.^[181]

α -Amino acids with nucleophilic side groups, such as lysine (**196**) and ornithine, react intramolecularly via the elusive cyclic intermediate (**197** in the case of **196**), affording the corresponding seven- (**198**) or six-membered α -aminolactam.^[182] Whereas serine and threonine are transformed intermolecularly to the corresponding iminodicarboxylic acid derivatives without participation of the hydroxy function, homoserine (**199**) as the α -amino acid component leads to the α -amino- γ -lactam **200** in the solvent trifluoroethanol.^[183] Hundreds of potential virostatics, for example **203**, were synthesized with nucleobase-derived aldehydes (**201**), α -amino acids (**193**), and isocyanides (**202**), and screened for their activity.^[184]

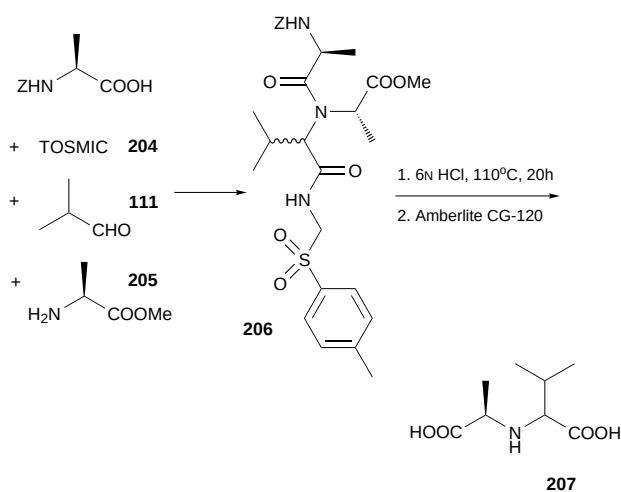
The methods impress with their simplicity: After the insoluble α -amino acid is dissolved through the reaction with aldehyde and isocyanide in alcohol, the conversion is finished and surplus solvent is removed. The reactions are fast and take place at room temperature. In this way many thousands of iminodicarboxylic acid derivatives were synthesized and screened.

With this reaction type, mixing libraries were synthesized in solution.^[185] Three each α -amino acids, aldehydes, and isocyanides, respectively, were converted in one reaction vessel. Of the expected 54 compounds ($3 \times 3 \times 3 \times 2$), 39 were identified by means of GC-MS analysis of the mixing library and corresponding daughter libraries.^[186]

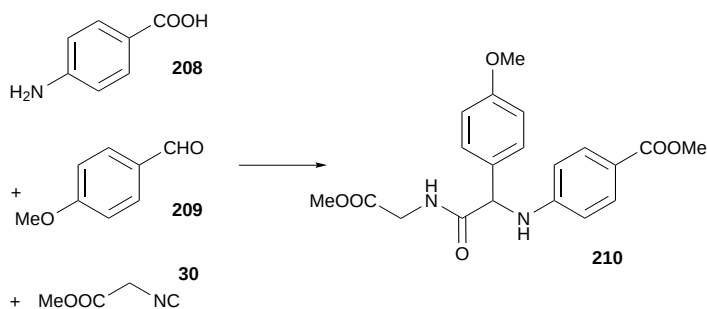
Besides the similarity of these Ugi products to commercial acetylcholine esterase (ACE) inhibitors, the iminodicarbox-



ylic acids are important natural compounds: The opines are constituents of many poisonous fungi. They are also isolated from virus-induced plant galls. Owing to the pharmacological importance, many efforts towards the synthesis of opines were made. An alternative, multistep synthesis of the opine **207** by means of a classical U-4CR with benzyloxycarbonyl(Z)-protected L-alanine and tosylmethyl isocyanate (TSMIC) **204** and subsequent secondary transformations was described by a Japanese group.^[187]

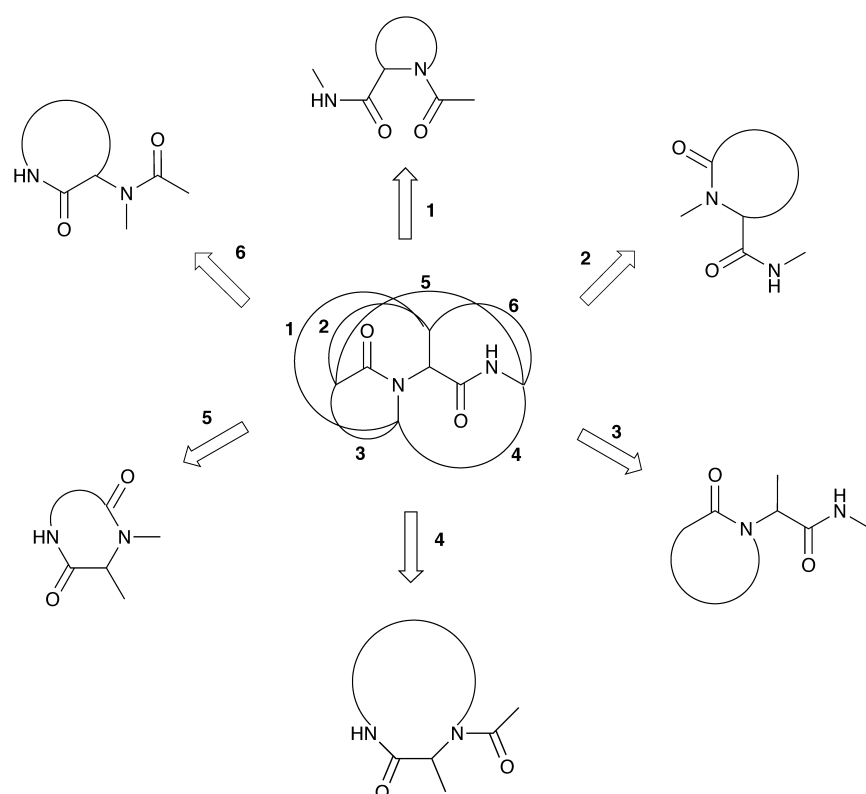


Another unnatural amino acid, 4-aminobenzoic acid (**208**), reacts with oxo compounds and isocyanides in methanol, affording *N*-carbamoylmethyl *p*-aminobenzoates, as shown for **210** (37% yield).^[188]



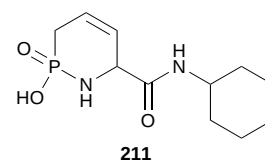
3.4. Cyclic Variants of the U-MCRs with Bifunctional Parent Compounds

Cyclic products can be obtained in U-MCRs with bifunctional starting compounds or in secondary reactions. Cyclic variants of the MCRs are interesting for several reasons. On the one hand, they lead to new types of structures, on the other hand they increase the stiffness of an open-chained basic structure so that the product often shows improved pharmacological properties. Formally, mono-, bi-, or oligocyclic compounds can evolve depending on the number of intramolecular binding sites (Scheme 11).

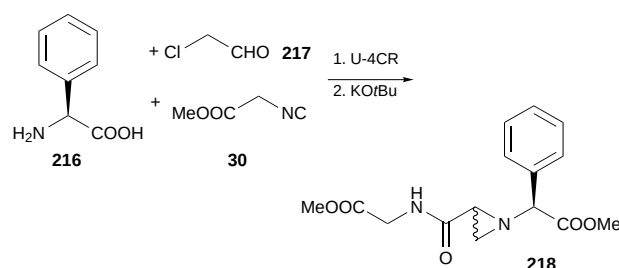
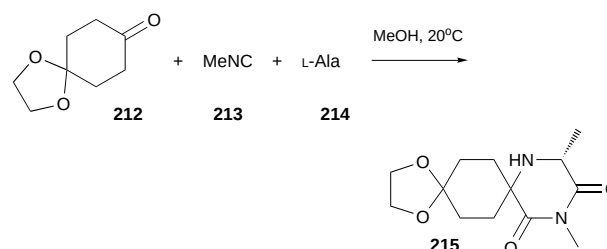


Scheme 11. All topologically feasible combinations of bifunctional parent compounds which can lead to cyclic structures in the carboxylic acid variant of the U-4CR. The variants 1 (cyclic imines), 2 (ω -oxocarboxylic acids), 3 (ω -aminocarboxylic acids), and 5 (ω -isocyanocarboxylic acids) have already been realized.

Phosphonic acids react as the acid component in the U-4CR. In a cyclic variant, ω -oxophosphonic acids as bifunctional parent compounds were treated with isocyanides and amines, leading, for example, to the aminophosphonate **211**.^[189]



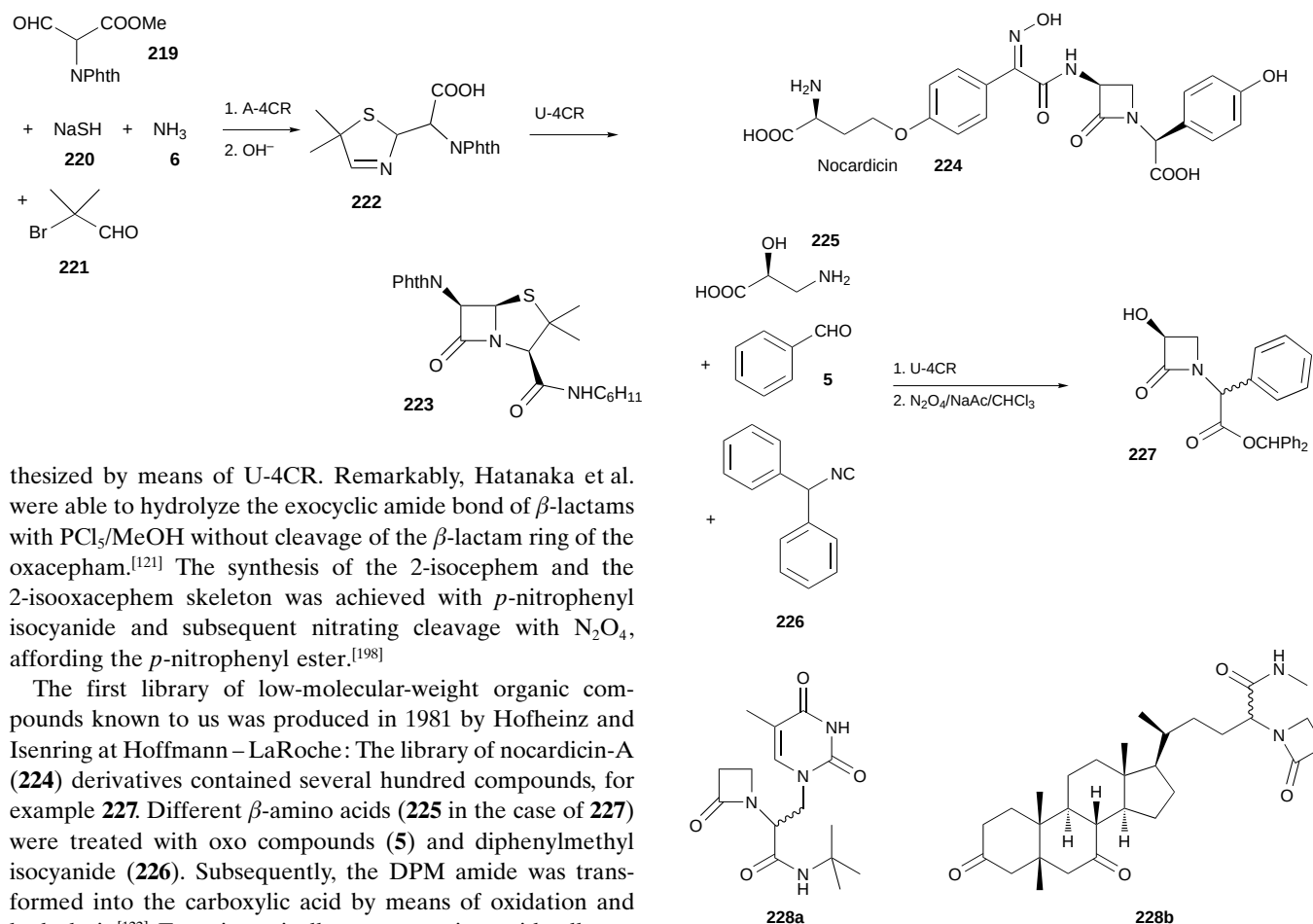
When α -amino acids (**214**) are treated with ketones (**212**) and isocyanide (**213**) in the presence of triethylamine in



methanol, the main products are bispir-cyclic 2,6-piperazine diones like **215**.^[190] The iminodicarboxylic acid derivatives formed with aldehydes can also cyclize to 2,6-piperazine diones in THF/*t*BuOK in good yields. In an interesting variant, α -amino acids like **216** react with α -haloaldehydes such as **217** and isocyanides to form aziridine derivatives (**218**).^[191]

The one-step formation of the β -lactam ring starting from β -amino acids, oxo compounds, and isocyanides is a very early and interesting application of the U-4CR.^[192, 193] Penicillin derivatives such as **223** were already produced by means of U-4CR in the early 1960s.^[194] A central parent compound, the thiazolidine derivative **222**, was generated by means of another MCR, the A-4CR, from NaSH (**220**), the α -bromoaldehyde **221**, the aldehyde **219** and ammonia (**6**). According to NMR studies, the product has the same relative configuration as natural penicillin G. This synthesis is therefore the shortest known synthesis of a penicillin derivative.

In a similar fashion, carbapenems,^[195] carbacephems,^[109, 196] carbacephems,^[197] and cephalosporin derivatives were syn-



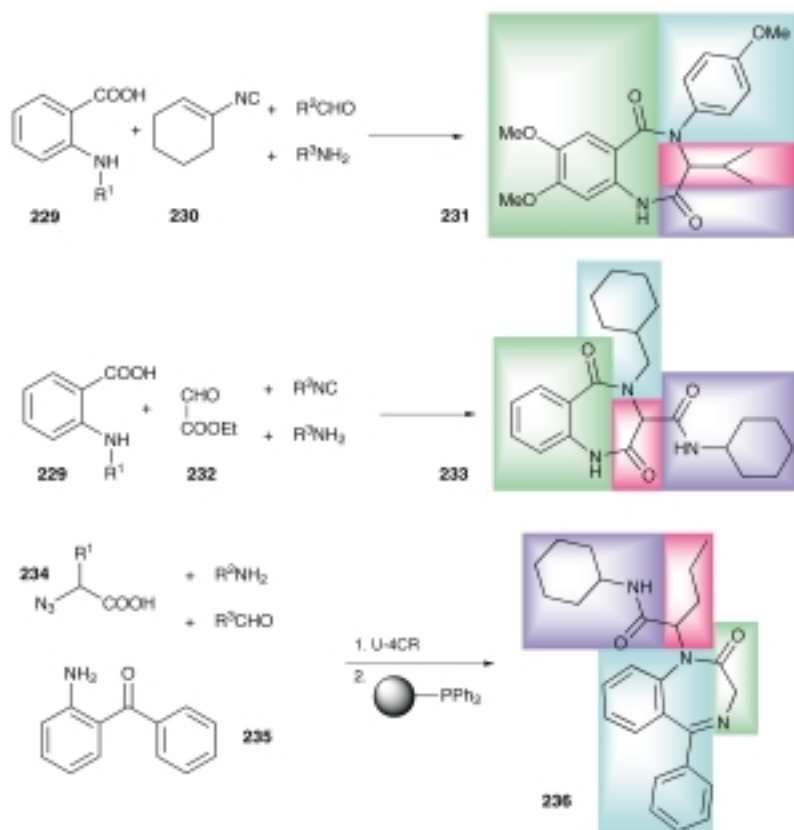
thesized by means of U-4CR. Remarkably, Hatanaka et al. were able to hydrolyze the exocyclic amide bond of β -lactams with PCl_5/MeOH without cleavage of the β -lactam ring of the oxacepham.^[121] The synthesis of the 2-isoxacephem and the 2-isooxacephem skeleton was achieved with *p*-nitrophenyl isocyanide and subsequent nitrating cleavage with N_2O_4 , affording the *p*-nitrophenyl ester.^[198]

The first library of low-molecular-weight organic compounds known to us was produced in 1981 by Hofheinz and Isenring at Hoffmann – LaRoche: The library of nocardicin-A (**224**) derivatives contained several hundred compounds, for example **227**. Different β -amino acids (**225** in the case of **227**) were treated with oxo compounds (**5**) and diphenylmethyl isocyanide (**226**). Subsequently, the DPM amide was transformed into the carboxylic acid by means of oxidation and hydrolysis.^[122] Enantiomerically pure α -amino acids allow a facile synthesis of stereochemically uniform monocyclic β lactams substituted in the 4-position.^[199]

Chimeric monocyclic β -lactams with nucleobases or steroid skeletons in the side chains, like **228a** and **228b**, respectively, were also prepared.^[200, 201] No other method allows such a fast and elegant synthesis of a multitude of unusual β -lactams.

Libraries of monocyclic β -lactams, which are of interest as potential serine protease inhibitors, were synthesized in solution with a U-4CR. In this way, 126 β -lactams were described that were characterized by means of EI-MS.^[202] Such β -lactams could be useful as inhibitors of the CMV protease, as HLE inhibitors, and as chymotrypsin inhibitors.^[203]

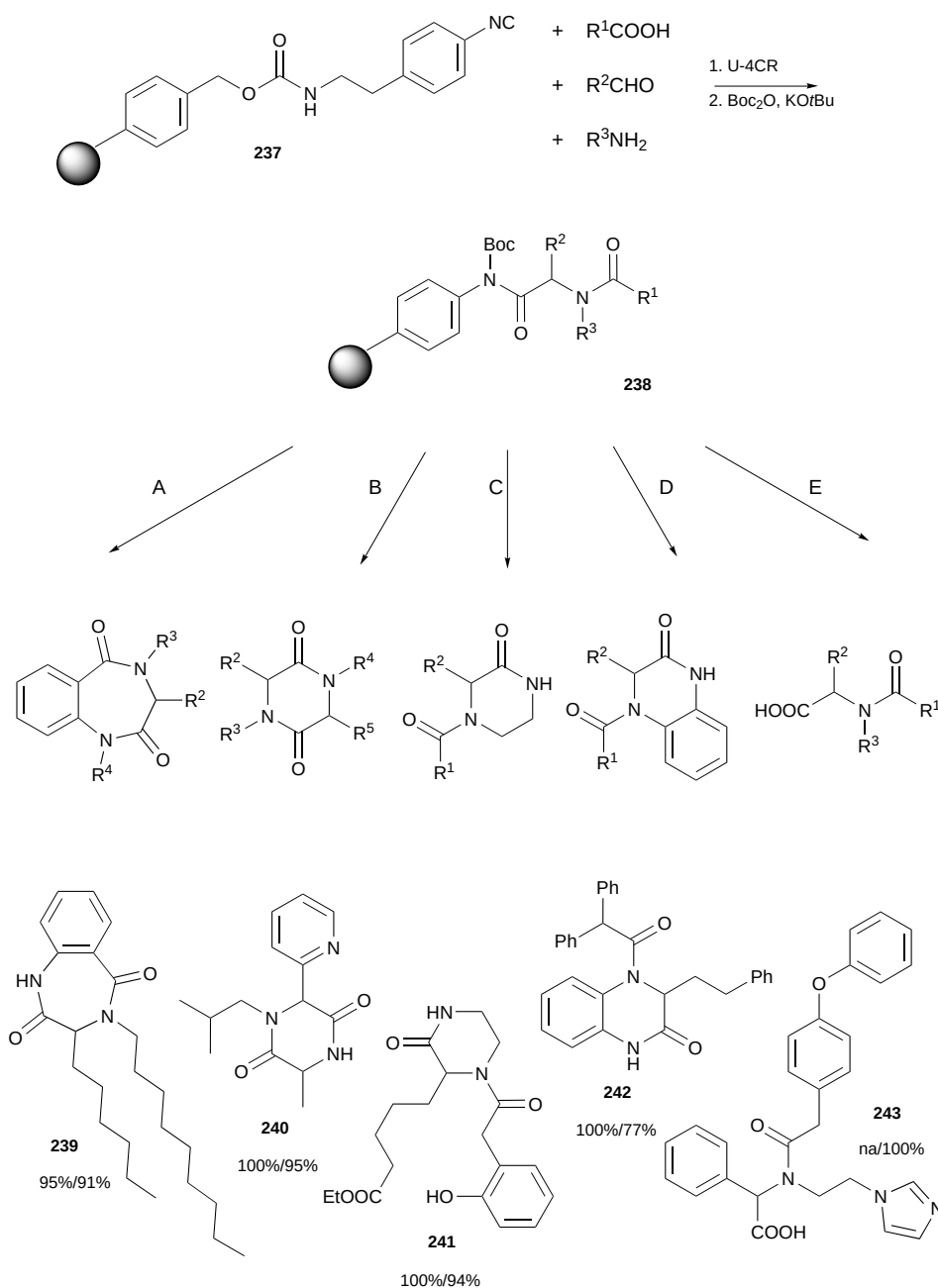
Hardly any other type of skeleton has been subject to more intense pharmacological investigations than that of the benzodiazepines. Armstrong et al. were first to describe the synthesis of 1,4-benzodiazepine-2,5-diones such as **231** by means of U-4CR. Anthranilic acids **229** react with oxo compounds, amines, and cyclohexenyl isocyanide to give the corresponding open-chained α -benzoyl aminoamides. In acid solution the cyclization to **231** takes place. The authors suggest münchnones as intermediates of the cyclization.^[204] Chemists from the company Affymax improved the method by linking silyl groups to the anthranilic acids, thereby rendering them soluble in organic solvents.^[205]



Alternatively benzodiazepine **233** as well as others described below can be prepared from glyoxal ethyl ester **232**. A group at Hoffmann–La Roche treated α -azidocarboxylic acids (**234**) and *o*-azidobenzoic acids with *o*-aminobenzophenones like **235**, isocyanides, and oxo compounds and then effected ring closure to the benzodiazepines (**236**) and the benzodiazocines, respectively, by means of an aza-Wittig reaction.^[206] In that reaction, polymer-bound triphenylphosphane was used, so that the product could be purified by means of resin-capture. It is noteworthy that there are different, complementary entries to the basic benzodiazepine skeleton.

A group at Rhône–Poulenc–Rorer reported the solid-phase synthesis of 1,4-benzodiazepines, dioxopiperazines, oxopiperazines, and dihydroquinoxalinones by means of U-MCR (Scheme 12).^[207] The application of Boc-protected building blocks and subsequent Boc cleavage allow all sorts of cyclizations. The isocyanide component is bound to Wang resin in this reaction (**237**). After the U-4CR with an oxo compound, an amine, and a carboxylic acid, the secondary amide of the former isocyanide is provided with a Boc group, affording **238**, and then intramolecularly cleaved by a functional group of the other components. Thus, Boc-protected anthranilic acids lead to benzodiazepines (route A, e.g. **239**), Boc-protected α -amino acids to dioxopiperazines (route B, **240**), singly Boc-protected ethylene diamines to oxopiperazines (route C, **241**), and singly Boc-protected 1,2-phenylenediamines to dihydroquinoxalinones (route D, **242**). If the primary Ugi product is removed from the resin with LiOH, N-alkylated and -acylated α -amino acids result (route E, **243**). The striking abbreviation UDC (*Ugi/DeboC/Cleavage*) was introduced for the sequence of Ugi reaction, linking of the Boc group and cleavage. The same strategy for the removal of an amide group from a U-4CR product by bonding of the Boc group and basic cleavage was already applied some years before in the synthesis of bicyclic β -lactams.^[109, 208]

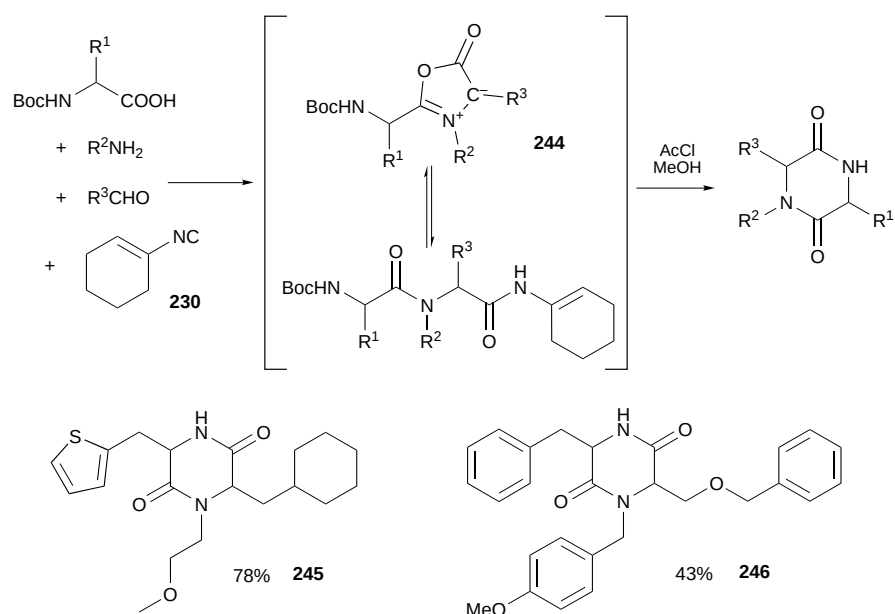
A variation for the preparation of dioxopiperazines in solution was described by the same group. In this reaction,



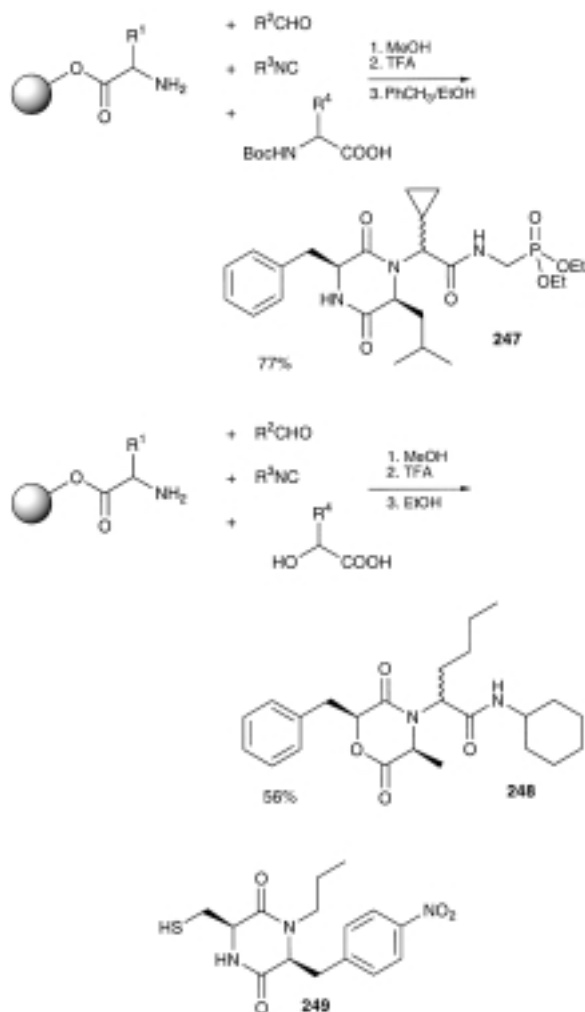
Scheme 12. By using protected starting materials and a bifunctional component, many interesting classes of heterocyclic compounds, such as benzodiazepines, oxopiperazines, dioxopiperazines, or dihydroquinoxalinones are accessible in a one-pot reaction. In each case the yield and HPLC purity is given. na = not available.

Boc-protected α -amino acids and cyclohexenyl isocyanide (**230**) were converted (Scheme 13). The U-4CR products were treated with acetyl chloride in methanol or with TFA, and the cyclization affords dioxopiperazines such as **245** and **246** in satisfactory to good yields. A possible substitute for the sensitive cyclohexenyl isocyanide (**230**), which is difficult to prepare and to store, is the stable and commercially available benzyl isocyanide. The cyclization takes place via the münchnone **244**. A side product is the noncyclized amine.^[209, 210]

Szardenings et al. from the company Affymax have described another route to dioxopiperazines by means of U-4CR (Scheme 14).^[211] An α -amino acid anchored to the resin with its carboxyl group as the amine component, a Boc-protected



Scheme 13. The reaction of Boc-protected parent compounds with cyclohexenyl isocyanide (**230**) to form dioxopiperazines in solution. A münchnone (**244**) is discussed as the intermediate of the acidic cyclization.



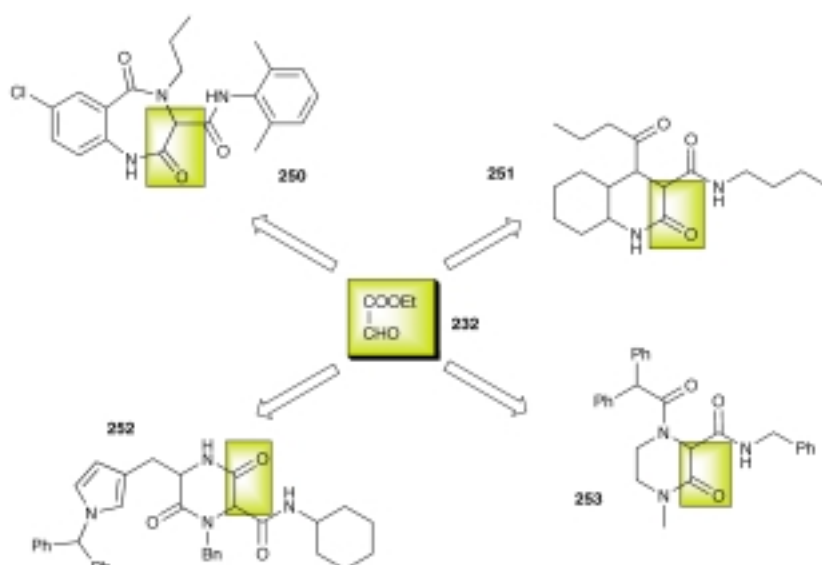
Scheme 14. Solid-phase synthesis of **247** and **248** as well as the collagenase I inhibitor **249**.

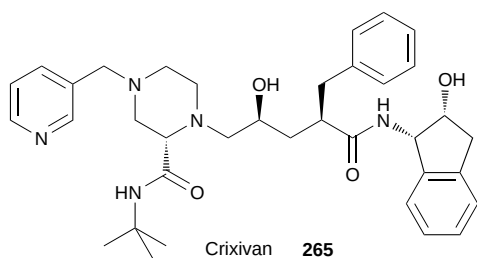
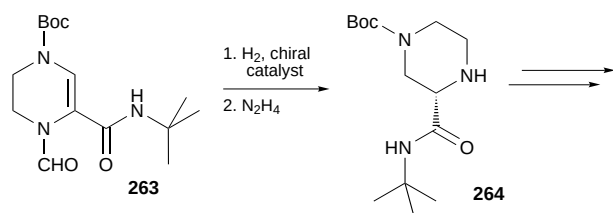
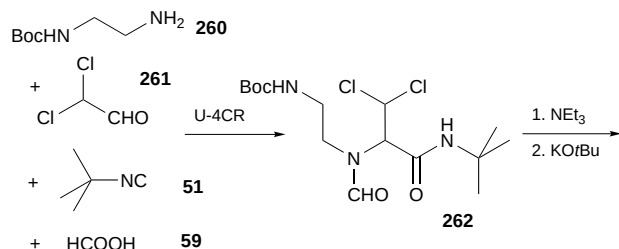
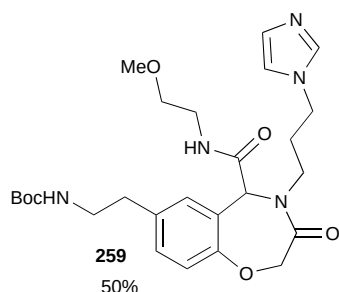
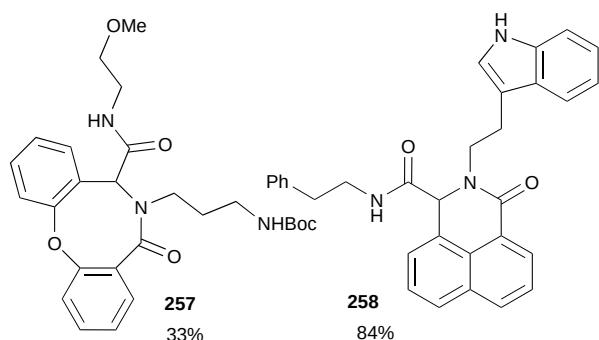
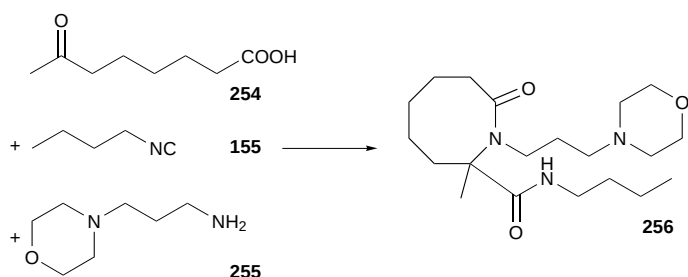
α -amino acid, an oxo component, and an isocyanide react in a U-4CR and can then be cyclized to dioxopiperazines such as **247** under acidic conditions. In contrast to the variants described before, which are only 3CRs, this is a genuine 4CR in which all four components, including the resin-bound amino acid, can be varied independently. When α -hydroxycarboxylic acids are used instead of the Boc-protected α -amino acids, dioxomorpholines like **248** are also accessible. With this Ugi variant, highly selective and potent collagenase I inhibitors were found. Thus, the dioxopiperazine **249** inhibits collagenase-I with an IC₅₀ value of 65 nM and a selectivity towards the enzymes gelatinase B (1) and stromelysin of 1:45: > 10³.^[212]

Oxopiperazines can also be synthesized from glyoxalic esters, diamines, carboxylic acids, and isocyanides in 60–90% yield. The corresponding

seven-membered rings are analogously available from 1,3-diaminopropane.^[167] The glyoxalic ester **232** was also used by another research group as the central component for the synthesis of benzodiazepines **250**, dioxopiperazines **252**, oxopiperazine **253**, and dihydroquinoxalines **251**.^[213]

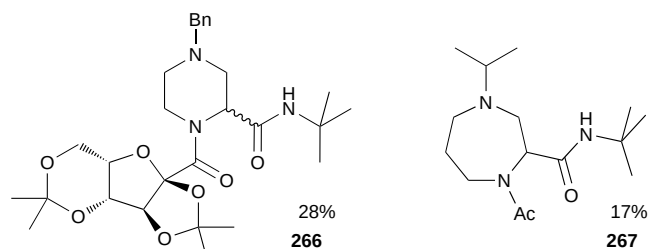
The behavior of glyoxylic acid derivatives and higher ω -oxocarboxylic acids in the U-4CR was already examined in the 1960s.^[171] Obviously in ignorance of the old paper, the reaction of the ω -oxocarboxylic acid **254** was since described three times by several research groups. The solid-phase synthesis, however, is new. Five- to eight-membered highly substituted lactams such as **256** are thus accessible in one step, which is achieved with no other method.^[214, 215] In the case of the eight-membered lactam, the U-4CR obviously proceeds via a nine-membered α -adduct. Chemists at the



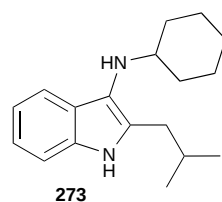
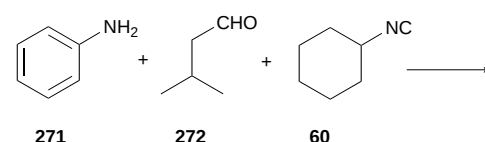
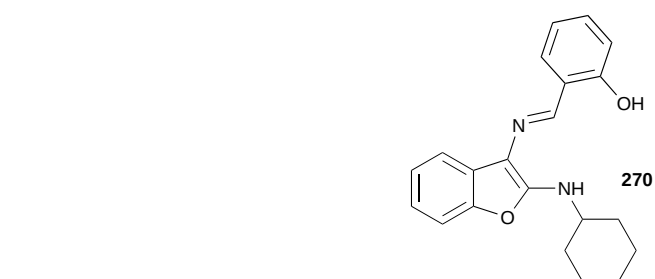
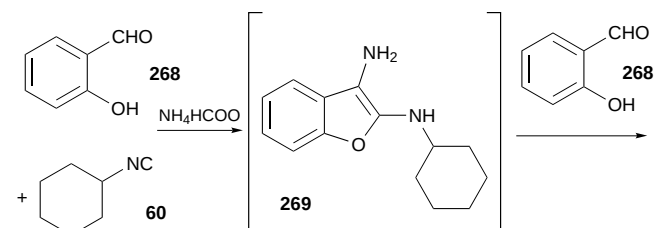


company Repligen have described many heterocyclic analogues, for example **257–259**, which contain exactly that lactam motif.^[216]

Piperazines such as **266** and their seven-membered analogues (e.g. **267**) can be synthesized from monoalkylated (-arylated) ethylenediamines and propylenediamines, chloroacetaldehyde, carboxylic acids, and isocyanides in one step and with great diversity. Piperazine-2-carboxamides are the central elements of many drugs.^[217]

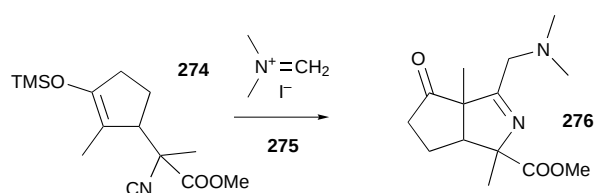


The enantioselective four-step synthesis of the precursor **264** of the HIV protease inhibitor crixivan (**265**) on a gram scale was described by a group at Merck. *tert*-Butyl isocyanide was treated with formic acid, dichloroacetaldehyde (**261**), and mono-Boc-protected ethylenediamine **260** to form **262**. After the Ugi reaction of **262**, base-supported cyclization of **263**, racemization, and enantioselective hydrogenation, the crixivan precursor **264** was isolated with good overall yield and

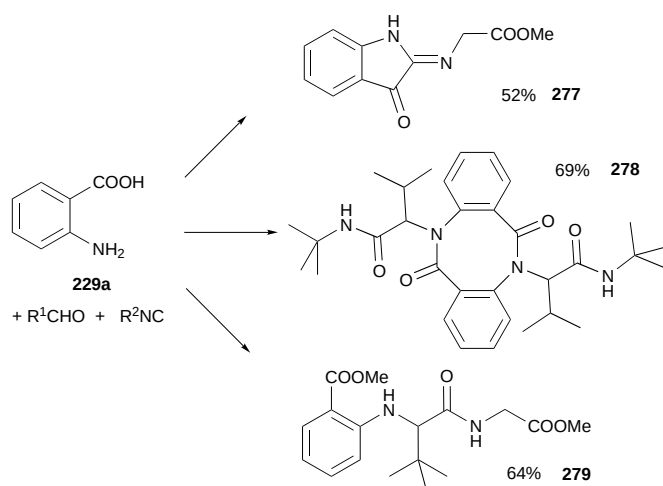


excellent enantioselectivity.^[218] The described synthesis is an advantageous alternative to the classic route.^[219]

The phenol moiety of salicylaldehydes can serve as the acidic function in the U-4CR. Salicylaldehydes like **268** react with ammonium formate and isocyanides (**60**) to form benzofurans. The intermediate 2,3-diaminobenzofuran (**269**) finally reacts with a further equivalent of salicylaldehyde to the Schiff base (**270**).^[220] Anilines (**271**), isocyanides (**60**), and aldehydes (**272**) react to form 3-aminoindoles (**273**). Interestingly, the acidic function here is the CH-acidic *ortho*-CH group in the aniline.^[221] A case of C-nucleophiles in the U-4CR with five examples was described for the reaction of 4-isocyano-1-silyl ethers like **274** (TMS = Me₃Si) with Eschenmoser's salt. The resulting pyrroline derivatives such as **276** can be isolated in 82–94 % yield.^[222]

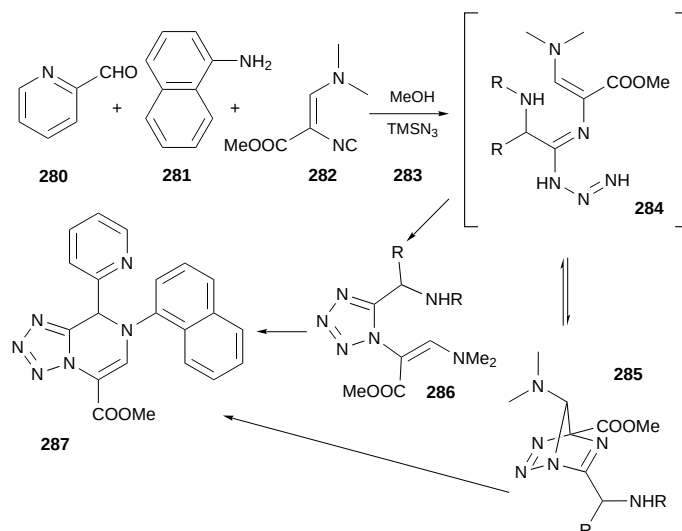


Since the beginnings of the U-4CR efforts have been made to elucidate how anthranilic acid **229a** reacts. Studies in our laboratory^[223] have shown that anthranilic acid is an unstable system in the U-4CR, leading to the formation of different products depending on the reaction conditions and the starting materials. Under reflux conditions in the presence of ketones, **229a** reacts with isocyanides to form 2-iminoindoline-3-ones such as **277**. The ketone is not incorporated into the product. Two equivalents each of anthranilic acid, isocyanide, and aldehyde lead to eight-membered 1,5-diazocine-2,6-diones, e.g. **278**. Finally, *N*-carbamoylmethyl anthranilic esters such as **279** can also be isolated with sterically hindered aldehydes.

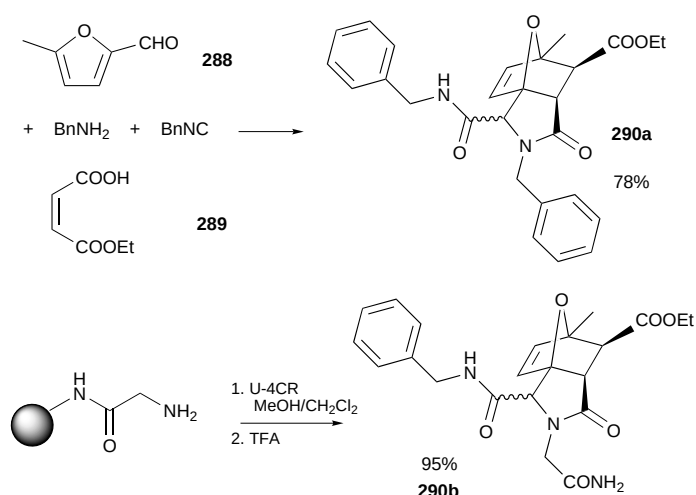


In a variant of Ugi's classic tetrazole synthesis, bicyclic, stiffened tetrazoles such as **287** were synthesized by utilizing the alkyl-β-(*N,N*-dimethylamino)-α-isocyanoacrylates (**282**) described by Schöllkopf. Depending on the starting materials,

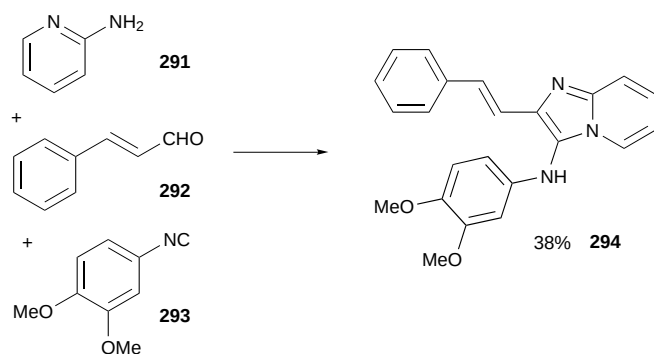
acyclic products (**286**), [3+2]cycloadducts (**285**), or bicyclic products (**287**) were isolated in varying ratios. The bicyclic compounds were purified in parallel by acidic extraction and isolated with a purity >80 % in most cases. TMSN₃ **283** in MeOH was applied as a convenient in situ source of HN₃. Thousands of conformationally limited bicyclic tetrazoles were synthesized this way and tested for their biological activity.^[177]



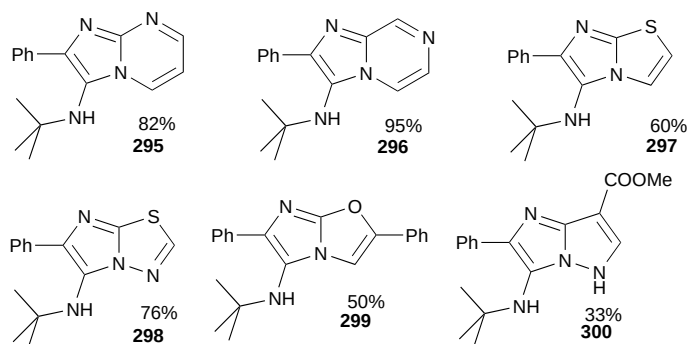
Chemists at the company Affymax treated furan aldehydes like **288** with maleic acid derivatives (**289**), amines, and isocyanides to obtain the tricyclic compounds **290a** and **290b**. The initially formed Ugi product is converted in one pot in an intramolecular Diels–Alder reaction. Variants in solution as well as on a solid phase (ArgoGel Rink resin) have been described.^[224] The mild conditions required for this transformation are remarkable.



Since 1996, Weber has reported on a new variant of the U-4CR at several congresses on medicinal chemistry.^[225] In this intramolecular reaction, heteroarenes such as **291** with an H₂N–C=N group are treated with oxo compounds like **292**

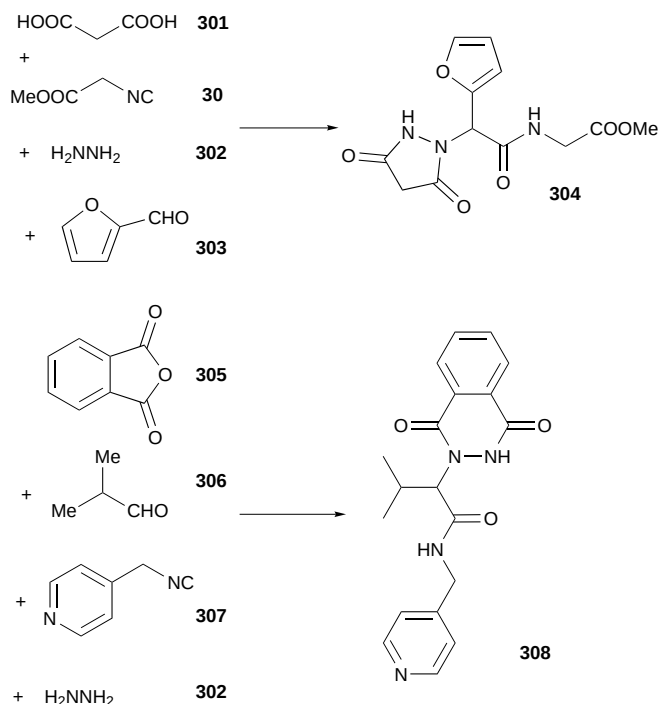


and isocyanides such as **293** to form annelated 3-aminoimidazoles (**294**).^[226] This reaction type was recently published simultaneously by three groups from the pharmaceutical companies Hoffmann–LaRoche, Millenium Pharmaceuticals, and Rhône–Poulenc–Rorer. The reaction can be carried out in solution or on solid phase (Wang resin). No limitations were found either concerning the isocyanide component or concerning the aldehyde component. The reaction is catalyzed by Lewis (Sc(OTf)₃; Tf = SO₂CF₃) or Brønsted acids (HClO₄, acetic acid). The range of applications for this reaction is very wide (see the different products **295**–**300**). Many tens of thousands of products were obtained from this versatile reaction and were tested.^[227]

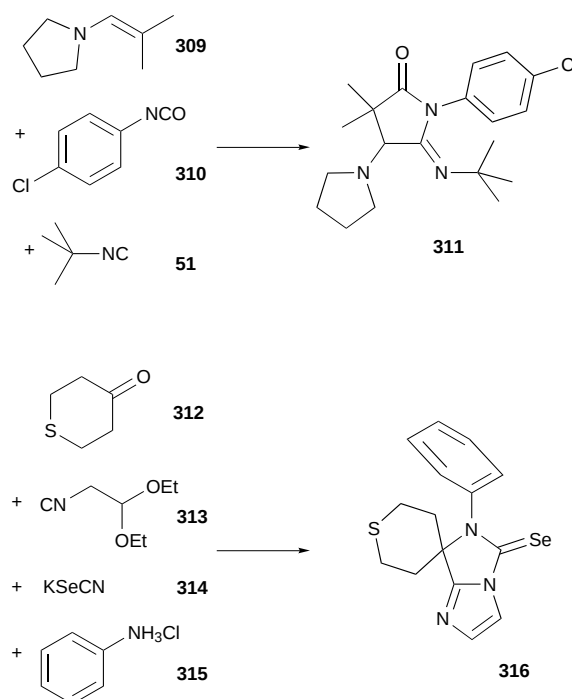


Another variant is the reaction of hydrazine with cyclic carboxylic acid anhydrides (**305**), oxo components (**306**), and isocyanides (**307**), leading to novel cyclic dioxohydrazine derivatives such as **308**. Malonic acid (**301**) reacts analogously to the cyclic anhydrides under formation of **304**. The reaction takes place at room temperature and affords the desired products from a wide range of starting compounds. Starting from malonic acid and five- to seven-membered anhydrides, the corresponding five- to eight-membered products are obtained. Accordingly, the intermediary cyclic α -adducts are eight- to eleven-membered rings.^[228]

Iminopyrrolidones such as **311** and -thiopyrrolidones can be obtained starting from isocyanides, enamines (**309**), and isocyanates (**310**) or isothiocyanates, respectively. The reaction proceeds with good yields and was used for the gram-scale production of the corresponding pyrrolidines.^[229] For example, **311** was obtained in 90% yield. A row of ketones like **312** and amine hydrochlorides like **315** was converted to the Ugi products with potassium cyanate, thiocyanate, and

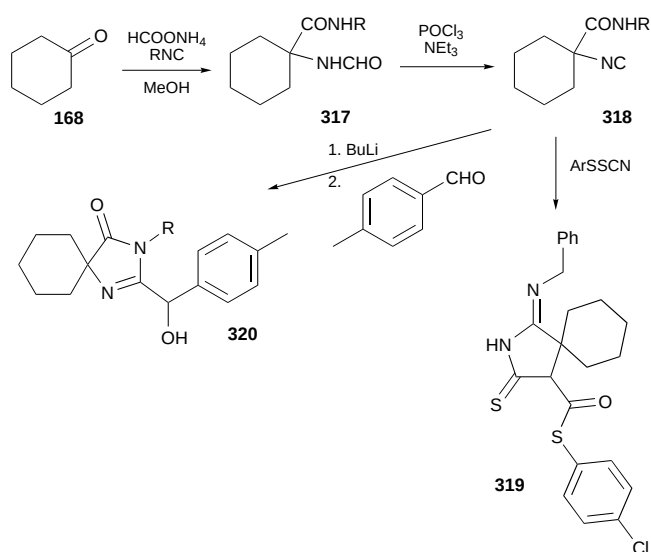


selenocyanate **314**, and 1,1-diethoxy-2-isocyanoethane (**313**) and subsequently cyclized under acidic conditions to the bicyclic imidazo[1,5-*a*]imidazoles (**316**).^[230]

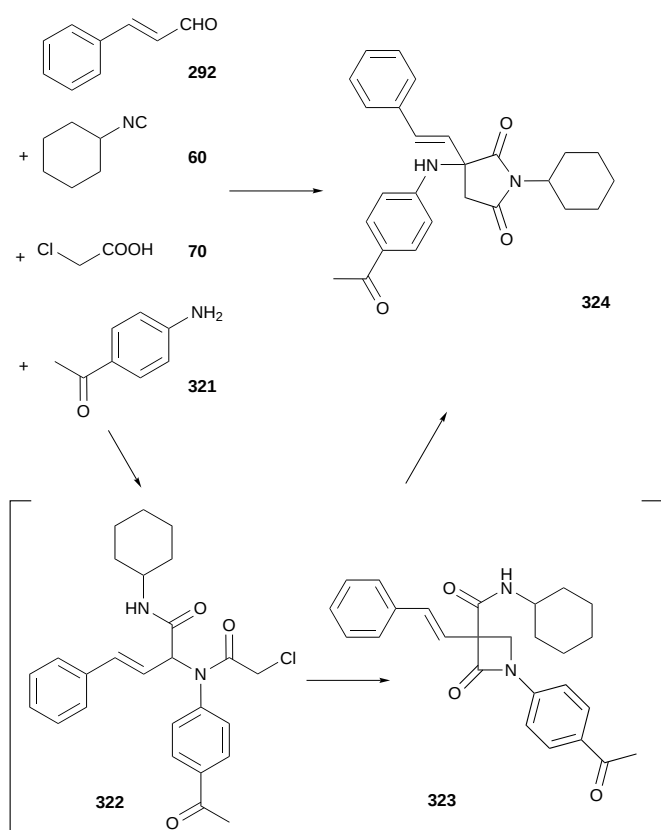


Cyclic ketones such as **168** react with ammonium formate and isocyanides to yield formamides such as **317**. Dehydration leads to α -isocyanocarboxylic acid amides (**318**), which form 1,3-diazaspiro-2-thiones (**319**) with arylsulfonyl isocyanates.^[231] When the α -isocyanocarboxylic amide is treated with butyllithium, the intermediately formed carbanion can

be trapped, for example, with aldehydes under formation of 2,3-disubstituted spiroimidazolones (**320**).^[232]



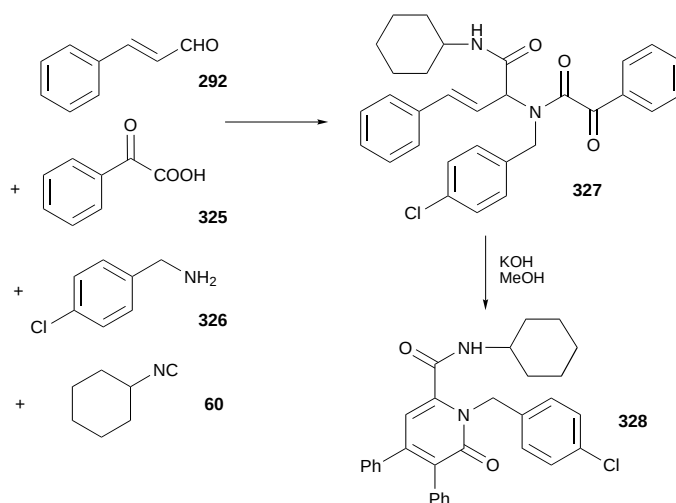
The reaction of cinnamic aldehyde **292**, chloroacetic acid **70**, amines like **321**, and isocyanides like **60** allows the one-



step synthesis of highly substituted succinimides like **324** in modest to good yields via the Ugi product **322**, and the elusive β -lactam **323**.^[233]

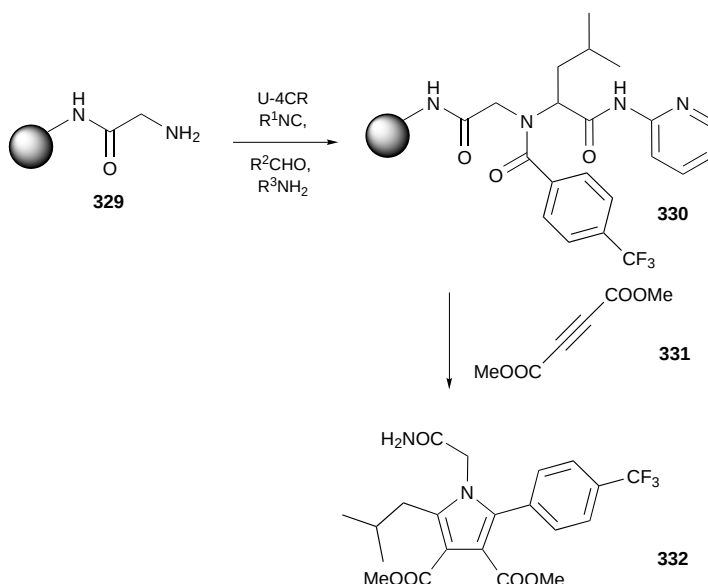
In contrast, **292** reacts with the α -oxocarboxylic acid **325**, the amine **326**, and the isocyanide **60** to form **327**, which

cyclizes to the 1,6-dihydro-6-oxopyridine-2-carboxylic acid derivative **328** with KOH in methanol. Corresponding structures occur in natural products such as acromelic acid.^[234]



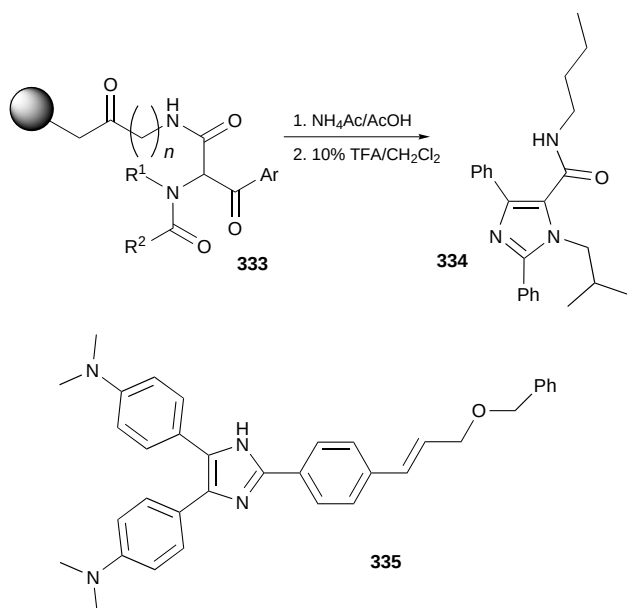
For the synthesis of α -acylamino esters and pyrroles on Rink and Wang resins, cyclohexenyl isocyanide was used and the carboxylic acid linked to the solid phase with succinic anhydride.^[119]

At the same time, chemists from the company Ontogen reported the synthesis of pyrroles such as **332** by means of U-4CR on NH_2 -functionalized Rink resin **329** and subsequent cycloaddition with alkynes (**331**) in good yields and with excellent purities after removal from the resin.^[120] They applied highly reactive 2-isocyanopyridine as a convertible isocyanide (see also Table 4, entry 2).

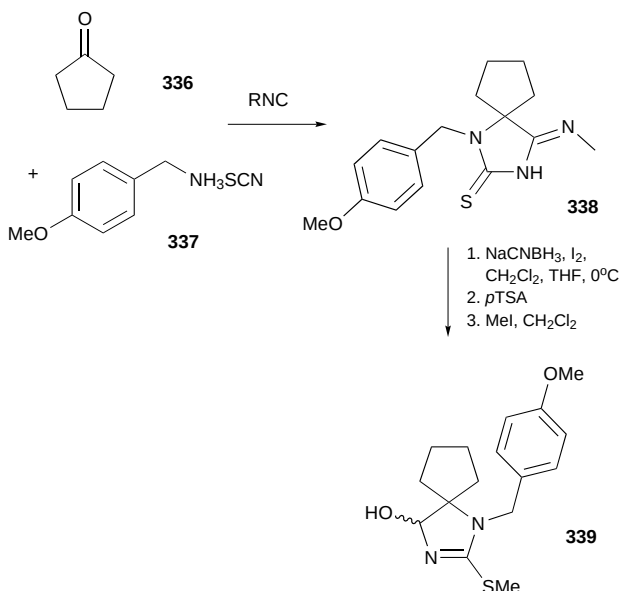


Tetrasubstituted imidazoles like **334** were synthesized by means of U-4CR of α -oxoaldehydes, amines, and carboxylic acids on isocyanide-functionalized Wang resin, affording **333**, and subsequent ring closure with ammonium acetate in 16–56% yield. In the first step an α -(N -acyl- N -alkylamino)- β -

oxoamide is formed. The corresponding two-step reaction to form **334** in solution proceeds with 47 % yield.^[235] It was shown that analogous compounds such as **335** were able to sensitize MDR cells for a treatment with chemotherapeutic agents.^[236]



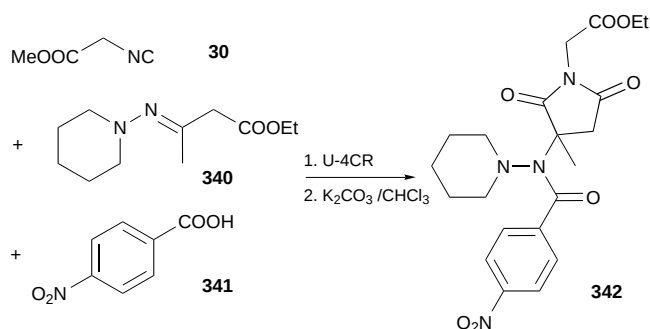
A method was introduced for the synthesis of spiro- α -hydroxyguanidines such as **339**, which occur in many natural products, for example tetrodotoxin or crambin B.^[237] The



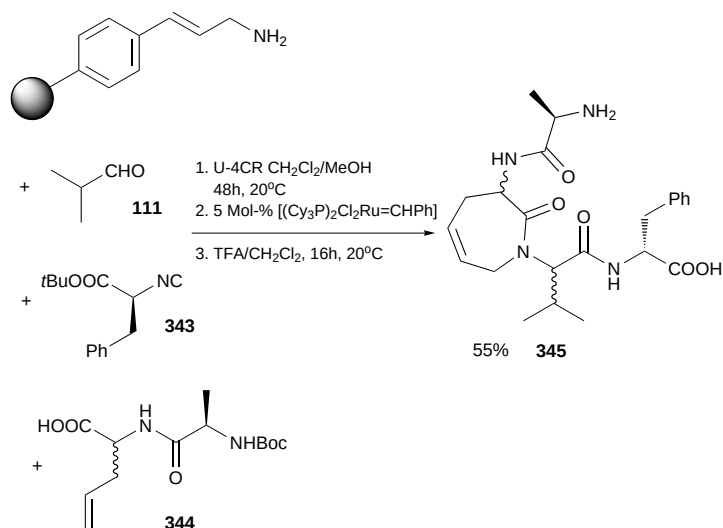
2-thiohydantoin-4-imines (**338**) synthesized from ketones, isocyanides and benzylaminothiocyanate **337** have to be reduced to the α -hydroxyguanidines with sodium cyanoborane ($p\text{TSA} = p$ -toluenesulfonic acid).

1,1-Disubstituted hydrazine react with acetoacetic ester to form imines such as **340**. These lead to the usual U-4CR

products with isocyanides and carboxylic acids such as **341**, which can then be cyclized to 2,5-dioxopyrrolidines (**342**) under mild basic conditions with good yields.^[162]



A topical example of how the choice of suitable parent compounds for a U-4CR and subsequent secondary reactions led to novel types of compounds is the synthesis of β -turn mimetics of the Freidinger lactam type (**345**). The cyclization by ring-closing metathesis takes place after the U-4CR. The resin-bound 3-phenylallylamine serves as traceless linker.^[238]

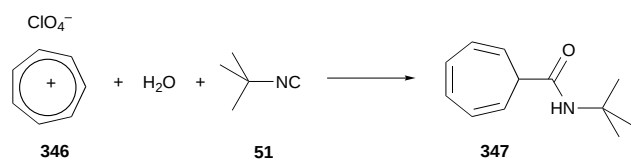


4. Other MCRs with Isocyanides

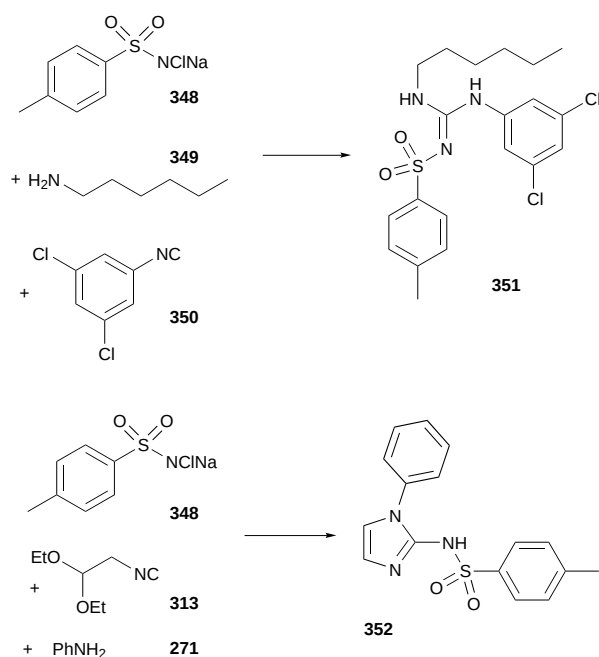
There are some IMCRs that cannot be classified as P- or U-MCRs.

Thus, the reaction of tropylium perchlorate (**346**) with isocyanide **51** and water to the tropylium derivative **347** has been described.^[239] This is a 3CR in which the tropylium ion serves as the electrophile instead of a Schiff base or an enamine.

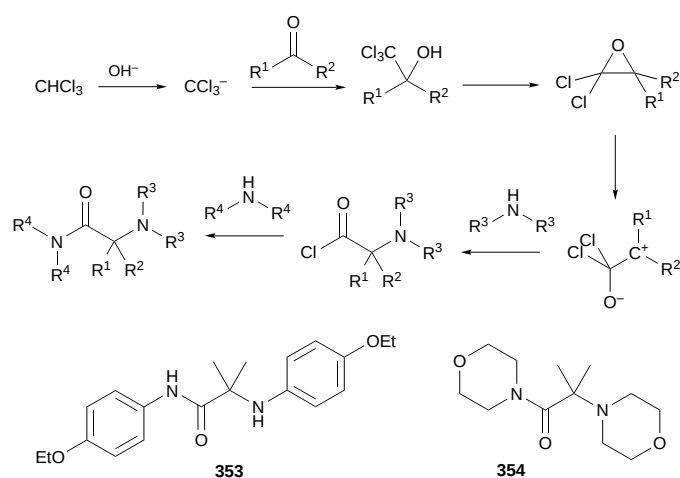
In a 3CR, chloramine T **348** and the amine **349** react with α -addition to the isocyanide, leading to the guanidine **351**.^[240] Analogously, the synthesis of highly substituted imidazoles is effected by the reaction of **348** with anilines (**271**) and 2,2-



diethoxy-1-isocyanoethane **313** and subsequent ring closure in boiling acetic acid. The two-step synthesis via the isolable *N*-tosylguanidines leads to the *N*-aryl-2-tosylamino-1*H*-imidazoles (**352**) in 40–75 % yield.^[241]



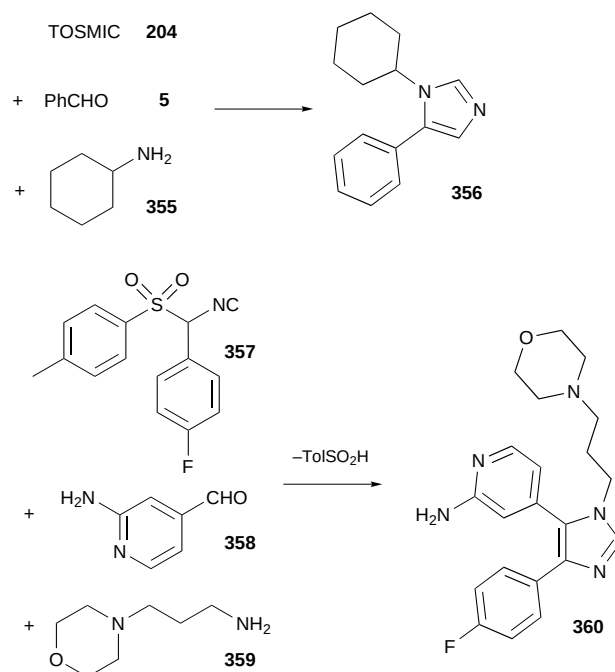
α -Amino amides are the U-3CR reaction products of amines, oxo compounds, and isocyanides in a mineral acidic medium. Interestingly, α -amino amides like **353** or **354** can be synthesized in a 4CR which obviously follows a completely different mechanism (Scheme 15): Chloroform is treated with two primary or secondary amines and an oxo compound in the



Scheme 15. Possible mechanism of a different MCR of amines, ketones, and chloroform which also leads to α -amino amides.

presence of a phase-transfer catalyst.^[242] As secondary amines (which cannot be converted to isocyanides) also react, the mechanism cannot proceed as for the Ugi reaction.^[243]

Van Leusen et al. have described the 3CR of TOSMIC **204**, aldehydes, and primary amines, leading to 1,4-disubstituted imidazoles (**356**).^[68] Alternatively, the intermediately formed α,β -unsaturated isocyanide can also be isolated and treated with amines.^[68] 1,4,5-Trisubstituted imidazoles (**360**) were



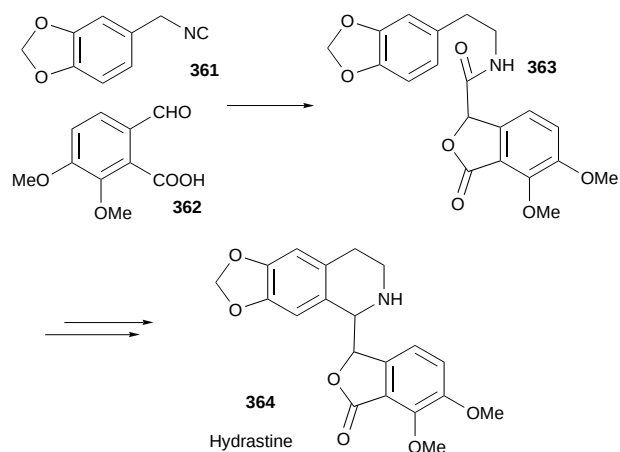
described by the same group: 2-Substituted TOSMIC derivatives like **357** are treated with primary amines and aldehydes. The reaction can be described as a dipolar [2+3]cycloaddition of the Schiff base and the isocyanide followed by sulfinic acid elimination (Tol = tolyl). Compound **360** and similar ones have been described as highly potent P38 MAP kinase inhibitors.^[270]

5. IMCRs in the Total Synthesis of Natural Compounds

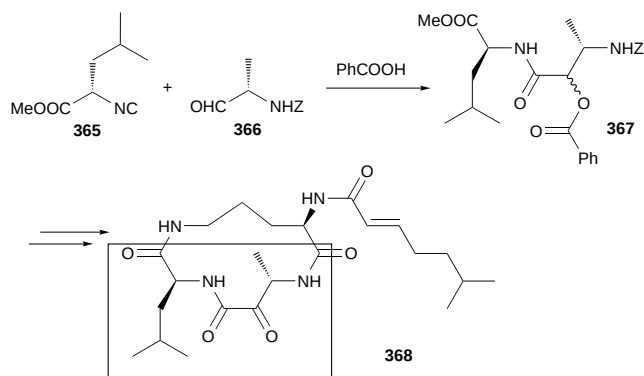
MCRs with isocyanides were often used in the total synthesis of natural products. This can be advantageous, as several starting materials are combined to very complex products in one step, which makes analogues of the corresponding natural compounds available. In the following, several total syntheses are introduced in which MCRs were used as the key steps.

Based on the lactone variant of the P-3CR to **363** with the bifunctional ω -oxocarboxylic acid **362** and the isocyanide **361**, a two-step total synthesis of the alkaloid hydrastin **364** was carried out.^[244]

The synthesis of the natural product and prolyl endopeptidase inhibitor eurystatin A **368** by Schmidt et al. uses the

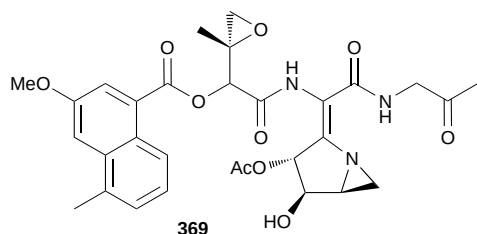


P-3CR as a build-up reaction. Eurystatin A, like the prolyl endopeptidase inhibitors postatatin,^[245] rapamycin, and FK 506 contains an essential α -oxoamide unit. In the P-3CR, the enantiopure α -isocyano ester **365** derived from an α -amino acid and the amine-protected α -amino aldehyde **366** are treated with benzoic acid. No diastereoselectivity was observed. As the hydroxy group formed here is oxidized to a



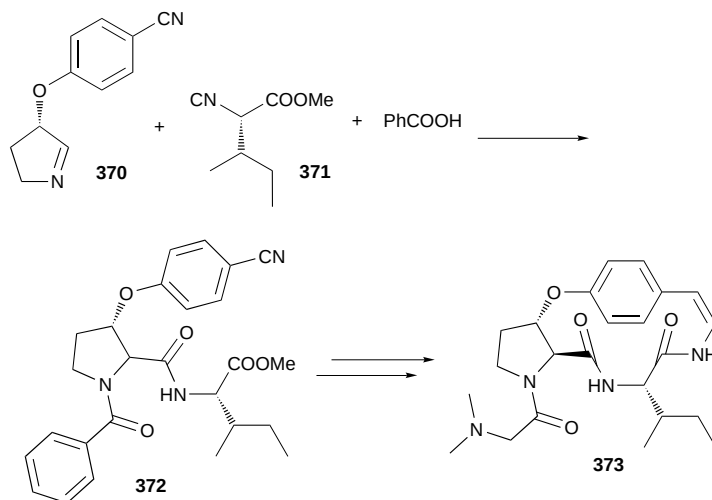
carbonyl function later in the synthesis, the missing diastereoselectivity of the Passerini reaction is of no importance. Overall, the complicated 13-membered natural product was synthesized in a short sequence.^[246]

Armstrong et al. have described the synthesis of the DNA-binding and alkylating antibiotic azinomycin **369** and of libraries of analogues by means of the P-3CR of appropriate α,β -unsaturated isocyanides, aldehydes, and carboxylic acids.^[8b]

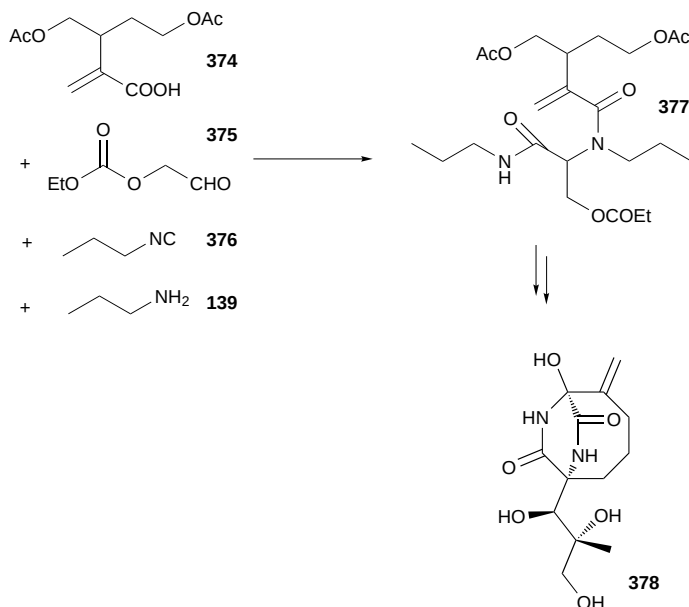


The large group of cyclic peptide alkaloids has been the object of numerous synthetic efforts since the first description in 1963. They usually are 13- to 15-membered macrocycles

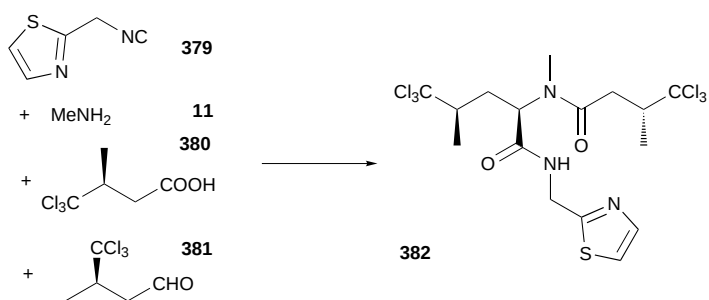
with para- or metacyclophane units and characteristic aryl ether bonds. Joullié et al. chose the U-4CR as the central step in the total synthesis of the 14-membered cyclopeptide alkaloid **373**.^[247]



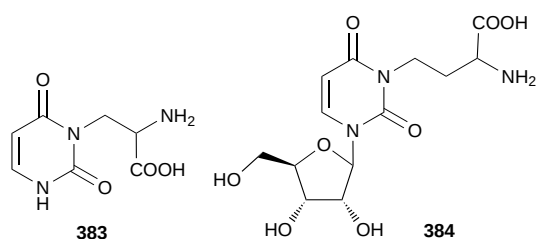
The antibiotic bicyclomycin **378**, which was isolated from *Streptomyces sapronensis* and is very active against gram-negative bacteria, and many derivatives were synthesized by Fukujama et al.^[248] A key position in the synthesis of numerous analogues is taken by the U-4CR of the α,β -unsaturated carboxylic acid **374**, the amine **139**, the α -hydroxy aldehyde **375**, and the isocyanide **376** to form **377**.



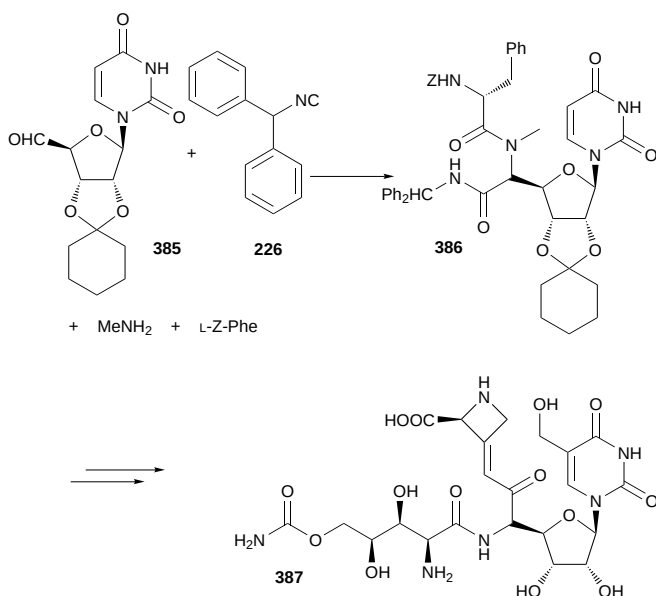
Dysidenin, isolated from the sponge *Dysidea herbacea*, is an unusual, highly chlorinated toxin which in its demethyl variant **382** could be synthesized in virtually one step by U-4CR from the appropriate starting compounds **379**–**381** and **11**. The two possible diastereomers are formed in a ratio of 17:13 and can easily be separated chromatographically.^[249]



Naturally occurring antimetabolites such as isowillardine (**383**), nikkomycin (**384**), and sinfugin were synthesized by Japanese research groups on a 20 g scale, a U-4CR being the key step.^[133, 250]

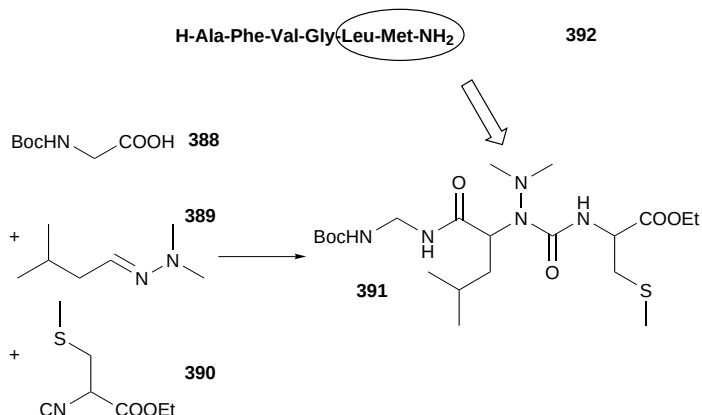


The total synthesis of various highly active fungicidal polyoxins like **387** was described by Boehm et al. A U-4CR with diphenylmethyl isocyanide and the aldehyde **385** was used as the central step. The resulting amide was oxidatively converted to the diphenylmethyl ester with N_2O_4 .^[251] The product of the U-4CR with amino-protected α -amino acids and diphenylmethyl isocyanide (**226**) was transformed to the corresponding oxopiperazines by means of hydrogenation. In another variant, cyclohexenyl isocyanide was used, which was transformed to the carboxylic acid via the primary amide.^[252]

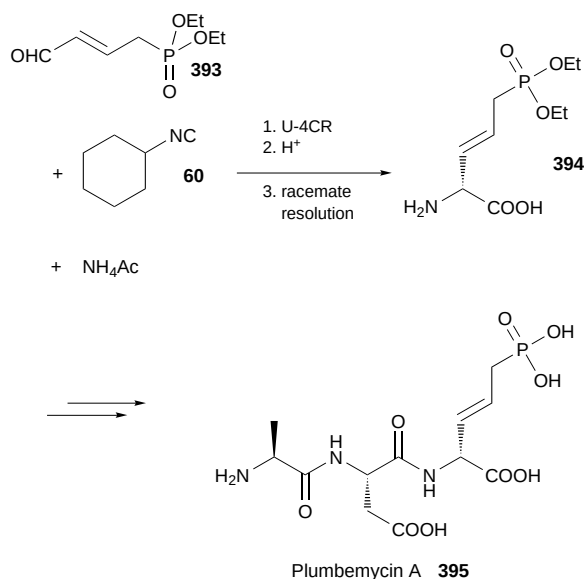


The modified analogue **391** of the antihypertensive hexapeptide elodoisin (**392**) was synthesized in a U-4CR and screened pharmacologically. For this reaction, the dimethyl-

hydrazine Schiff base **389** as the amine component was treated with N-protected glycine **388** and methionine isocyanide (**390**).^[253]

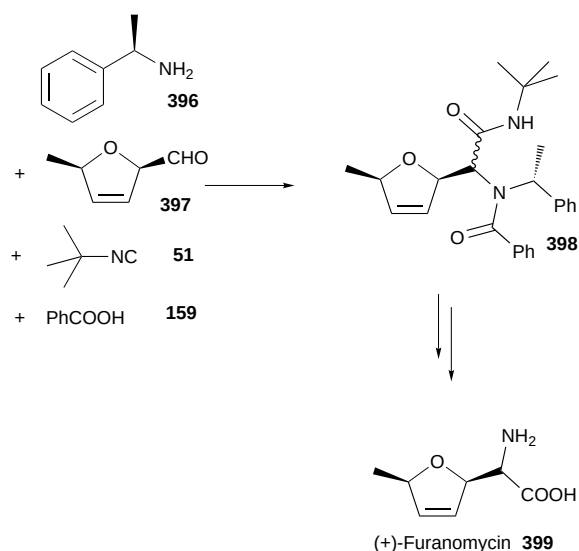


The antibiotics plumbemycin A (**395**) and B, as well as some analogues, were obtained in a synthesis which used the U-4CR as its key reaction. The unusual amino acid 3,4-didehydro-5-phosphono-D-norvaline (**394**) was obtained in a one-pot synthesis by means of U-4CR of cyclohexyl isocyanide, ammonium formate, and the aldehyde **393** and subsequent hydrolysis in 80% yield.^[189] The resolution of the racemate was carried out enzymatically.

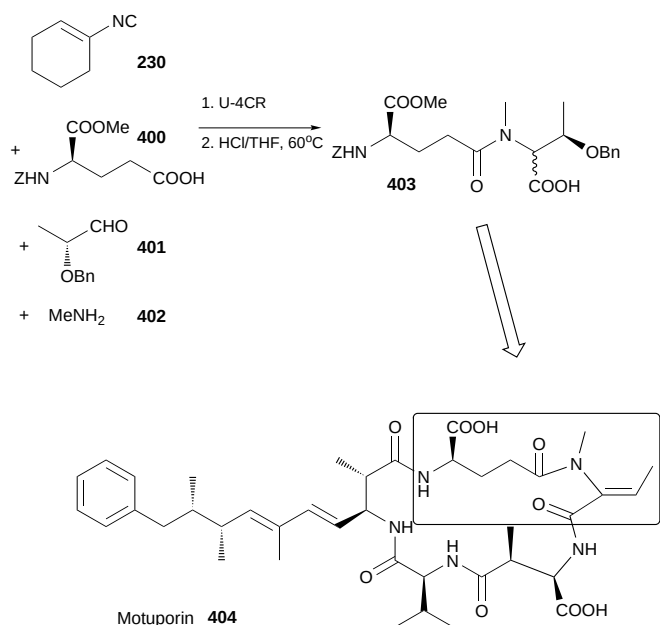


Furanomycin is a rare example of a triketide consisting of two acetate and one propionate unit. The stereoselective total synthesis of (+)-furanomycin (**399**) and its stereoisomers by means of U-4CR of **396**, **397**, **51**, and **159** to form **398** was achieved by Joullie et al.^[254]

Recently, the total synthesis of the complex protein phosphatase (PP) inhibitor motuporin (**404**) was described.^[255] The part of this cyclic peptide, consisting of a didehydro amino acid, an α -, two β -, and one γ -amino acid, which is of



interest for the bonding to PP1, was synthesized by means of a U-4CR and is therefore easily accessible for diversification. Both of the diastereomers **403** formed in the U-4CR in a ratio of 1:1 are converted into the didehydro amino acid fragment in a later stage of the total synthesis.



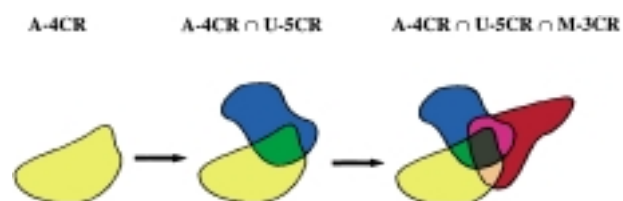
In this section, several examples for the application of the U-4CR in the synthesis of unnatural α -amino acids were presented. A series of different auxiliaries which can be removed in mild conditions is available today so that the IMCR should always be considered as a possibility for the synthesis of α -amino acids.

6. The Union Concept

Of all the combinatorial techniques, only split-and-combine methods^[256] and MCRs give the chemist the opportunity to examine very large structural space. With the entirety of all

organic-chemical reactions (two-component reactions), far fewer compounds can be synthesized than with MCRs by several orders of magnitude. If conventional reactions are to lead to large, highly diverse libraries, sequential work is necessary and the chemist inevitably has to go over to solid-phase syntheses—with all their advantages and disadvantages. Not so in MCRs, which often also proceed well in solution, but nevertheless open large structure and substance space.

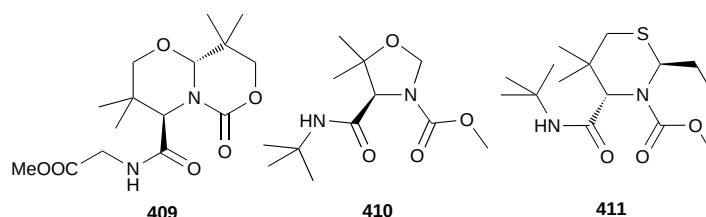
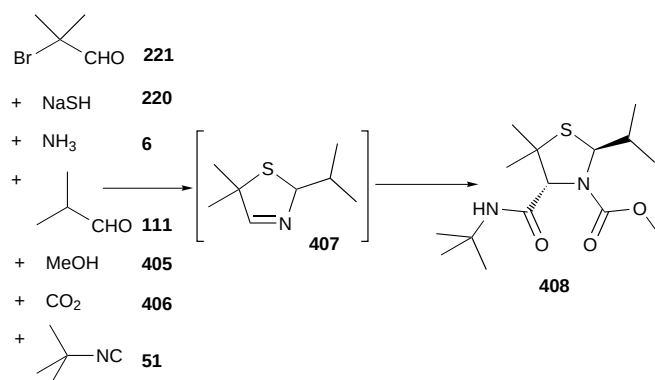
A few years ago, we asked ourselves how many starting materials can at most be converted to a product in a one-pot reaction. Our answer was: seven.^[257] We were able to show that the A-4CR can be combined with the U-5CR: $A-4CR \cap U-5CR \equiv AU-7CR$ (Scheme 16).^[258] The concept of unions of



Scheme 16. Schematic representation of the combination concept. The colored areas correspond to MCRs and the overlaps to new combinations of MCRs.

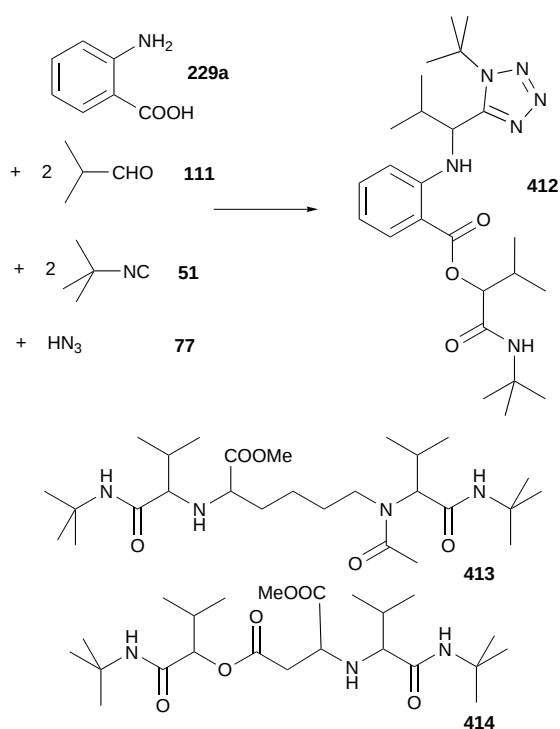
two or more (M)CRs proved to be productive in the following years, and further examples were described. It has turned out that many such combinations of MCRs are possible if the starting materials are well-chosen. Ideally, the elementary steps of the reactions take place according to a program. It is to be expected that many more examples are to come in the next few years. The reactions resulting from the union concept offer a greater structural variety than the simple MCRs.

A remarkable “seven-component reaction” is, for example, the transformation of the α -bromoaldehyde **221** with NaSH (**220**), another oxo component (**111**), and ammonia (**6**) in an



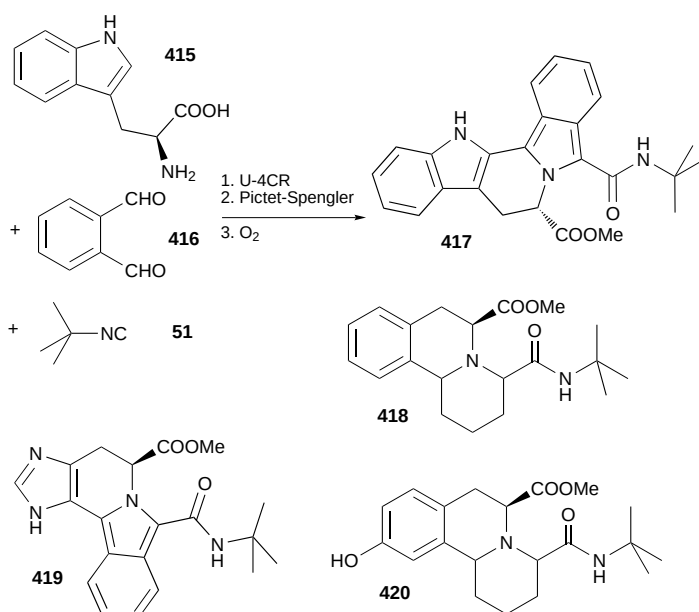
Asinger four-component reaction (A-4CR) to form the thiazoline **407**, which undergoes a U-4CR with the isocyanide **51** in methanol (**405**) and under CO₂ (**406**), affording the thiazolidine **408**. After optimization of the reaction, **408** was obtained in up to 45 % yield.^[257] Oxazine, oxazolidine, and thiazine derivatives (**409–411**) were also synthesized in this fashion.

Further such combinations of MCRs were achieved with multifunctional parent compounds. Thus, anthranilic acid reacts with two equivalents of isobutyric aldehyde, two equivalents of isocyanide, and hydrazoic acid in a Passerini and Ugi reaction (U-4CR $\cap$ P-3CR $\equiv$ UP-5CR) to form **412**. Compound **413** is obtained from lysine and a further six components (U-4CR $\cap$ U-4CR $\equiv$ UU-7CR) and **414** from glutamic acid and five other components (U-4CR $\cap$ U-5CR $\equiv$ UU-6CR).^[259]

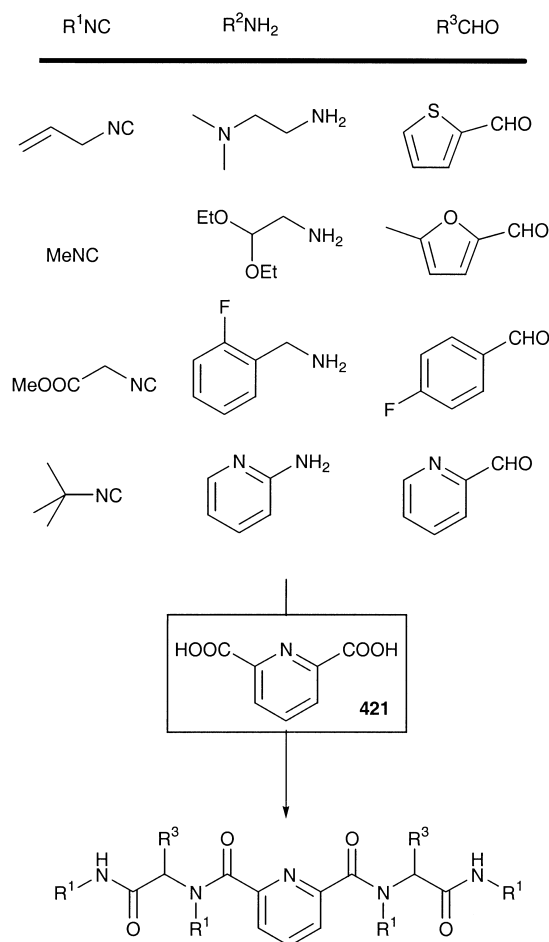


Recently the combination of the Pictet–Spengler reaction with the Ugi reaction succeeded: U-4CR $\cap$ PS-2CR. Here, electron-rich aromatic ethylamine derivatives react with dialdehydes and isocyanides, forming structures similar to alkaloids. In the example shown, tryptophane **415** first reacts with phthalic aldehyde **416** and the isocyanide **51** in an Ugi reaction, the α -amino acid variant, to a nonisolable intermediate which finally affords the pentacycle **417** after a Pictet–Spengler reaction and subsequent oxidation. Other aromatic amino acids, such as phenylalanine, histidine, 3,4-dihydroxyphenylalanine (DOPA), or tyrosine, were also converted to the products **418–420** in this fashion.^[260]

An interesting example of how the combination of U-4CRs can lead to very many products is the reaction of a dicarboxylic acid with four each amines, aldehydes, and isocyanides. In view of the construction of pseudo-C₂-sym-



metric protease inhibitors, pyridine-2,6-dicarboxylic acid (**421**) was treated with aldehydes, amines, and isocyanides. In this way, 13 starting materials lead to 8256 products (Scheme 17).^[186]

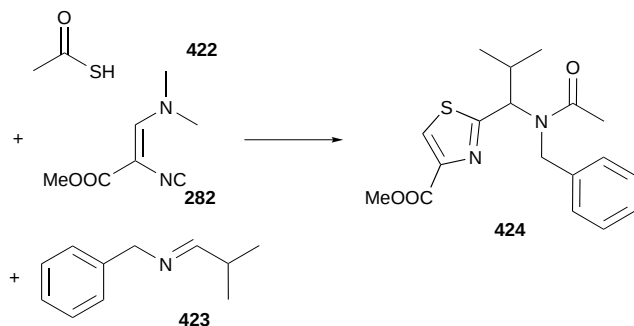


Scheme 17. Construction of pseudo-C₂ symmetric protease inhibitors from pyridine-2,6-dicarboxylic acid (**421**), aldehydes, amines, and isocyanides. A total of 8256 compounds are accessible from only 13 starting materials.

7. Outlook or Can New MCRs Be Found?

Basically, new MCRs can be found in different ways: by reflection (reaction design) or by applying combinatorial techniques (combinatorial reaction finding).^[261]

Some time ago we discovered that thiocarboxylic acids react in a U-4CR. Interestingly, only one of the two possible regioisomers α -amino thioacylamide and α -aminoacyl thioamide is formed. X-ray analyses prove that the α -amino thioacylamide is the exclusive product. This is in harmony with the different bond strengths (C-S < C-O). Thioamides are known as parent compounds for diverse thiazol syntheses. However, the thioamide obtained in this U-4CR variant is not suitable for any of the known thiazole syntheses due to its substitution pattern. Nevertheless, when methyl β -dimethylamino- α -isocyanoacrylate **282** as the isocyanide component is treated with Schiff bases and thiocarboxylic acids, 2,4-disubstituted thiazols such as **424** are formed in one step.^[262] It is of great importance that only one of the two possible



isomers is formed in the rearrangement in the U-4CR. The other theoretically possible isomer cannot cyclize with β -dimethylamino- α -isocyanoacrylic acid derivatives to form a thiazole, as this would lead to a tertiary thioamide and no aromatization could take place. Thus, by choosing a suitable bifunctional parent compound in combination with a new selective acid component, a multicomponent thiazole synthesis was designed and carried out.

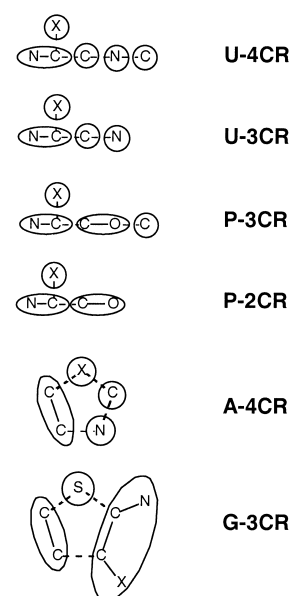
Can the chemist be given a set of rules or even algorithms and therefore programs which enable him to use MCRs for the total synthesis of natural and medicinal products and in the search for new drugs?

The intuitive method of synthesizing desired target molecules by means of MCRs is shown in Scheme 18. The smallest common structural element of a MCR is searched for in the molecule to be synthesized.

A combinatorial way of finding new MCRs was presented by Weber et al.^[263] A collection of ten compounds with different functional groups was provided (Scheme 19). Then, all MCR combinations of the ten parent compounds were pipetted together automatically, a 10CR variant, ten 9CR variants, etc. In the HPLC analysis, the signals for the starting substances and those from obvious 2CR products were ignored in the evaluation. Reaktionen whose product signals passed a predefined threshold value (here: 30%) were repeated on a preparative scale and analyzed in detail. In

this way, many classic MCRs were found, but many new MCRs were also discovered. For example, cyclohexanone reacts with benzyl isocyanide, acetic acid, and *p*-methoxyphenyl hydrazine hydrochloride to form 2,3-dihydrocinnoline **425**.

The chemistry of the MCRs is more varied than that of the 2CRs by several orders of magnitude. Whereas mainly the chemistry of 2CRs was examined ever since the beginnings of organic chemistry, MCRs were only researched sporadically and only by a few groups. Therefore, the chemistry of the MCRs presents itself rather like a map of the world before the times of the great discoverers: full of white areas. Thus, MCRs offer a large field for diverse and important new discoveries.^[264–282]



Scheme 18. In the products of every MCR, common basic structures can be found. These smallest common atom connectivities can help the synthetic chemist discover an MCR for his target molecule that can simplify its total synthesis.

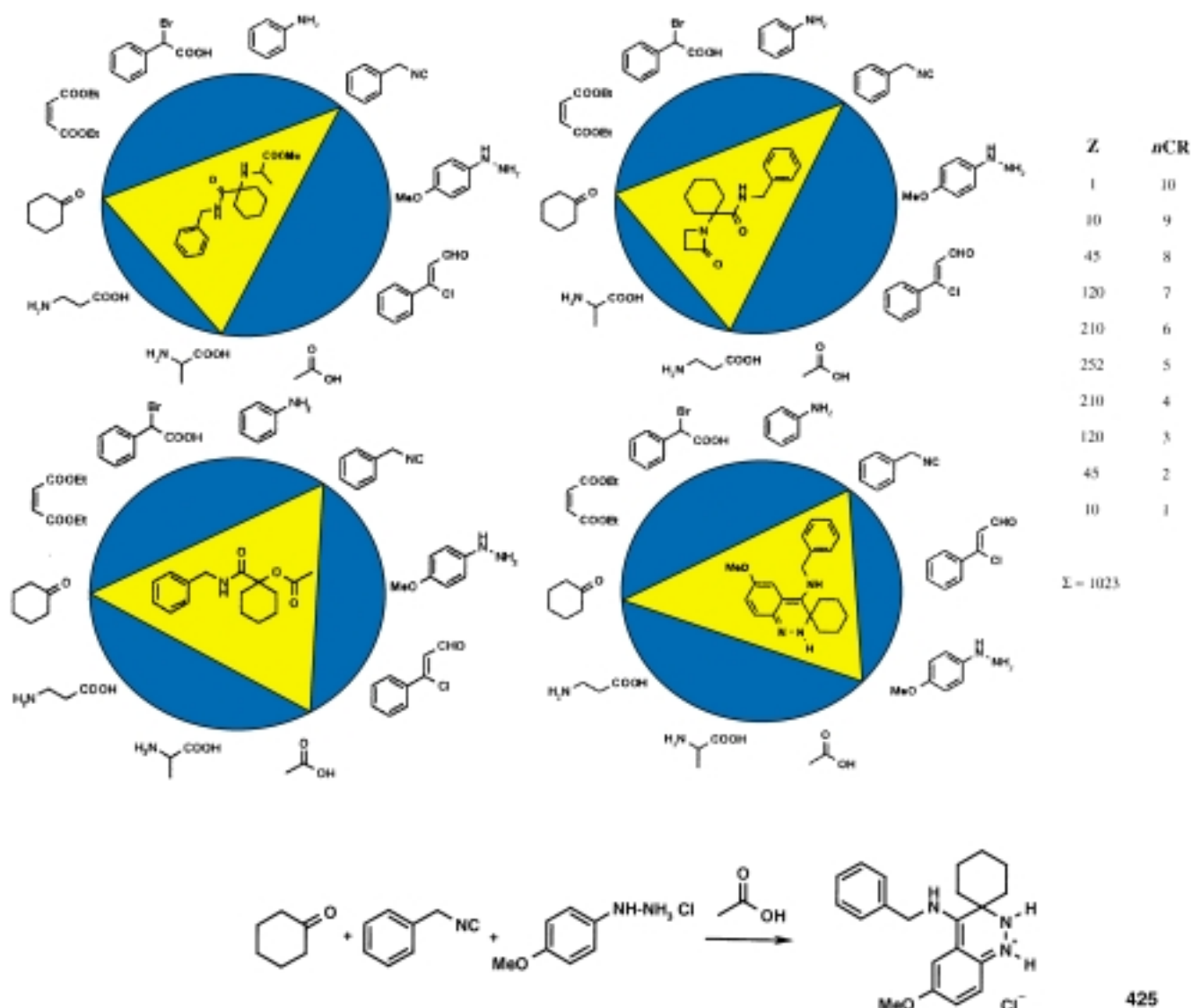
8. Appendix: Abbreviations

MCR	multicomponent reaction
IMCR	isocyanide MCR
M-3CR	Mannich three-component reaction
A-4CR	Asinger four-component reaction
PS-2CR	Pictet–Spengler two-component reaction
U-4CR	Ugi four-component reaction
P-3CR	Passerini three-component reaction
BB-4CR	Bucherer–Bergs four-component reaction
UM-5CR	Ugi–Mannich five-component reaction
G-3CR	Gewald three-component reaction
4CC-SSS	four-component condensation with stereoselective segment coupling
4CC-SC	segment coupling by means of four-component condensation
HLE	human leukocyte elastase
MDR	multiple drug resistance
CMV	cyto-megalo virus

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Scheme 19. Finding MCRs by means of combinatorial methods: Ten parent compounds with different functional groups are depicted on the rim of a circle. In principle there are 1023 possibilities for these compounds to react with each other if each compound is to react only once. Interestingly, there are many more possibilities for MCRs than for 2CRs with a maximum at 5CRs. Analysis of all combinatorial reaction possibilities led to the discovery of new MCRs, but also the rediscovery of old ones: the MCR of β -amino acids, ketones, and isocyanides to β -lactams (top right), the reaction of α -amino acids, isocyanides, and ketones to give iminodicarboxylic acid derivatives (top left), the classic Passerini reaction of isocyanides, carboxylic acids, and ketones (bottom left) and an example of a new 3CR of aromatic hydrazines, ketones, and isocyanides, which cyclize to dihydrocinnolines in the presence of acetic acid (bottom right).

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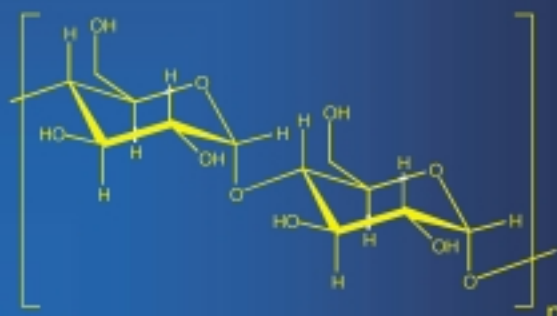
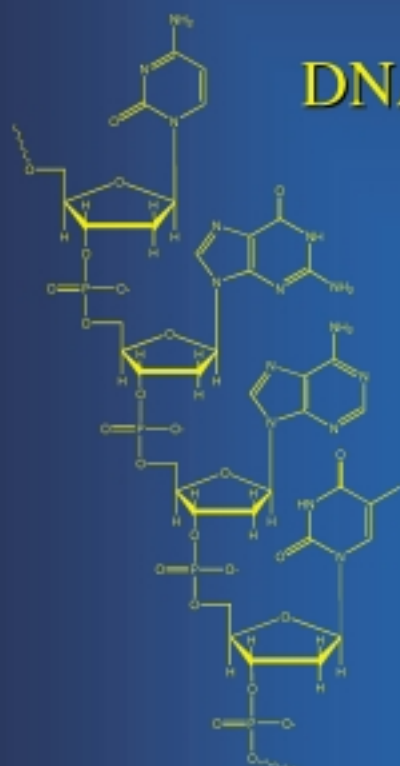
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Force Spectroscopy

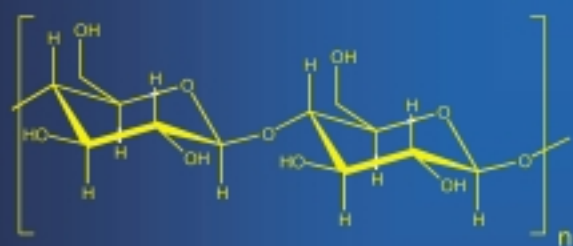
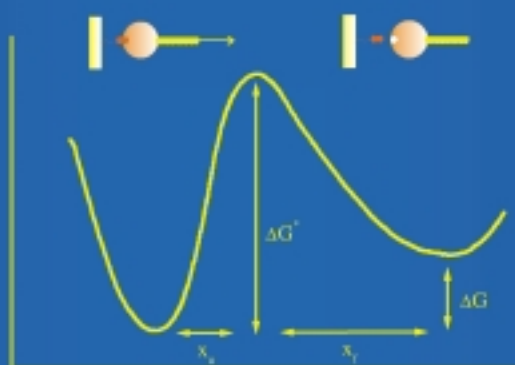
Wormlike chain

$$F(x) = \frac{k_B T}{\ell_p} \cdot \left[\frac{1}{4} \cdot (1 - x/L)^{-2} + x/L - \frac{1}{4} \right]$$

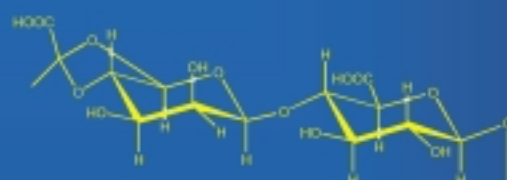
DNA



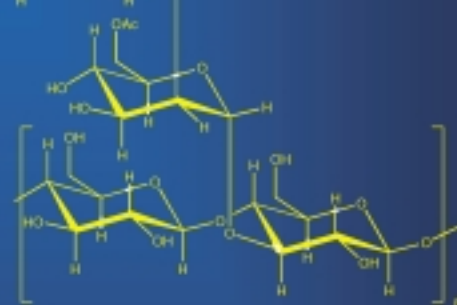
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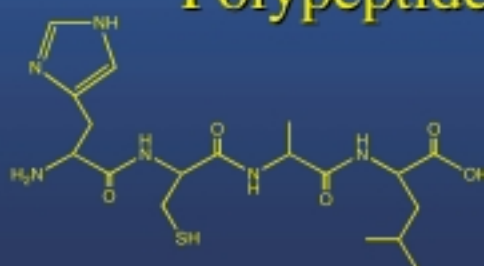
Cellulose



Xanthan



Polypeptides



Force Spectroscopy of Molecular Systems—Single Molecule Spectroscopy of Polymers and Biomolecules

Andreas Janshoff, Marcus Neitzert, York Oberdörfer, and Harald Fuchs*

How do molecules interact with each other? What happens if a neurotransmitter binds to a ligand-operated ion channel? How do antibodies recognize their antigens? Molecular recognition events play a pivotal role in nature: in enzymatic catalysis and during the replication and transcription of the genome; it is also important for the cohesion of cellular structures and in numerous metabolic reactions that molecules interact with each other in a specific manner. Conventional methods such as calorimetry provide very precise values of binding enthalpies; these are, however, average values obtained from a large ensemble of molecules without knowledge of the

dynamics of the molecular recognition event. Which forces occur when a single molecular couple meets and forms a bond? Since the development of the scanning force microscope and force spectroscopy a couple of years ago, tools have now become available for measuring the forces between interfaces with high precision—starting from colloidal forces to the interaction of single molecules. The manipulation of individual molecules using force spectroscopy is also possible. In this way, the mechanical properties on a molecular scale are measurable. The study of single molecules is not an exclusive domain of force spectroscopy; it can also be performed with a

surface force apparatus, laser tweezers, or the micropipette technique. Regardless of these techniques, force spectroscopy has been proven as an extraordinary versatile tool. The intention of this review article is to present a critical evaluation of the actual development of static force spectroscopy. The article mainly focuses on experiments dealing with inter- and intramolecular forces—starting with “simple” electrostatic forces, then ligand–receptor systems, and finally the stretching of individual molecules.

Keywords: DNA recognition • force spectroscopy • polymers • scanning probe methods

1. Introduction: Microscopic Forces

*Geschrieben steht:
Im Anfang war der Sinn.
Bedenke wohl die erste Zeile,
dass deine Feder sich nicht übereile!
Ist es der Sinn,
der alles wirkt und schafft?
Es sollte stehn:
Im Anfang war die Kraft!*

Johann Wolfgang von Goethe (1749–1832), *Faust I*

The physical unit of force is named after Sir Isaac Newton (1643–1727), who was the first to mathematically describe the concept of force precisely in his axioms. Moreover, Newton

was not only interested in long-range gravitational forces but also—ahead of time—in microscopic forces: “The parts of all homogeneous hard bodies stick together very strongly. I had rather infer from their cohesion, that their particles attract one another by some force...”. To the best of our present knowledge, Newton already referred to intermolecular forces at that time. The radius of action of intermolecular forces is in the microscopic range, and extends from a few micrometers to several angstroms. Intermolecular forces determine the properties of gases, behavior of colloidal solutions, growing of crystals, and interaction of biomolecules. It is not only the radius of action of these forces which is on the microscopic scale but also its magnitude relative to the forces that are present in daily life. In general, its magnitude is only some fractions of a nanonewton. The direct measurement of such forces is an experimental challenge and was first possible in the late 1970s with the invention of the *surface force apparatus* (SFA).^[1] This apparatus can be used to monitor forces between two transparent, atomically smooth and curved mica surfaces covered with the system under investigation by optical interference techniques. The SFA has a force resolution of approximately 10 nN and a vertical resolution of

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0.1 nm. A drawback of this method is its limitation to atomically flat transparent substrates and the lack of lateral resolution. In addition, contaminations on the large surfaces may influence the measurements considerably.

With the development of the atomic force microscope (AFM) in the mid 1980s an instrument became available for the first time that was distinguished not only by its enormous force resolution down to the piconewton range but also by its lateral and vertical resolution.^[2] Scanning force microscopy gained high significance in research fields where in situ imaging of surfaces with a submicrometer resolution was required, such as in materials and life sciences. The versatility of this method is based on the large number of contrast mechanisms. Besides the topography, elastic moduli, friction

coefficients, chemical residues, surface charges, and energy loss during the oscillation of the cantilever can be imaged with high spatial resolution.

The difference between static force spectroscopy and dynamic force spectroscopy is that in the former the interaction between the tip and the sample is calculated from the amplitude signal as well as the frequency and phase shift of the cantilever's oscillation.^[3]

By chemical functionalization of the tip the force microscope has developed as a new tool for materials and life sciences. For the first time it was feasible to measure the forces between two individual molecules locally. A milestone in this regard was the direct observation of a single bond rupture of a distinct streptavidin–biotin complex in 1994 as reported



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Andreas Janshoff was born in 1966. He attended the Westfälische Wilhelms-University (WWU) Münster, where he received a B.S. in Biology and Chemistry. He received his M.S. in 1994 and his Ph.D. in 1997 for studies on the biofunctionalization of gold surfaces and its applications in bioanalytics, which he carried out at the Institute for Biochemistry at the University of Münster under the supervision of Prof. Hans-Joachim Galla. As a postdoctoral fellow, he worked in the group of Prof. M. Reza Ghadiri at the Scripps Research Institute, La Jolla (CA), USA for 1½ years. During this time he worked on scanning force microscopy imaging of lipid membranes. In 1999 he returned to Germany to join the group of Prof. Harald Fuchs at the WWU Münster starting his habilitation in close collaboration with the Institute for Biochemistry (WWU). His research fields include the development of new sensor systems based on micropatterned surfaces, force spectroscopy of extracellular matrix proteins and the application of acoustic resonators to investigate biological relevant questions.

Marcus Neitzert was born in 1967 in Barcelona, Spain. He moved to Germany in 1986 to study Physics at the University of Heidelberg. He worked at the Laboratory for Surface Science and Technology at University of Maine and returned to Germany in 1994 to start his Ph.D. thesis at the Westfälische Wilhelms-University of Münster under the supervision of Prof. Harald Fuchs. He graduated in 1999 with a thesis on the force spectroscopy of biopolymers. Currently, he is working as a scientific journalist for the public relation office of the Deutsche Physikalische Gesellschaft (DPG).

York Oberdörfer was born in 1973 in Hamm-Heessen, Germany. From 1993 to 1999 he studied Physics at the Westfälische Wilhelms-University of Münster. He is currently a graduate student in the group of Prof. Harald Fuchs and his work is concerned with the force spectroscopy of modular proteins and micropatterning of surfaces.

Harald Fuchs, born 1951, studied Physics at the University of Saarbrücken. After his diploma thesis in theoretical physics he worked at the Institute of Physiology of the University of Saarbrücken until 1980 on transport processes in biological membranes. He graduated in 1982 in the group of Prof. Gleiter at the University of Saarbrücken with a Ph.D. thesis on nanocrystalline systems. Until 1984 he worked as a research associate at the Institute of Material Science. During his postdoctoral stay at IBM Rüschlikon from 1984 to 1985 he worked in the group of Binnig and Rohrer on scanning tunneling microscopy. From 1985 to 1993 he was the head of a research group at BASF AG. In 1993 he became full professor for Experimental Physics at the Westfälische Wilhelms-University of Münster and the head of the institute in 1998. In 1994 he received the Philip-Morris-award for his outstanding work on nanostructuring. His main research fields are nanotechnology, organic/inorganic interfaces, and the development of new scanning probe microscopy techniques.

independently by the groups of Gaub^[4] and Colton.^[5] Quasi-static force spectroscopy has become established as a particular technique of single molecule spectroscopy and has now found numerous fields of applications. It should be emphasized that not only static but also dynamic information is available, such as during the separation of a single ligand–receptor couple upon applying an external force with different pulling velocities. From these measurements information about the dynamics of the bond rupture of noncovalent complexes and, thus, about the energy barrier which needs to be overcome during bond rupture is gathered. Conformational analysis of polymer strands and biomolecules has also been developed as an additional independent research field. Here, long-chain molecules such as DNA, synthetic polymers, polysaccharides, and filamentous proteins experience folding–unfolding cycles upon application of an external force. The characteristic force–extension curves can be utilized to analyze the different conformations of the biomolecules by applying statistic polymer models.^[6] In summary, with scanning force microscopy and related techniques, methods are available that allow imaging and spectroscopic characterization of surfaces on a molecular scale as well as the manipulation of single molecules.

2. General Overview: The Force Microscope as a Sensor of Molecular Forces

Since its invention (1986) by Binnig, Quate, and Gerber^[2] force microscopy has evolved from being a predominantly imaging technique^[7–9] to a method that allows the investigation of molecular forces at interfaces in great detail.^[4, 5] The cantilever of the microscope acts as a sensor for the local interaction between the tip and the sample (Figure 1).

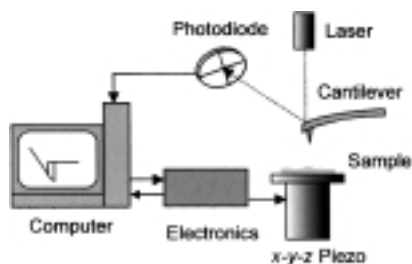


Figure 1. Schematic drawing of a force microscope with a photodetector. The deflection of the cantilever is detected by the laser deflection reflected from the cantilever. The sample is positioned on a piezoelectric device (movable in three directions). The piezo allows the sample to be moved in the vertical direction (*z*-piezo controlling unit) and scanning the surface in the *x*-*y* direction (*x*-*y* piezo controlling unit). There are two different modes: in the constant-height mode the cantilever deflection is detected without feedback control (applicable to very smooth surfaces), while in the constant force mode the *z*-piezo unit compensates for any possible cantilever deflections by changing the *z* position.

A so-called force–distance curve can be determined by detecting the cantilever deflection^[10] (which reflects the dependence of the force on the movement of the piezo device) when the tip of the force microscope is moved toward and away from the surface. The applied force F can be

deduced from the cantilever deflection z_F using Hook's law [Eq. (1)]. Force resolution (under ambient conditions) is in

$$F = k z_F \quad (1)$$

the range of piconewtons and restricted to the thermal fluctuation of the cantilever.^[11, 12] The entire measurement (approach and withdrawal) typically takes a couple of seconds. In some cases, the retraction curves exhibit various complex features from which conclusions about the interaction between the tip and samples can be drawn (Figure 2). The approach curve also contains valuable information about surface forces.

The interactions observed range from van der Waals forces in the nN scale (Figure 2A) to entropic forces of several hundred pN—the latter ones caused by the stretching of single molecules fixed between the tip and the substrate (Figure 2E). Thus, the interactions occurring are strongly dependent on the experimental conditions, such as the tip and sample material, chemical modification of the surfaces, and the surrounding medium. Therefore, it is in general possible to image surfaces in a chemically sensitive fashion.^[13–19] The interactions dominating the course of the experimental force curves depend on the medium. Van der Waals forces, which are always present in air, are in general superimposed by stronger capillary forces, which are caused by the condensation of water vapor in the area where the tip contacts the sample (capillary condensation). These capillary forces are negligible if the measurements are performed in fluids.

Force spectroscopy experiments in an aqueous solution are of major importance since only this environment allows the study of native biological processes and mechanical behavior of single molecules, such as nucleic acids and proteins, in situ. A proper functionalization of the tip and sample is very important for these kinds of experiments. Electrostatic forces are predominant in aqueous solution;^[20–23] they can be mainly attractive or repulsive (Figure 2B, C)—depending on the surface potential, the ionic strength, and pH. In addition to electrostatic interactions, the use of molecules with appropriate functional groups leads to short-ranging dipole–dipole interactions, hydrogen bonding,^[11, 24–26] or in the case of ligand–receptor couples highly specific complex combinations of different binding types as well as covalent bonding.^[4, 5, 27–30]

The forces between the tip and the sample can be investigated by force spectroscopy on a spatially restricted area. The upper limit is given by the contact radius of the tip with the substrate, which is typically of the order of some ten nanometers,^[31, 32] while the lower limit is in the range of single molecules or atom groups.

2.1. Force–Distance Curves

The cantilever deflection is a measure of the interaction force. The relationship between the cantilever deflection and the distance between the tip and sample is shown in Figure 3A with $s = l + d - z_F$. As deduced from Equation (1), attractive forces ($F < 0$) lead to a negative cantilever deflec-

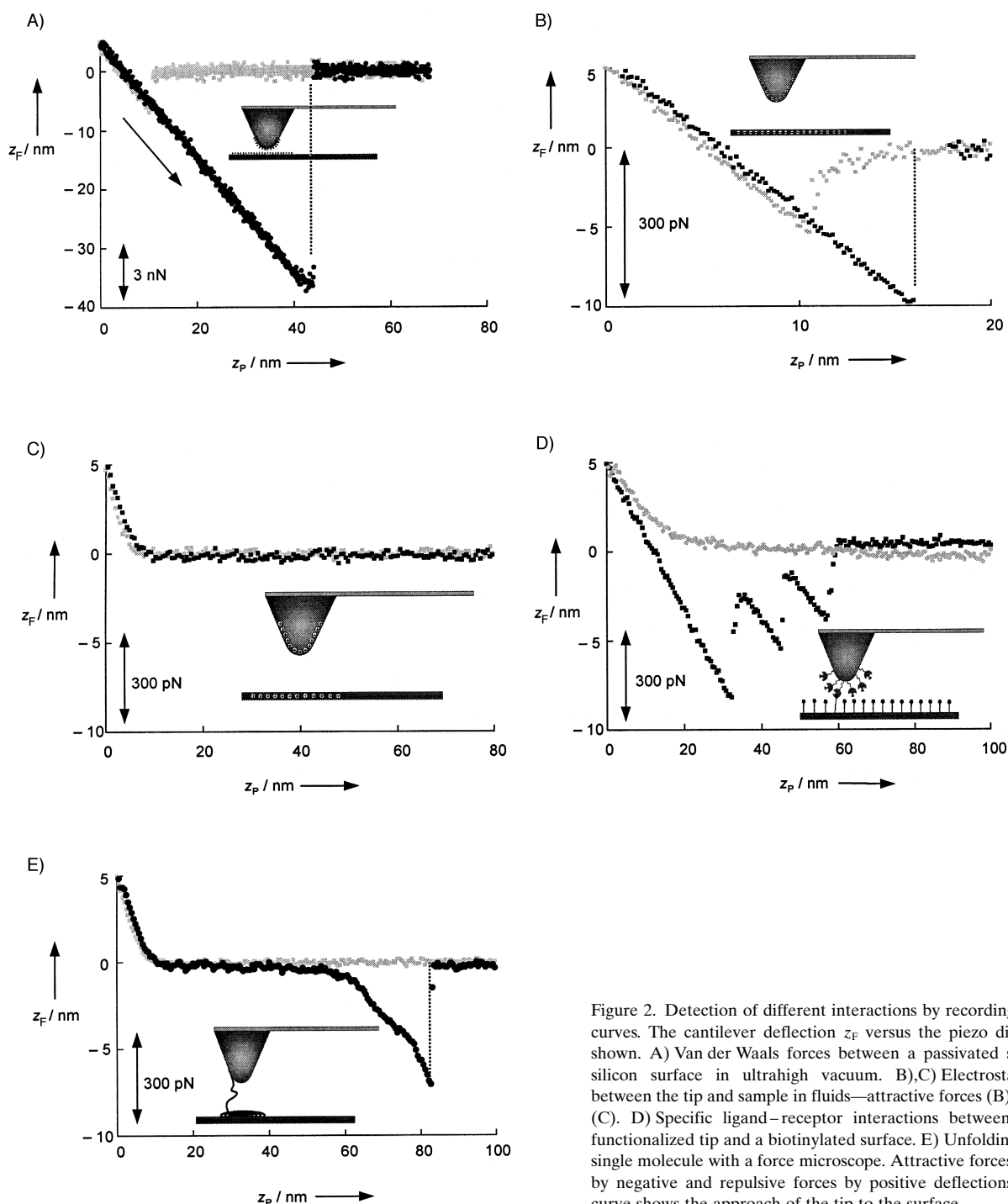


Figure 2. Detection of different interactions by recording force–distance curves. The cantilever deflection z_F versus the piezo displacement z_p is shown. A) Van der Waals forces between a passivated silicon tip and a silicon surface in ultrahigh vacuum. B), C) Electrostatic interactions between the tip and sample in fluids—attractive forces (B), repulsive forces (C). D) Specific ligand–receptor interactions between a streptavidin-functionalized tip and a biotinylated surface. E) Unfolding of a long-chain single molecule with a force microscope. Attractive forces are represented by negative and repulsive forces by positive deflections. The light gray curve shows the approach of the tip to the surface.

tion ($z_F < 0$). For a movement of the sample as a result of the displacement of the piezo device Δz_p , $\Delta s = \Delta z_p$ is valid.^[33] From this, the relation $\Delta z_p = \Delta d - \Delta z_F$ follows ($z_F < 0$ at attractive forces and $\Delta l = 0$). The slope of the force curve can be determined from Equation (2), where Δz_F is the change in

$$\frac{\Delta z_F}{\Delta z_p} = \frac{1}{k/F'(d) - 1} \quad (2)$$

cantilever deflection, and Δz_p is the change in the sample position or displacement of the piezo device. k is the spring constant of the cantilever and $F'(d)$ the force gradient, which

is a function of the tip–sample distance d . Equation 2 provides a stability criterion ($k/F'(d) - 1 \neq 0$). The characteristic features of an approach and retraction curve is given in Figure 3 B.

If the tip is far away from the surface forces are absent and the cantilever is not deflected (1). Attractive surface forces become apparent when the tip approaches the substrate: if the value of the positive force gradient is the same as the spring constant ($F'(d) = k$) a mechanical instability occurs according to Equation (2), known as snap-on (2). The tip jumps into a close repulsive contact with the sample (2→3),

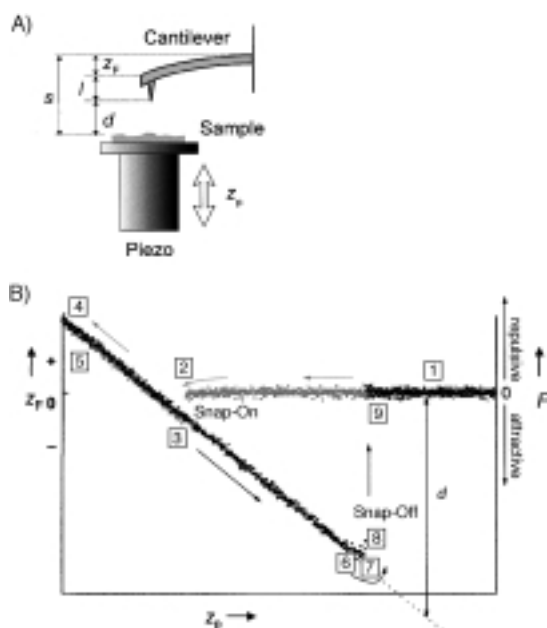


Figure 3. A) Relevant parameters for calculating the transfer function between the sample displacement z_p and the cantilever deflection z_F ; l is the length of the tip, d the distance between the tip and the sample. B) Simple force curve; the numbers and arrows illustrate the course of the measurement. A detailed description is given in the text.

and consequently the surface experiences deformation. Even if the cantilever was infinitely stiff, a snap-on would always be present on the atomic scale, since the gradient of the surface forces becomes larger than the elastic restoring force at any small separation ($1\text{--}2\text{ \AA}$).^[34] Although the net force is negative, repulsive forces also act on the cantilever.^[35] The deflection of the cantilever ($\text{[3]} \rightarrow \text{[4]}$) is now determined by the viscoelasticity of the substrate.^[36] Applications of force microscope range from the measurements of deformation of elastomers^[37] or thin organic layers^[38] to mechanical experiments on individual cells.^[39] A quantitative determination of the elastic moduli is, however, difficult in most cases as the contact area needs to be known precisely.^[40, 41] This is rarely the case since on the one hand the tip geometry varies from cantilever to cantilever and on the other hand the contact radius is a function of adhesion.

The pressure that the tip exerts on the surface increases as the sample displacement increases. In the case of a hard substrate ($|F(d)| \gg k$) the slope of the force curve converges to -1 ([4]), sample displacement and cantilever deflection are proportional ($\Delta z_p = -\Delta z_F$, here $z_p = -z_F$). The distance between the tip and the sample remains constant along this contact line. For example, this is the case for glass and passivated silicon. This fact is used to calibrate the cantilever deflection in the case of force microscopes using a laser deflection signal, which means that the deflection is directly obtained from the signal of the photodiode (voltage).

The repulsive forces decrease continuously ($\text{[5]} \rightarrow \text{[6]}$) upon retraction of the z-piezo device. The separation from the sample surface occurs at the so-called snap-off ([8]), which is visible immediately after the minimum of the curve ([7])—at this point the maximum attractive force can be measured (adhesion force). According to the most common contact

models^[37, 42] the adhesion force F_{Adh} is proportional to the tip radius and the surface energy between the tip and the sample. The snap-on and the snap-off are both characterized by a mechanical instability ($F(d) = k$); the contact to the sample surface is suddenly interrupted ($\text{[8]} \rightarrow \text{[9]}$). The cantilever reacts sensitively to disturbances, such as thermal noise or vibrations, in the vicinity of the curve minimum ([7]). Therefore, the snap-off already occurs at the minimum. Large regions of the attractive interaction potential are not accessible experimentally because of the snap-on and snap-off. This becomes more pronounced for softer cantilevers. Under appropriate conditions the adhesion force, that is, the snap-on/off, might vanish in fluids. The interaction between the tip and the sample is then mainly repulsive.

A force curve is a plot of the measured force versus the displacement of the z-piezo device. The spring constant has to be determined most accurately for a quantitative evaluation of the force. A very elegant method of calibrating is to monitor the spectral density of the thermal noise of the cantilever. The determination of the average amplitude of the vibrational resonance allows the calculation of the spring constant.^[43] In most cases it makes more sense to display the force as a function of the tip–sample distance. The displacement of the piezo device can be translated into a tip–sample distance by subtracting the contact line from the cantilever deflection^[44]—after this normalization one obtains a so-called force–distance curve. It is possible to directly determine persistence and contour lengths of polymers and elasticities of single-molecule chains from the tip–sample distance.^[45]

The approaching and retracting path of a force curve does not necessarily match (hysteresis). The enclosed area reflects the energy that is transferred from the cantilever to the sample and the environment during the entire cycle. The hysteresis is a measure of the viscosity of the sample. The sample gains deformational energy during the snap-on. If the sample is retracted and the tip is still on the surface, mechanical energy is stored in the strain of the cantilever. When the tip jumps off the surface, the energy is mainly transformed into vibrational energy of the cantilever.

3. Interactions Between the Tip and the Sample

3.1. Forces in a Vacuum

Van der Waals forces act between all particles on a nanometer scale. They emerge from the interplay of permanent and induced or fluctuating dipole moments and exhibit a $1/r^6$ distance dependency for two pointlike particles. The interaction between identical particles in a vacuum is always attractive. Van der Waals forces between different particles embedded in a third medium might also be repulsive.^[20, 37] A single van der Waals bond is relatively weak and of the order of -1 kJ mol^{-1} , which is equivalent to approximately 10 meV per molecule.^[20]

The interaction energy between macroscopic bodies such as a tip and a sample, that is, a sphere–plate model is given for small distances d between the sample and tip [Eq. (3)]:^[46] H is

$$W(d) = -HR/6d \quad (3)$$

the conventional Hamaker constant (a typical value in a vacuum is $H \approx 10^{-19} \text{ J}^{[20]}$). Figure 4 shows numerical simulations of the interaction of a silicon tip with a silicon sample in ultrahigh vacuum. The tip–sample interaction $F(d)$ that is

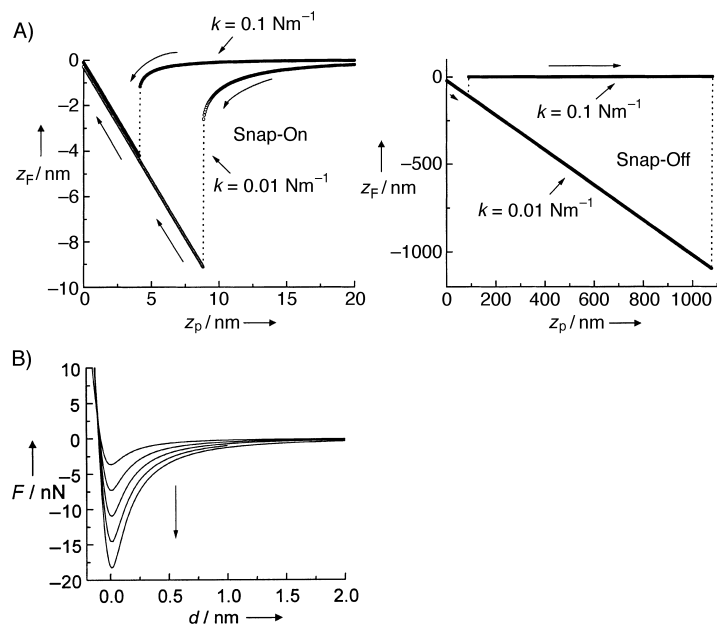


Figure 4. Influence of the spring constant and the tip radius on the appearance of the force–distance curve. A) Simulated force plots (sample approach) using different spring constants k (MYD/BHW potential, tip radius $R = 30 \text{ nm}$). B) Interaction of a silicon tip with a silicon substrate according to the MYD/BHW theory (based on van der Waals forces).^[3] Elastic modulus $Y = 179 \text{ GPa}$, Hamaker constant $H = 2.5 \times 10^{-19} \text{ J}$. An increase of the tip radius R (arrow) leads to a deeper potential with longer range.

needed for the numerical simulation of the force curve was determined following the approach of Muller–Yushchenko–Derjaguin/Burgess–Hughes–White (MYD/BHW). This model describes the entire course of the potential. The starting point is again the van der Waals force.^[47] The interfacial energy of the composite (Si/SiO_2) can be estimated from the obtained adhesion force to be $\gamma \approx 90 \text{ mJ m}^{-2}$. This value is in the range expected for van der Waals forces. The interfacial energy of approximately 1200 mJ m^{-2} ^[48] for pure silicon is larger since covalent bonds can be formed through dangling bonds.^[49]

3.2. Forces in Air

The predominant adhesive force in air is the capillary or meniscus force. A distinct hysteresis between the approach and retraction curve can be observed in the experimental force curve; during the approach the adhesion force is considerably larger than other attractive forces. The adhesion force is approximately $10\text{--}100 \text{ nN}$ and is therefore the prevailing force. In principle, the capillary force depends on the surface tension of the sample, tip, and fluid as well as on the tip radius, air humidity, and the thickness of the water layer. Quantitative measurements are mostly performed in a

vacuum, a dry gas atmosphere, or directly in liquids. Besides the dominating capillary forces, Coulombic forces may occur if a voltage is applied between a conductive tip and sample. These forces are mainly used to determine the geometry of the tip.^[50]

3.3. Electrostatic Forces in Aqueous Solutions

Van der Waals forces are weaker in aqueous solution than in a vacuum.^[20, 51, 52] This fact is the result of the high dielectric constant of water ($\epsilon \approx 80$)—van der Waals forces are of electrodynamic origin and are thus shielded from water dipoles. Though weak, van der Waals forces among particles of the same kind in nature are always attractive, even in electrolytes. Colloidal particles aggregate and precipitate as long as van der Waals forces are present. However, dissolved particles carry charges on their surface, which leads to repulsive forces that prevent aggregation. For example, red blood cells have negative surface charges that protect them from agglutination.^[53]

These electrostatic forces are dependent on the ion concentration and the pH of the solution. In simple terms, the pH influences the surface charge, while the number of ions in solution affects the radius of action of the electric field. Figure 5 depicts schematically the course of the potential at an interface. Electrostatic interactions among charged surfaces in fluids can be classically treated with the Poisson–Boltzmann equation,^[54] which describes the potential and charge density of the counter ions at every spot x except for the surface.

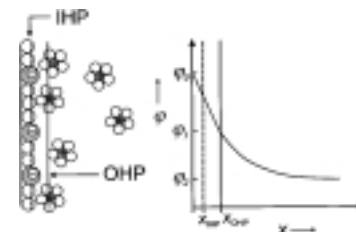


Figure 5. Left: Schematic drawing of the electrochemical double layer according to Stern. The first part of the double layer (Helmholtz layer) is characterized by a linear decrease in the potential up to the outer Helmholtz plane (OHP) followed by the diffuse double layer (Gouy–Chapman layer). The separating line at the inner Helmholtz plane (IHP) is positioned at the center of specifically adsorbed ions (here negatively charged). Ions carrying a solvation shell can approach the electrode only up to the outer Helmholtz plane because of the finite extension of the adsorbed ions. The graph on the right-hand side depicts the potential in the corresponding regions.

The theory of Derjaguin, Landau, Verwey, and Overbeck (DLVO)^[20, 52, 54] deals with the interplay of electrostatic interactions (which result from the charge distribution described above) and short range van der Waals forces. Only once the surface force apparatus was established could a number of the occurring surface forces be experimentally investigated, such as attractive van der Waals forces, repulsive electrostatic double-layer forces, solvation and structural forces, hydration forces, steric interactions, and hydrophobic

forces. Such interactions have also been shown by force spectroscopy experiments to be present in electrolytes.^[21, 22, 55–59]

The force F resulting from the approach of charged surfaces (namely, the tip and the sample) as a function of the distance d reads to a first approximation (for $d > \kappa$) as Equation (4).^[21, 60] ϕ_s and ϕ_p are the surface charges on the tip and sample,

$$F(d) \propto \phi_s \phi_p \exp(-d/\kappa) \quad (4)$$

respectively, and κ the Debye length, which is a measure of the range of the electric field^[61] or the extension of the electrostatic double layer.^[54] The situation is rather complex and depends on the surface charge.^[62] For the symmetric case, that is, the overlap of two identical double layers, there is a repulsive (long-range) interaction, whose origin is not electrostatic.^[63] In such a situation an electrostatic interaction would always favor an association. In reality, the effect is of entropic nature. The dissociation of surface molecules and the release of ions in solution result in a gain in entropy. Upon approaching the two double layers these ions are pushed (against the entropy) back to the surface. Consequently an osmotic pressure becomes generated—a repulsive force becomes apparent.

The DLVO theory describes the entire interaction as a superposition of the exponential repulsion and the attractive van der Waals force. Hence, the van der Waals interaction dominates at small distances below approximately 5 nm.^[64] If the Debye length decreases because of an increase of the ion concentration the long-range repulsion also decreases and the interaction becomes overall attractive.

When nonequally charged surfaces come close together^[52, 65, 66] the interaction is either repulsive or attractive depending on the ratio of the charges. If the charges are equal but opposite, the interaction is attractive. Figure 6 shows the

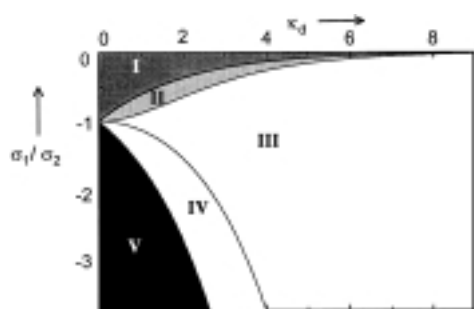


Figure 6. Regions of attractive and repulsive forces as a function of the Debye length κ_d and the ratio of the charge density σ_1/σ_2 of the approaching surfaces.^[62] I: Repulsion with both surface potentials positive; II: attraction with both surface potentials positive; III: attraction with surface potentials of different signs; IV: attraction with both surface potentials negative; V: repulsion with both surface potentials negative.

different areas of attractive and repulsive interactions occurring between planar and oppositely charged surfaces. Long-range electrostatic interactions can be monitored spatially resolved using force microscopy. Here, the pioneering work dealing with force spectroscopy at electrochemical double layers was performed by Butt et al.^[21, 53, 67] They demonstrated

for the first time the influence of pH and ionic strength on the surface potential using scanning force microscopy. Figure 7 shows the dependence of the force curves obtained experimentally on the ionic strength for a silicon nitride tip approaching a self-assembled film of 3-sulfanylpropionic acid

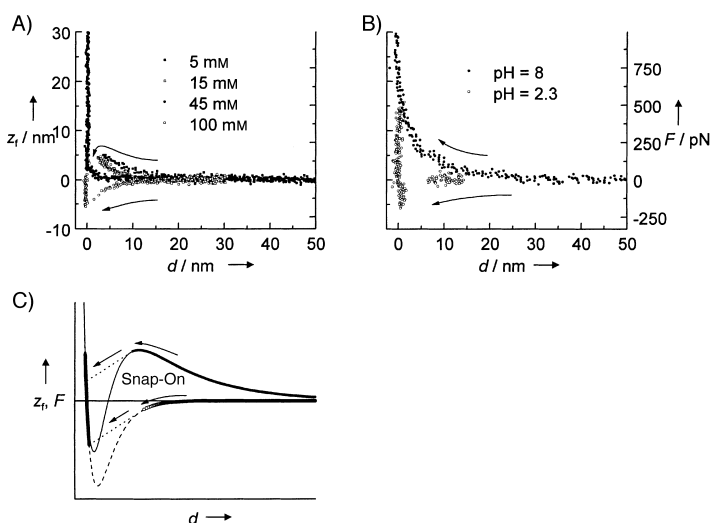


Figure 7. Analysis of electrostatic interactions by means of force-distance curves (only the approach curves are shown). A Si_3N_4 tip in contact with a self-assembled film (3-sulfurylpropionic acid) at pH 7.7: the tip and sample are both negatively charged. A) Variation of the ion concentration (KCl): repulsion is clearly observable before snap-on in the approach curve. Long-range electrostatic repulsion is well resolved at low ion concentrations. At 45 mM, snap-on occurs very close to the surface and the jump-to-contact is not resolved. B) Variation of the pH at low ion concentrations: at pH 8, both the tip and sample are negatively charged, which results in repulsive interaction; whereas at pH 2.3 the interaction is attractive. C) Numerical simulation of force-distance curves (qualitative) with underlying interaction forces (assuming a superposition of van der Waals forces and exponential repulsion) for low (—) and high ion concentrations (---).

chemisorbed on gold.^[68] At concentrations of 100 mM KCl the Debye length is already so small that attractive van der Waals forces prevail, and therefore the attraction is discernable (Figure 7A). A numerical simulation supports this result (Figure 7C). Likewise, the dependence of the surface charges on the pH value of the solution is shown. If both surfaces are negatively charged (pH 8), repulsion is predominant at an ionic strength of 5 mM. At pH 2.3, attraction is favored. Surface potentials can be imaged and surface charges, for example, of biomembranes, such as the purple membrane of the halobacterium *Halobacterium salinarum*, can be determined with this method.^[67]

3.4. Solvation Forces

If two surfaces approach as close as a couple of nanometers the forces occurring cannot be sufficiently ascribed with continuum theories, such as attractive van der Waals and repulsive double layer forces. Short-range forces resulting from the molecular structure of the fluid are summarized to so-called solvation or structural forces.^[20] The geometric organization of the solvent molecules at the solid surface

plays a crucial role in the strength of these forces. The attractive forces between the solvent molecules and the solid surface lead to an organization of the molecules into quasidiscrete layers. In the case of a fluid enclosed between two solids, one can show that rearrangements are induced as those two surfaces approach, which in most cases provide an exponential decay of an oscillating force. Monotonously declining attractive or repulsive components can also occur, and these are dependent on the geometry of the molecule and the interaction potential with the surface. A special case of the solvation force is the repulsive hydration force observed in aqueous solution. It describes the force necessary to remove the hydration shells of surface-confined molecules and bring the tip closer to the surface. In the case of two approaching lipid bilayers composed of zwitterionic phospholipids, very short-range forces of entropic nature with a range of 1–2 nm are observable because of the overlap of the head groups and hydrophobic chains. Forces which occur from the adsorption of highly charged ions can be determined from those hydration forces caused by steric effects. Butt demonstrated that an exponentially decaying repulsive force with a decay length of approximately 3 nm occurs if one eliminates the classical double-layer repulsion by adding CaCl_2 or SrCl_2 .^[69]

3.5. Chemical Force Microscopy (CFM)

This concept is a particular case of force spectroscopy where the tip and the substrate are derivatized so that interaction between defined functional groups can be clearly distinguished. In general, the immobilized molecules are low molecular weight compounds which in most cases contain only one functional group so that the interpretation of the results is facilitated.^[13, 14] The topics that can be addressed by CFM range from the spatially resolved determination of surface energies to locally determined pK values, which are obtained by mapping adhesion forces. Functionalization is mostly achieved by covering the tip and sample surface with gold and subsequently chemisorbing thiolated molecules. As an alternative, classical silane chemistry using mono- and trialkoxysilanes or trichlorosilanes is utilized. The environment (atmosphere, electrolyte concentration, pH) plays a central role in CFM. The following sequence of adhesion forces between amine- and carboxy-terminated functional groups was determined by force spectroscopy in a dry gas atmosphere to be in accordance with their binding strength: $\text{COOH}/\text{NH}_2 > \text{COOH}/\text{COOH} > \text{NH}_2/\text{NH}_2$.^[70] There is a vast number of data available for different interactions in various organic solvents, water, and gases. The interested reader is referred to the comprehensive review article of Noy et al.^[70]

Adhesion forces are generally larger among functional groups that have the ability to form hydrogen bonds than those without. Force spectroscopy is of particular interest if high surface energies need to be determined, such as which occur with carboxylic acid monolayers, which cannot be determined conventionally by contact angle measurements.

Mechanical models are the theoretical foundations for calculating surface energies using adhesion forces.^[71] In 1881 H. Hertz described the deformation of two elastic spherical bodies; however, adhesion was not considered.^[72] An analytical contact model including adhesion was developed by Johnson, Kendall, and Roberts (JKR model).^[73] To simplify this model it is assumed that adhesion is established by surface energy implying only forces in close contact to the surface. Derjaguin, Muller, and Toporov (DMT model) postulated a distance-dependent potential.^[74] For the sake of simplicity they assumed, that deformation of the surface is, apart from external forces, dependent on adhesion forces, which can be determined by integrating the energies of all the atoms in one body with all the atoms in the other. A complete solution of the contact problem of two elastic adhesive bodies is given by the MYD/BHW model.^[75] In the first case, the surface forces are, for example, described by a Lennard–Jones potential. A consequence of the MYD/BHW-model is that the pull-off force is dependent on the elasticity of the contact body and is in between the limits of the DMT and the JKR model. Depending on the elasticity of the body and the attractive forces a mechanically stable (DMT model) or a “jump to contact” behavior (JKR model) is conceivable.^[3]

In most cases the JKR or DMT model is sufficient to describe the experiment. While the JKR model is well suited for modeling soft and very adhesive bodies with large radii the DMT model describes bodies with the opposite properties best. From both the JKR and DMT model, a direct proportionality between adhesion force and adhesion energy can be obtained if a spherical tip and a flat surface is assumed: $W_{\text{ad}} (F \propto R W_{\text{ad}})$, with $W_{\text{ad}} = \gamma_{\text{SM}} + \gamma_{\text{TM}} - \gamma_{\text{ST}}$. γ_{ST} , γ_{TM} , and γ_{SM} are the free surface energies with the following indices M: medium, S: sample, and T: tip, and R is the radius of the tip. Large adhesion forces result from large interaction energies between the tip and the sample. To obtain γ_{ST} , first the tip and the sample are functionalized equally and γ_{TM} and γ_{SM} determined at the same time. Afterwards, γ_{ST} can be obtained by measuring the adhesion force between the tip and the sample when they are differently functionalized.^[76]

The free surface energy reflects the ionization state of the surface-confined molecules. Thus, the dependency of the state of the surface charges on the pH of the solution can be determined using contact angle measurements that lead to the pK values of the functional groups on the surface. These values can also be obtained using adhesion force measurements with appropriately functionalized CFM tips, especially if high surface energies need to be determined and contact angle measurements fail. A titration of the adhesion forces of a COOH-functionalized tip and sample is shown in Figure 8.^[70] The obtained surface pK value of approximately 5.5 agrees well with those determined in solution, which indicates that neighboring group effects can be neglected.

The basis for determining surface energies in the fashion described above are macroscopic models, which do not consider the discrete nature of the contact problem. If large forces occur, spontaneous rearrangements on the atomic scale and plastic deformations, which already occur at low forces, play a crucial role. Moreover, the assumption of an ideal

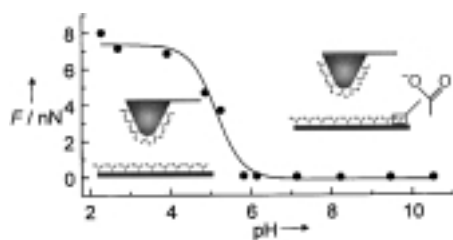


Figure 8. The dependence of the adhesion force F between a tip functionalized with 3-sulfanylpropionic acid and a similarly prepared substrate on the pH of the solution. At the point where the $\text{pH} = \text{pK}$ of the immobilized carboxylic acid the adhesion force decreases, since deprotonation abolishes formation of hydrogen bonds and as a consequence adhesion declines.^[70]

smooth surface as required by contact models is not fulfilled in reality. Even an insignificant roughness of some nanometers diminishes the adhesion considerably.

4. Force Spectroscopy of Ligand–Receptor Interactions

Molecular recognition of biomolecules plays a pivotal role in nature. Ligand–receptor interactions occur during the formation of double-stranded Watson–Crick DNA, in enzymatic reactions, in antigen–antibody recognition events, hormone–receptor reactions, signal transduction, the initial step of the infection of viruses and bacteria, inflammatory reactions, muscle function, adhesion and migration of cells, and in the cohesion of the cytoskeleton. Binding forces are generally a combination of electrostatic, hydrophobic, dipole–dipole interactions, and hydrogen bonding and depend on the conditions such as pH and ionic strength. Besides the thermodynamic and kinetic data, the complex interplay of forces is of paramount interest for the understanding of structure–function relationships of biomolecules.

For example, the consequences of simple point mutants (exchange of a single amino acid) on the specificity and binding strength of proteins can be investigated. Force spectroscopy is complementary to established methods which measure the properties of large ensembles of molecules. It is possible to estimate the shape of the potential describing the transition from the bound to the free state by means of statistical analysis of binding/rupture events. Accompanying the experiments, molecular dynamics (MD) simulations provided various supporting information that facilitated the understanding of binding and rupture on a molecular level.^[77] Evans and Richie, for example, managed to close the gap between MD calculations and experiments of dynamic bond dissociation under an external force by means of Monte Carlo simulations.^[78]

A typical experiment to investigate molecular recognition events comprises the functionalization of the probe (namely, the tip) and substrate with their respective binding partners. Here, it is important that the binding of the molecule to the tip or sample is much stronger than the intermolecular bonding being investigated. Two techniques dominate the chosen approaches, the chemisorption of thiols on gold and silanes on

siliconoxide surfaces. These covalent or quasi-covalent bonds ensure that the weakest bonds are the noncovalent ones between the biomolecules. Cross-linking between anchor molecules and various biomolecules can be achieved by common bioconjugation chemistry such as through the formation of amide bonds or imines. A contact between the sample and the tip is accomplished by moving the z-piezo device with the mounted sample towards and away from the tip. Retraction with constant velocity provides the desired information about bond-rupture events. Modern instruments permit complete control during these approach and retraction cycles through the sweep velocity, duration the tip is in contact, and resolution. Retraction is usually accompanied by several distinct rupture events. It is most probable that the last rupture event under an external force is the breakage of one single bond, since only then are few connections between the molecules left. Ideally, only one single bond of the ligand–receptor pair ruptures.

Statistical analysis of the data obtained shows the number of rupture events versus rupture force. By definition, the maximum peak represents the binding strength. Distributions occurring at higher binding forces can also be meaningful if the maxima are described in terms of nF (a multiple n of the actual binding force F , see Figure 13B—the shoulder at 556 pN represents the simultaneous rupture of two bonds). Coincident breakage of several bonds results in multiples of the binding force.^[97]

It should be noted that as an alternative approach, the binding strength can be resolved by applying the Poisson distribution as introduced by Beebe and co-workers. This approach enables the binding rupture to be analyzed without resolving single rupture events.^[79]

A simple kinetic model can be used to explain basic observations in force experiments, such as the probability distribution of rupture forces and their dependency on retraction velocity. The model is also successful in describing the force-driven unfolding of polymers, as will be described in more detail in Section 5).

4.1. Kinetics of Ligand–Receptor Separation

The lifetime of noncovalent bonds in the absence of external forces is considerably high. However, pulling long enough at one or the other end with an external force may eventually overcome the potential barrier arising from thermal fluctuations, and thus break the bond. The following presentation will show that adhesive and cohesive forces do not only depend on binding enthalpy but also on the pulling velocity. From a chemical point of view pulling a ligand–receptor couple apart can be considered as an infinite dilution of both compounds. The system is far away from thermal equilibrium and from a kinetic viewpoint the rate constant of association is negligible. Only the rate constant of dissociation is meaningful, the probability of rebinding is zero. The binding strength is thus a result of the kinetic stability of the corresponding complex. Binding strength depends on the pulling velocity relative to the thermal equilibrium conditions. As the external force decreases, the potential of the barrier,

dissociation that occurs on the time-scale of the experiment, is a result of thermal fluctuations. Bell introduced a simple model of binding dissociation under external forces that was based on the transition-state theory.^[80] He assumed that the free enthalpy of the transition state ΔG^* is reduced by the external force $\Delta G^* - Fx_u$, in which F denotes the mechanical, external force and x_u the width of the potential of the bound state.

Figure 9 illustrates the two-state model which allows the basic observations made in force spectroscopy to be explained. In analogy to radioactive decay or a monomolecular

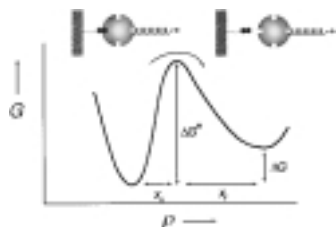


Figure 9. Classic two-state model of bond rupture of a ligand–receptor couple (left diagram) and the unfolding of a polymer (right diagram). ΔG^* is the free activation enthalpy, which is reduced by an external force until bond rupture occurs as a result of thermal noise, ΔG is the binding enthalpy, x_u the width of the potential of the bound state, and x_t the potential width of the unbound state.

reaction the probability of bond breakage after time t can be expressed by Equation (5), where k_{off} denotes the rate constant of dissociation. Notably, the rate constant depends, in

$$p_{\text{off}} = 1 - \exp\left(-\int_0^t k_{\text{off}}(t') dt'\right) \quad (5)$$

contrast to classical kinetics, on the time t [Eq. (6)]. x_u represents the dependence of the distribution of rupture

$$k_{\text{off}} = \nu_0 \exp\left(-\frac{1}{k_B T} \left(\Delta G^* - \int_0^{x_u} F(t) dx\right)\right) = \alpha \exp\left(\frac{Fx_u}{k_B T}\right) \quad (6)$$

forces on the shape of the potential, ν_0 denotes the frequency of the bond (the reciprocal of a diffusive relaxation time), and α denotes the dissociation rate in the absence of external forces. The time-dependence arises from the external force given by $F(t) = kvt$, in which v is the pulling velocity and k the spring constant of the cantilever.

Equation (6) illustrates that the rate of dissociation increases exponentially with the external force and therefore with the pulling velocity of the cantilever, whereas the association rate decreases exponentially with the external force. The probability density w that bond rupture occurs under an external force ramp is given in Equation (7). $w(F)$ describes the frequency of rupture events for a particular

$$w(F) = \frac{dp_{\text{off}}}{dF} = \frac{\alpha}{kv} \exp\left(\frac{Fx_u}{k_B T} \left(\frac{k_B T}{kvtx_u} \alpha \left(1 - \exp\left(\frac{Fx_u}{k_B T}\right)\right)\right)\right) \quad (7)$$

force and is usually illustrated by a histogram. By definition the maximum of the distribution is identical to the rupture force of the binding [Eq. (8)]. Increasing the pulling velocity results in a shift of the distribution to higher binding forces as shown in Figure 10.

$$F = \frac{k_B T}{x_u} \ln\left(\frac{x_u}{k_B T \nu_0} kv\right) + \frac{\Delta G^*}{x_u} \quad (8)$$

The width of the distribution depends on the width of the potential x_u and the thermal energy. A more elaborated description of this issue is given by Evans and Ritchie.^[78] Bell lumped the whole energy topology in one single parameter x_u , while Evans and Ritchie proposed a model in which bond breakage can be either classified as ductile or brittle. When the frictional losses arising from intrinsic and hydrodynamic damping are considered the rate of dissociation can be expressed as Equation (9), in which $g(F)$ and $\Delta\Delta E^*(F)$

$$k_{\text{off}} = \alpha g(F) \exp\left(-\frac{\Delta\Delta E^*(F)}{k_B T}\right) \quad (9)$$

represent the deformation of the potential under the influence of the external force and molecular friction, respectively. Hence, increasing the pulling velocity results in a shift in the distribution of the probability density to higher binding forces and is accompanied by a broadening of the function.

4.2. Hydrogen Bonding and Complementary DNA Strands

Among the vast number of molecular recognition events in nature the base pairing of the DNA duplex is the most recognized one. Colton and co-workers were the first to describe an experimental approach using force spectroscopy to determine the binding strength of a DNA duplex by using single-stranded DNA immobilized on a probe and sample.^[81] Silica beads with 60–120 nm diameters were derivatized with trimethoxysilanes and covalently linked to oligonucleotides with the sequence (ACTG)₅ (tip) and (CAGT)₅ (sample). In this way 20, 16, 12, 8, and 4 base pairs could be formed theoretically. Lee et al.^[24] found four populations with force maxima at 1.52, 1.11, 0.83, and 0.48 nN. The adhesion force between noncomplementary base pairs was determined to be merely 0.38 nN. Thus, the obtained forces correspond to the breakage of double stranded DNA with 20, 16, and 12 base pairs (the maximum at 0.48 nN was ascribed to a nonspecific interaction). The pulling velocity varied between 0.1 and 10 nm s^{−1}. In addition, C₂₀-single strands immobilized on the tip and sample were connected through poly(I)-single-strands with different lengths (Figure 11).^[81]

Lieber and co-workers determined the binding strength of complementary DNA strands (14 bp) to be 0.45 nN. The nonspecific adhesion force was 0.1 nN.^[82] Boland and Ratner chose a more general approach to directly quantify the binding strength of single base pairs such as adenine and thymine as well as cytosine and guanine.^[83] Their starting point was a well-ordered self-assembled film of the corre-

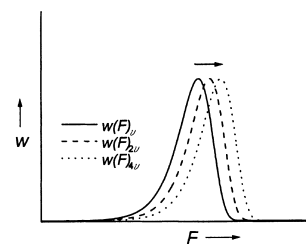


Figure 10. Numerical simulations of the probability density w as a function of the applied force F at three different pulling velocities (increasing in the direction of the arrow). According to Equation (7), the rupture force increases logarithmically with the pulling velocity.

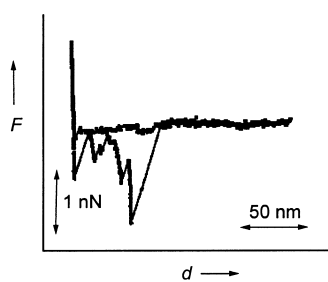


Figure 11. Force-induced rupture of double-stranded DNA. The surface was functionalized with cytidine (C_{20}) and hybridized using poly(I). The tip was also derivatized with C_{20} , so that the bridging of both cytosine single strands with poly(I) becomes possible during the approach.^[81]

for G-C was obtained from the approach curve, whereas the rupture forces on retraction were 3.24 nN for A-T and 3.10 nN for G-C (different nonspecific adhesion forces were also identified). It was shown that the distribution of the attractive forces display increments of 54 pN, which is indicative of the formation of single hydrogen bonds. A simple estimation yields 125 pN for the A-T base-pair by assuming an enthalpy of $1.8 \text{ kcal mol}^{-1}$ for hydrogen bonds and a base separation of 0.2 nm. The calculation becomes more cumbersome when the weaker Hoogsteen base-pairing, which occurs between AA, GG, CC, TT, and AG, is taken into account. Florin et al. observed a dependence of the adhesion forces on the duration of contact between a poly-dT-functionalized tip and a poly-dA-coated sample.^[84] The maximum of adhesion was reached after a contact time of 2 min. This can be explained in terms of the slow reorientation of partially hybridized DNA double strands.

Recently, Rief et al.^[85] reported on the rupture of single-stranded hairpin loops consisting of alternating A-T and G-C sequences; They found values of 9 pN for the A-T sequence and 20 pN for the G-C sequence. In the course of the experiment the strands melted, which was induced by the external force enabling the molecule to adopt a hairpin conformation. The rupture of the hairpin loop takes place in quasi-thermal equilibrium (reversible) and is thus independent of the duration of the applied force. This observation implies that formation of base pairing and rupture occurs faster than the approach–retraction cycle of the force measurement. A similar time-range was found by Essevaz-Roulez et al. for the unfolding of double-stranded DNA (10–15 pN).^[86]

Strunz et al. investigated the dynamics of the unwinding of complementary DNA strands at different pulling velocities ($16\text{--}4000 \text{ pNs}^{-1}$) under physiological conditions.^[87] Both 5'-termini of the double-stranded DNA were linked to the sample and probe through a polyethyleneglycol (PEG) spacer in order to suppress nonspecific interactions in the vicinity of the substrate. Strunz et al. showed that the binding force is dependent on the number of base pairs and scales logarithmically with the pulling velocity according to a simple two-state model. The elasticity of the DNA (about 2 pN nm^{-1}) changes only moderately with increasing numbers of base pairs. The

corresponding sulfuranyl derivatives of adenine (A), cytosine (C), thymine (T), and 2-amino-6-hydroxy-8-sulfuranylpurine (G), a guanine analogue, on crystalline gold. They studied the interaction with the corresponding bases immobilized on the tip and 16 combinations were quantified with respect to the attractive forces occurring during the approach. An average attractive force of 0.63 nN for the base-pair A-T and 1.08 nN

cooperative melting of the DNA duplex also depends on the frequency ν and thus on the number of base pairs, since the degree of freedom increases with an increasing length of the molecule. Strunz et al. demonstrated that the force which is needed to melt DNA converges with increasing length of the duplex to approximately $1.2 k_B T / 0.7 \text{ \AA}$ (70 pN). This value is, however, independent of the pulling velocity of the cantilever. In this context, the work of Bockelmann et al. should be mentioned. They discovered the occurrence of sequence-dependent molecular stick–slip motion while separating a DNA double strand.^[88, 89] Essevaz et al. estimated the energy (by force spectroscopy) which is necessary to melt λ -phage-DNA to be $1.9 \text{ kcal mol}^{-1}$. This value is in good agreement with classical DNA melting experiments that revealed $1.5 \text{ kcal mol}^{-1}$ for the A-T and 3 kcal mol^{-1} for the G-C base-pairs.^[90] It should be mentioned that besides hydrogen bonding, base stacking has a considerable influence on the stability of DNA duplexes.

Recently, McKendry et al. showed that the chirality of low molecular weight compounds can be detected sensitively by means of force spectroscopy using a tip functionalized with acetylphenylglycine (T) and a sample coated with mandelic acid (M).^[91] Both compounds were immobilized on gold substrates using thiol anchors. The sample was additionally structured by means of microcontact printing. Different combinations of enantiomers and racemic mixtures were quantified with respect to their adhesion forces. The strongest interaction was observed by using the T(S)/M(R) and M(S)/T(R) enantiomeric pairs (1.1 nN). The results reveal that local force spectroscopy may become well suited for pharmacological applications (pharma screening).

4.3. Ligand–Receptor Interactions of Proteins

Noncovalent ligand–receptor interactions of proteins are of widespread interest for immunologists, physiologists, and biochemists. Beside gathering information about the different interacting forces, the determination of the overall binding strength can provide valuable data for understanding the interaction of proteins with high and low molecular weight compounds. For example, the consequences of point mutations near the active center can be quantified and the shape of the binding potential may become available. A number of noncovalent ligand–receptor couples such as antigen–antibody, biotin–streptavidin/avidin and P-selectin–glycoprotein have so far been investigated by force spectroscopy (Table 1).

The first experiments on molecular recognition were conducted by the groups of Gaub^[4] and Colton,^[5] both investigating the streptavidin–biotin system. Figure 12 displays a typical force–distance curve of a streptavidin-functionalized tip retracting from a biotinylated surface (BBSA). Biotin blocks the binding sites of streptavidin in solution, thus no rupture forces are observable.^[68]

Particularly interesting are investigations of the force-induced rupture of ligand–receptor bonds in the case of selectin–glycoprotein and –lipid couples because of their significance in the inflammatory response of mammals. In response to an inflammatory signal, leukocytes in the blood-

Table 1. Binding parameters of antibody–antigen complexes

Complex components	F [pN]	ΔG [kJ mol ⁻¹]	ν [μm s ⁻¹]
IgG (goat), biotinylated BSA ^[95]	111.5 ± 98.6	–	–
single-chain F _V (wild-type), fluorescein ^[12]	50 ± 4	– 50.8	1
single-chain F _V (His(H58)-Ala), fluorescein ^[12]	40.3 ± 3	– 44.8	1
human serum albumin (HSA), polyclonal anti-HSA ^[27]	240 ± 48	–	–
biotin, avidin ^[4, 93, 96]	170 – 280	–	–
streptavidin, biotin ^[5]	340 ± 120	–	–
Hb-A1c-antibody, anti-Hb-A1c-antibody ^[97]	278 ± 83	–	< 2
<i>Micronotona proliferata</i> (MAF), anti-MAF ^[30]	50	–	–
adhesion molecule (ICAM-1), anti-ICAM antibody (F10.2) ^[151]	100 ± 50	–	–

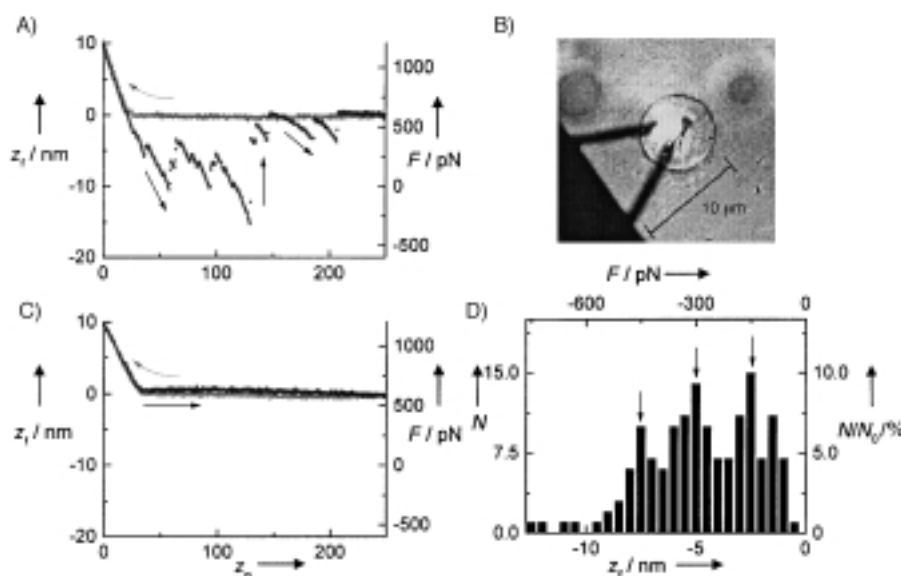


Figure 12. A) Typical force curve of a tip functionalized with streptavidin interacting with a biotinylated substrate. The separation of the tip occurs in several steps in which discrete bonds between the tip and the sample break one after the other. The tip was covered with biotinylated bovine serum albumin (BBSA) and subsequently incubated in a streptavidin solution. Biotinylated agarose beads, adsorbed onto glass, serve as the substrate. B) Optical image of a V-shaped cantilever above an agarose bead. C) Control experiment, in which biotin was added to the solution to block the binding sites of streptavidin. No bond ruptures were observed. D) Histogram of the rupture forces of the biotin–streptavidin couple. In the histogram three maximums separated by a distance of 150 pN are discernible—and correspond to the rupture of one, two, and three ligand–receptor bonds.^[68]

stream start to roll on the surface of the endothelia in which adhesion is mediated by selectins produced by the endothelial cells. This movement, generated by an external force, the bloodstream, can be simulated in a force experiment. P-selectin strongly binds to its ligand, the P-selectin-glycoprotein-ligand-1 (PSGL-1), presented by leukocytes. Binding sites are located far away from the cellular membrane at the N-terminus of the polypeptide chains. Fritz et al. studied this system using recombinant fusion proteins with a constant IgG region.^[92] Probes (Si₃N₄) and samples (glass) were derivatized by silane chemistry and coated with avidin, which served as the anchor for biotinylated PSGL-1/IgG (tip) and biotinylated P-selectin (sample). The authors managed to determine not only the binding strength of the complex ($F = 110–165$ pN), which depends on the pulling velocity of the cantilever, but also the adhesion probability as a function of the duration of contact. It was shown that the binding strength increases logarithmically with the pulling velocity, as expected from the two-state model [Eq. (8)]. In contrast to typical antigen–an-

tibody and streptavidin–biotin reactions the adhesion probability decreases exponentially with interaction time. The width of the potential was 0.25 nm and the rate of dissociation in the absence of an external force 0.022 s⁻¹. A Monte Carlo simulation revealed that rupture forces are mainly determined by the dissociation constant of the complex and scale exponentially with the pulling force [Eq. (8)].^[92] However, the association rate is not influenced by the rupture force but by the adhesion probability. The results reveal that the kinetics of the binding/dissociation process are important and binding forces are a function of the pulling velocity. The fact that both binding force and adhesion probability depend on the pulling velocity indicates that the rate constants are in the same timescale as the experimental pulling velocity of the cantilever.

The success of a force experiment depends on two factors, force resolution, which is limited by the thermal noise of the cantilever, and suitable functionalization of the tip and sample. In particular, steric hindrance arising from the immobilization of molecules can lead to considerable differences between binding constants in solution and on surfaces. Therefore, derivatization of the surface must involve flexible spacer molecules and linkers. For instance, the limited mobility of an antibody with a length of merely 10 Å connected directly to the tip can be improved by using spacer molecules,

thus increasing the binding probability. Hinterdorfer et al.^[27] immobilized the antibody polyclonal anti-human serum albumin (anti-HSA) covalently through a PEG spacer to the probe and measured a binding strength between HSA and anti-HSA of $F = (240 \pm 48)$ pN. Ludwig et al.^[93] used a protein cushion consisting of bovine albumin to study the interaction of biotin with avidin which was bound to a biotinylated Si₃N₄ tip derivatized by nonspecifically adsorbed biotinylated bovine albumin (BBSA) in order to avoid denaturing the avidin. They determined a binding strength of $F = 200$ pN for the biotin–avidin complex.^[94] Ros et al.^[12] used aminopropyltriethoxysilane to couple the antigen fluorescein (Fluor-NHS5000) to the tip. The authors found a binding force of $F = (50 \pm 4)$ pN and a free enthalpy of $\Delta G = -50.8$ kJ mol⁻¹ for fluorescein and single-chain F_V (wild-type) fragments, while single-chain F_V fragments (His(H58)-Ala mutants) displayed a binding strength of $F = (40.3 \pm 3)$ pN and a free enthalpy of $\Delta G = -44.8$ kJ mol⁻¹. Dammer et al. utilized thiol–gold chemistry in conjunction with activated carboxylic

acids to study the binding strength of anti-biotin–immunoglobulins (ABIO) and biotin–BSA.^[12, 95]

Besides gold, mica and silicon surfaces have been extensively used as they are atomically flat and can easily be derivatized by silane chemistry.^[12, 17] Nonspecific interactions between the molecules on the tip and sample have to be taken into account. Protein-functionalized agarose-beads have been utilized in order to minimize the nonspecific interactions. These polymer beads are known to suppress nonspecific interactions efficiently.^[4, 93, 96] Two different types of control experiments are conceivable. Either one prevents binding between the two partners through the addition of an inhibitor to the solution which blocks the binding sites of the receptor, preferably with low molecular weight compounds,^[4, 27, 30, 127] or one may remove one binding partner from either surface quantitatively.^[1] In either case the maximum of the force spectrum should vanish if it represented a specific interaction. The remaining forces most likely represent nonspecific interactions and result in a background that can hardly be avoided. Examples are given in Figure 13. In this experiment the binding strength of Hb-A1c antibodies has been determined to be $F = (278 \pm 83)$ pN and rupture events were suppressed by adding antibodies to the solution.^[97]

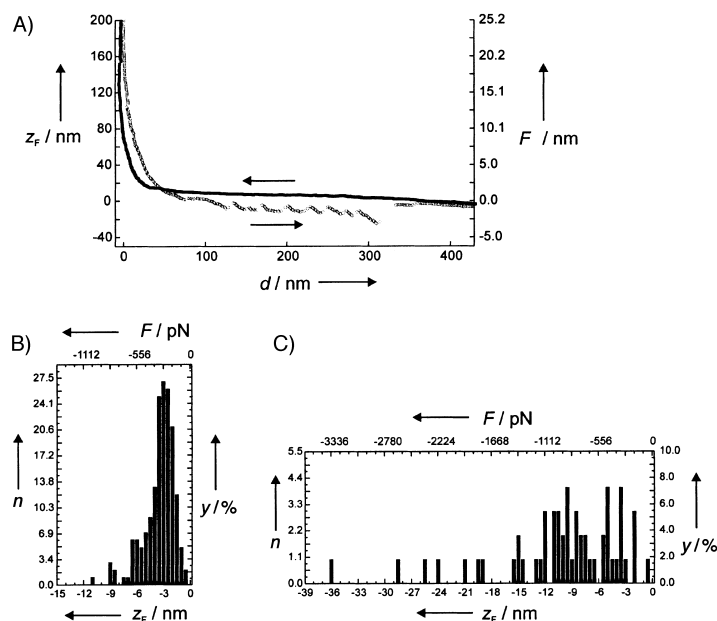


Figure 13. Force curves and the histogram of bond rupture events of an antigen–antibody complex. A) Force curves display the typical picture of a long filament, which is at first stretched and then strips off the tip at a defined force. B) The histogram is composed of 171 curves (171 of 300 force curves exhibited bond rupture events). The number of ruptures (as n and as a percentage of the total number of ruptures (y)) is depicted as a function of rupture length and rupture force. C) Control experiment with a tip in which the antigens were blocked with antibodies from solution. The histogram shows the last ruptures of the force–distance curves, which were obtained with a tip that is blocked with antibodies. Only 55 of 300 curves show rupture events.^[97]

Possible changes in binding characteristics that result from the immobilization of proteins on the surfaces have to be taken into consideration if force spectroscopy is performed. Effects range from slight conformational changes up to the entire denaturation of proteins.

4.4. Covalent Bonds

Determining the binding strength of covalent bonds by force spectroscopy is cumbersome and requires caution since the anchoring of the molecules can give rise to a variety of different types of bonding. Although most polymers exhibit only one type of bonding immobilization on the tip and surface adds on several heterogeneous bonds. Either the bond under investigation needs to be the weakest member in the chain or weaker bonds have to be compensated for by designing parallel bonds of the same kind to enhance the strength, as provided by the use of trichlorosilanes and a subsequent cross-linking. So far only one convincing report regarding this difficulty has been published and provides an approach to determine the strength of two types of covalent bonds. Grandbois et al. anchored an amylose chain covalently between the tip and the sample surface and measured the binding strength of the Si–C (2 nN) and Au–S (1.4 nN) bond.^[98] The immobilization chemistry was chosen to be identical at the tip and the sample in order to facilitate the analysis of bond breakage. To determine the Si–C bond strength the glass surface and the tip were both functionalized with *N*-(3-trimethoxysilylpropyl)diethylenetriamine, while the carboxymethylated amylose was activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) which would then be covalently linked to the substrate. Contact with the tip gave rise to the desired second linkage to the probe from a molecule suspended between the tip and the sample.

Since amylose exhibits a reversible conformational transition at 275 pN, which is independent of the pulling velocity, the binding forces could be quantified without prior calibration of the cantilevers. The determination of the binding strength of the Au–S bond was performed in the same manner. Activated carboxymethylated amylose was covalently linked to a self-assembled film of cysteamine on gold, while immobilization on the tip was accomplished by silane chemistry as described above. In both cases multiple irreversible ruptures occurred but only a single polymer chain was stretched. From this result one may assume that bond breakage took place at the tip where multiple bonds were formed by the silane anchor. Four conceivable bonds had to be considered: Si–O, Si–C, C–C, and C–N. The authors deduced from computer simulations that the Si–C bonds most likely break, although there is room for speculation about the Au–S bond. It remains to be elucidated whether the Au–S bond ruptures or gold atoms are removed from the substrate and give rise to the observed rupture events.

5. Intramolecular Interactions: Stretching of Single Long-Chain Molecules

The biological function of proteins is directly related to their structure. Polypeptides do not fold randomly but adopt a certain structure based on specific intramolecular interactions. The structure of biomolecules can be studied by circular dichroism spectroscopy, IR spectroscopy, X-ray analysis, and NMR spectroscopy, while their stability can be analyzed by calorimetry. In most cases, the energy hypersurface is

unknown, which makes a direct measure of intramolecular forces necessary. This measurement can be done using force spectroscopy on single molecules. The polymer is immobilized on the substrate and interacts with the tip specifically or nonspecifically. On retraction of the cantilever the molecules are stretched revealing important information about their conformation. The forces of interest are mostly weaker than those associated with bond rupture, thus a special treatment of the probe can be avoided.^[45, 99, 100]

Statistically valid results are only obtained if many single-molecule experiments are performed and a large number of experimental data obtained. Combining the data with computer simulation can be very useful in determining a variety of microscopic parameters, for example, mechanical quantities such as the elasticity of the molecule, and the thermodynamic and kinetic parameters such as the energy barrier between the folded and unfolded state as well as between different conformational states of a biopolymer. The following section deals with a general discussion of different polymer models.^[101]

5.1. Thermodynamics of Polymers

Even a single polymer chain made up of hundreds to thousands of monomers has to be treated by means of statistical physics because of its large number of degrees of freedom. Since all experiments described here are performed in solution the temperature T can be assumed to be constant—the molecule can be described as a canonic ensemble. How does a molecule react on the pulling force or more precisely, what kind of restoring forces occur?

Stretching a molecule causes two kinds of restoring forces.^[102, 103] At small displacements, *entropic* forces dominate. If the polymer adopts a random coil conformation the Brownian molecular motion causes a permanent fluctuation of the molecule. For instance, DNA, a relatively rigid polymer, can be considered as linear on a length scale of $0.05\ \mu\text{m}$.^[102, 104] The force experiment requires long strands of several micrometers thus DNA can be regarded as a random coil.^[105, 106, 108] The number of conformations is drastically reduced by extending the molecule. This loss in conformational entropy is reflected in the so-called elastic entropy.^[102]

Large displacements leads to a tension of the molecular backbone, in which bonds are stretched in the direction of the pulling force. This effect is called *enthalpic* elasticity.^[102, 107] The high external force can cause deformations of bonds^[45] and can induce rupture of salt bridges and intramolecular hydrogen bonds, and eventually bring about conformational changes of the entire molecule.^[99, 108] This is illustrated by the transition from *B*-form DNA to the *S*-form.^[102, 106, 108] Polysaccharides can also change their conformation as a result of high external forces.^[45, 99, 109, 110]

Force–distance curves of polymers can be recorded by applying different boundary conditions. It is possible to measure the force necessary to maintain a certain length of the molecule. From a thermodynamic point of view this is an isothermal–isochore process, since the volume represents the length of the molecule and the pressure can be substituted by

the force. The other possibility is to provide a constant force and measure the length of the molecule, which is an isothermal–isobar process.^[111]

The force F of a polymer stretched in the x direction for an isothermal–isobar process can be expressed independently of the microscopic mechanism as Equation (10).^[103] It is decisive

$$F = \left(\frac{\partial A}{\partial l} \right)_T = \left(\frac{\partial U}{\partial l} \right)_T - T \left(\frac{\partial S}{\partial l} \right)_T \\ = -kT \left(\frac{\partial \ln Z_r}{\partial l} \right)_T \quad (10)$$

that the change in the free Helmholtz energy A can also be determined. In this case the derivative is determined under isothermal conditions, in terms of the index T . Z_r is the configurational partition function for a chain with a fixed distance between the ends. The Helmholtz energy can be expressed by the internal energy U and the entropy S , which represents the first term of the energetic contribution to the force, the second one shows the influence of the entropic force.^[103] Stretching a molecule leads in general to a reduction in the entropy by restricting the number of possible configurations. The derivative of the internal energy plays an important role in the stretching of the molecule as well as in the increase in energy caused by the stretch. From both considerations an attractive—positive—force results. In the purely entropic region only the second term on the right-hand side of Equation (10) remains.^[102] It follows that in this case the force is proportional to the temperature.^[103]

5.2. Polymer Models from Statistical Mechanics

What does the function $F(x)$ actually look like that determines the force–distance curve? $F(x)$ can be obtained from a mathematical modeling of the real molecule (Figure 14). Among the large number of models^[103, 112–116] (Table 2) two general approaches dominate: the freely jointed and the wormlike chain. Prior to the discussion of these models the Gaussian chain, a model valid for low pulling forces, will be described.

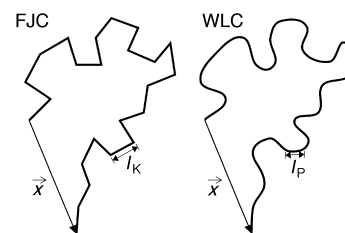


Figure 14. Schematic representation of different polymer models: the freely jointed chain (FJC) is composed of n rigid segments (with the Kuhn length l_K), which are coupled by flexible joints. By replacing the parts of the chain by elastic springs (spring constant κ) enthalpic effects can be included (FJC⁺). The image on the right-hand side shows the wormlike chain (WLC); it describes the polymer as an irregular curved filament, which is, however, linear on the scale of the persistence length l_p . The introduction of a specific stiffness Φ results in the backbone becoming elastic, that is, stretchable (WLC⁺). The point to point distance vector $\vec{x}$ connects the starting and the end point of the polymer chain.

Table 2. Elastic models for polymers.

Model	Force law	Fit parameters
<i>freely jointed chain</i>		
nonelastic (FJC)	$x(F) = L \left[\coth\left(\frac{F l_K}{k_B T}\right) - \frac{k_B T}{F l_K} \right]$	l_K
elastic (FJC ⁺)	$x(F) = L \left[\coth\left(\frac{F l_K}{k_B T}\right) - \frac{k_B T}{F l_K} \right] \left(1 + \frac{F}{\kappa L} \right)$	l_K, κ
<i>wormlike chain</i>		
nonelastic (WLC)	$F(x) = \frac{k_B T}{l_p} \left[\frac{1}{4} \left(1 - \frac{x}{L} \right)^{-2} + \frac{x}{L} - \frac{1}{4} \right]$	l_p
elastic (WLC ⁺)	$F(x) = \frac{k_B T}{l_p} \left[\frac{1}{4} \left(1 - \frac{x}{L} + \frac{F}{\Phi} \right)^{-2} + \frac{x}{L} - \frac{F}{\Phi} - \frac{1}{4} \right]$	l_p, Φ

5.2.1. The Gaussian Chain (GC)

The conformation of a random coil can be described by a random-walk approach.^[117, 118] A Gaussian^[102, 103, 112–114] distribution^[119] is assumed which is independent of the microscopic structure of the molecule. By assuming an extension x which is smaller than the contour length L of the molecule the random-walk character is preserved. The contour length L is the length of the linearly extended molecule without stretching the molecular backbone.^[100, 113, 120] However, a measure for only part of the molecule is usually given for force spectroscopy rather than for the entire length of it.^[99] Thus, L represents merely a section of the molecule, since the tip randomly picks up a certain part of the polymer chain.^[121] The variable x denotes the distance between tip and sample. By assuming $x \ll L$ the force–distance relationship can be expressed as Equation (11). The force $F(x)$ is a linear function of x and thus the polymer reacts like an ideal spring in the Gaussian model. k_B is the Boltzmann constant and l_K the Kuhn length, which is a measure for the stiffness of the molecule.

$$F(x) = 3 \frac{k_B T x}{l_K L} \quad (11)$$

5.2.2. The Freely Jointed Chain (FJC)

At higher forces the sections of the chain are no longer oriented in a random fashion and the molecule is predominantly aligned along the direction of the external force. This situation can be described by a generic polymer model such as the FJC model. Here, the polymer is divided into n rigid elements, each exhibiting a length of l_K connected through flexible joints without any long-range interactions (Figure 14).

The extension x can be expressed as a function of the pulling force F [Eq. (12)]. $\mathbf{L}$ is the Langevin function^[122] and

$$x(F) = L \left[\coth\left(\frac{F l_K}{k_B T}\right) - \frac{k_B T}{F l_K} \right] = L \mathbf{L}\left(\frac{F l_K}{k_B T}\right) \quad (12)$$

the contour length is defined as $L = n l_K$. For a large number of segments n , Equation (12)^[123] can be written as Equation (13). $\mathbf{L}^{-1}$ is the inverse Langevin function.^[124] For small extensions ($x \ll L$) Equation (13) still obeys Hook's law.^[125] Large extensions, however, ($x < L$) result in a nonlinear behavior.

$$F(x) = \frac{k_B T}{l_K} \mathbf{L}^{-1}\left(\frac{x}{L}\right) \quad (13)$$

Since the segments of the freely jointed chain are assumed to act independently of each other, the FJC model describes flexible molecules reasonably well. Examples of the successful application of the FJC model are oligonucleotides,^[24] polydimethylsiloxane,^[126] and poly(methacrylate)^[116] (Table 3).

Table 3. Mechanical parameters of different molecules according to the FJC model.

Polymer	Kuhn length [Å]
polyinosine ^[24] in aqueous solution	3.0
polydimethylsiloxane ^[126] in heptane	2.5 ± 0.4
polymethacrylic acid ^[116] in aqueous solution	3.3 ± 0.5

5.2.3. The Extended FJC Model: Combination of Entropic and Enthalpic Elasticity

Both the Gaussian and the FJC models solely consider entropic effects (see Section 5.1). Since elastic deformations are neglected, the polymer chain cannot be stretched by more than the contour length. For large extensions, the Gaussian approach is not valid as the molecules do not behave like an ideal spring. The FJC model also fails to describe the stretching of molecules for $x = L$. According to Equation (13) a molecule would be infinitely rigid at this point. Since this situation is not realistic, one has to take into account deformation of bonds and bond angles. An exact description would require the knowledge of intramolecular potentials and the molecular structure, which in some cases is provided by computer simulations.^[45] However, in general one has to choose a generic approach: by modeling the molecule as n identical elastic springs in series the FJC model can be expressed as Equation (14).^[108] κ is the spring constant of a

$$x(F) = L \mathbf{L}\left(\frac{F l_K}{k_B T}\right) \left(1 + \frac{F}{\kappa L} \right) \quad (14)$$

single segment that can be directly determined by force spectroscopy. Typical values for real polymers are above 1 Nm^{-1} . For comparison, the spring constant of the atomic-force microscope is commonly in the range of 0.1 – 0.01 Nm^{-1} . The additional enthalpic term in Equation (14) gives rise to a linear increase in the force for large extensions ($x \sim L$) since the chain behaves elastically. Figure 15 illustrates that the force can be stretched beyond the contour length.

This extended FJC* model has been successfully utilized to describe a variety of different polymers (see Tables 4 and 6) such as polysaccharides,^[110] ligand–receptor complexes,^[127] as well as synthetic polymers such as polystyrene^[128] and polyvinyl alcohol^[129] (Table 4).

5.2.4. The Wormlike Chain (WLC)

The model of Kratky and Porod describes a polymer as a homogenous string of constant bending elasticity.^[130] The conformation of the molecule can be described as a continuous curve, a wormlike chain. Although entropic and enthalpic contributions are combined in this approach,^[109] extension is limited by the contour length of the polymer.^[131]

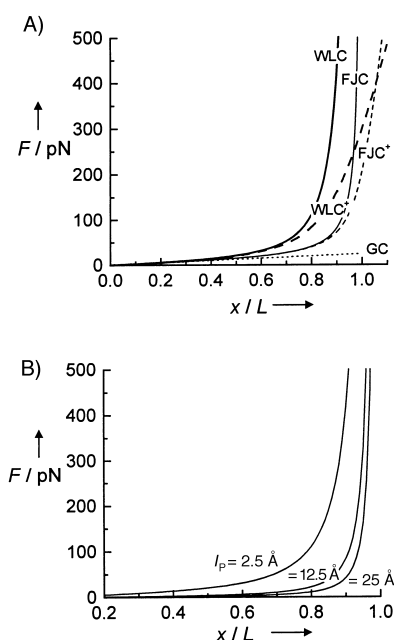


Figure 15. A) Comparison of the force laws of different polymer models: the Gaussian chain (GC), the freely jointed chain (FJC), the wormlike chain (WLC), and the enthalpic extensions FJC⁺ and WLC⁺. The significant bending rigidity of the WLC polymer leads to the storing force occurring at lower extensions than in the FJC model. As the individual segments of the FJC polymer can be arranged independently, this chain is extraordinarily flexible—upon unfolding of the random coil loops of the polymer strand are evenly stretched without any effort. If the models include a finite stiffness of the segments, then a slower increase in the force occurs at large displacements. This is a result of the elasticity of the chain, which can then be stretched beyond the contour length. Parameters: $T = 293.15$ K, $l_K = 5$ Å, $l_p = 2.5$ Å, $\kappa = 50$ N m⁻¹, $\Phi = 2500$ pN. B) Force–distance curves according to the WLC model. The chain can be more easily stretched (lower restoring force at the same extension) by increasing the persistence length. The tendency for coiling is reduced and, thus, it becomes easier to stretch the polymer to form a linear chain. However, it is noteworthy, that the WLC polymer cannot be stretched beyond its contour length.

Table 4. Mechanical parameters of different molecules according to the FJC model (FJC⁺).

Polymer	Kuhn length [Å]	Segment elasticity [N m ⁻¹]
polystyrene ^[128] in toluene	12.2	2.1
polyvinyl alcohol ^[129] in aqueous solution	6.2	17
polyethyleneglycol ^[100] in hexadecane	7	150
P-selectin/PSGL-1 ^[127] in aqueous solution	7 ± 1	0.8 ± 0.2

The following approximation gives an expression for the force–distance relationship^[132] [Equation (15)],^[105, 133] which

$$F(x) = \frac{(k_B T)^2}{B} \left[\frac{1}{4} \left(1 - \frac{x}{L} \right)^{-2} + \frac{x}{L} - \frac{1}{4} \right] = \frac{k_B T}{l_p} \left[\frac{1}{4} \left(1 - \frac{x}{L} \right)^{-2} + \frac{x}{L} - \frac{1}{4} \right] \quad (15)$$

is accurate up to several 100 pN. The persistence length, like the Kuhn length, represents the flexibility of the molecule. B denotes the bending module of the polymer.^[134] The chain direction is preserved on the length scale of the persistence length, and below the persistence length the polymer can be considered to be linear.^[135] In other words: molecules with high persistence length have a tendency *not* to form coils. Strictly speaking, the persistence length is a function of the

external force.^[109, 136–138] This fact explains why recombinant titin fragments exhibit a persistence length of 8 Å at low forces, whereas the molecule shows a persistence length of merely 4 Å at forces higher than 50 pN (see Table 7). The WLC model has been successfully applied to describe the elastic behavior of DNA, poly(methacrylic acid), muscle, and adhesion proteins (Table 5). For very small extensions ($x \ll L$)

Table 5. Mechanical parameters of different molecules according to the WLC model.

Polymer	Persistence length [Å]
polydimethylsiloxane ^[126] in heptane	2.3 ± 0.2
polymethacrylic acid ^[116] in aqueous solution	2.8 ± 0.5
proteoglycane ^[30] in aqueous solution	4.8

the difference between the WLC and the FJC model is negligible, since the equilibrium conformation of the molecule is Gaussian-like. At low forces, the persistence and Kuhn length are related by: $l_K = 2l_p$.^[139]

It needs to be emphasized that the discussed models are independent of the molecular composition of the polymer. The WLC model assumes a continuum without fine structure. Usually, no simple relation between the model parameters l_p and l_K and real structural quantities such as bond length or the length of a monomer unit can be found: for DNA, l_p is approximately 500 Å and thus considerably larger than the helical pitch of the duplex (34 Å) or the diameter of the helix (25 Å). In the case of dextran, however, the Kuhn length (ca. 4.4 Å) and the length of a monomer (ca. 5 Å) are rather close.^[110] The structure of a polymer depends on the environment and hence, also on the Kuhn and persistence length. The approach, which is most suited to describing the experimental data, depends on the polymer and the force regime.

5.2.5. The Extended WLC Model: Enthalpic Stretching

Although the standard WLC model already includes bending elasticity, the extended WLC model includes the stiffness of the chain [Eq. (16)]. The parameter Φ denotes the specific stiffness of the polymer.^[140] Equation (16) describes the elastic properties of DNA at elevated forces more realistically than the classic WLC model.^[141]

$$F(x) = \frac{k_B T}{l_p} \left[\frac{1}{4} \left(1 - \frac{x}{L} + \frac{F}{\Phi} \right)^{-2} + \frac{x}{L} - \frac{F}{\Phi} - \frac{1}{4} \right] \quad (16)$$

5.2.6. Modular Polymers: A Two-State Model

Force-induced conformational changes are often accompanied by a lengthening of the molecule backbone. A spontaneous structural change is extremely improbable since it is energetically unfavorable. Without the presence of an external force, the polymer is in the ground state. An external force, however, can give rise to a repeated unfolding of protein domains as shown for spectrin, titin, and tenascin. The subunits of the modular proteins change from a folded (length l_1) to an unfolded, extended state (length l_2).

Following the FJC model, the number of subunits n is constant during extension but every subunit has two possible

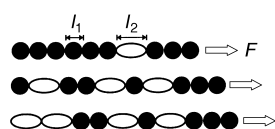


Figure 16. Part of a polymer chain whose subunits may show two different length states (l_1 and l_2). An external force F favors the transition of the coiled state 1 to the stretched state 2. This conformational change occurs randomly along the chain.

range of seconds. The final state—thermal equilibrium—is reached instantaneously. Therefore, the distribution of the states can be expressed in terms of a Boltzmann distribution: $n_2/n_1 = \exp(-\Delta G(F)/k_B T)$. The free enthalpy ΔG depends on the external force, which causes a continuous reduction in the energy barrier of the conformational transition $\Delta G(F) = (E_2 - E_1) + F(l_2 - l_1)$. In the case of the extended FJC model [Eq. (14)] this leads to expression (17). Again the relevant length scale is the contour length of the polymer: $n = L/l_K$ at $F = 0$.

$$x(F) = L(F) \mathbf{L} \left(\frac{F l_K}{k_B T} \right) \left(1 + \frac{F}{\kappa L} \right) \quad (17)$$

$$= \frac{L}{l_K} \left(\frac{\exp(-\Delta G(F)/k_B T)}{\exp(-\Delta G(F)/k_B T) + 1} + \frac{l_2}{\exp(+\Delta G(F)/k_B T) + 1} \right) \mathbf{L} \left(\frac{F l_K}{k_B T} \right) \left(1 + \frac{F}{\kappa L} \right)$$

Figure 17 shows the applicability of the theory by stretching polyethyleneglycol in aqueous solution until an irreversible structural change occurs.^[100] The energy barrier between the all-*trans*-(ttt) state and the ttg conformation of the monomers

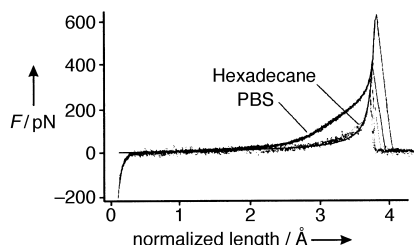


Figure 17. A number of normalized force curves are displayed (pulling forces are positive) for single polyethyleneglycol molecules (PEG) dissolved in hexadecane or PBS buffer and functionalized by a thiol or butoxy group on a gold substrate. Along the abscissa, the relative displacement is plotted multiplied by the monomer length. In an aqueous environment PEG adopts a helical conformation, which turns into a stretched state upon exerting an external force at one end. The stretching behavior at low forces can be well described with the modular FJC⁺ model [Eq. (17)]. However, in an organic solvent (hexadecane) formation of intermolecular hydrogen bonds which stabilize the helical conformation is abolished. In this case, the enthalpic FJC model is appropriate.^[100]

was determined to be $E_2 - E_1 = (3.0 \pm 0.3) k_B T$. However, hydrogen bonds are missing in organic solvents such as hexadecane and the PEG filaments can no longer be described by the enthalpic FJC model. The value of the energy barrier was predicted by quantum mechanics calculations. In a very similar approach it is feasible to analyze the folding of proteins quantitatively using the WLC model.

In many cases the stretching of molecules is irreversible as evident from the hysteresis between the approach and

states 1 and 2, which are independent of each other (Figure 16): $n = n_1(F) + n_2(F)$.^[106] Hence, the contour length becomes a function of the external force: $L(F) = n_1(F) l_1 + n_2(F) l_2$. If the force–extension curves are reversible, that is, the trace and retrace are identical, the system is in equilibrium. Structural changes are fast relative to the timescale of the experiments, which is usually in the

retraction curve. If the potential is asymmetric, that is, the potential width of unfolding is small relative to that of refolding, very different rate constants for folding and unfolding result. It was shown that in the case of titin, a muscle protein, refolding is very unlikely, thus the stretching of titin is an irreversible process. While the width of the potential for unfolding x_u is small (0.3 nm), the one for folding x_f is rather large (15 nm).^[109] If an external force is applied the rate of refolding is decreased as shown in Figure 18A. Therefore, the unfolding force depends on the pulling velocity. Small pulling velocities result in small unfolding forces. This observation is analogous to the results obtained from experiments on the force-induced rupture of ligand–receptor couples.

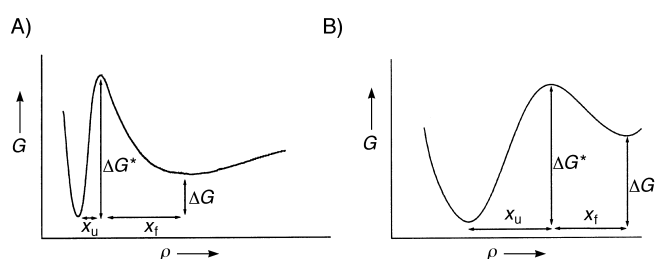


Figure 18. Schematic drawing of the two-state model of the unfolding of polymers. A) An asymmetric potential results in an irreversible unfolding/stretching of the polymer (for example, titin) at the timescale of the experiment. B) A symmetric potential with narrow potential widths, such as in the case of dextran filaments, however, causes a reversible conformational change during expansion and compression cycles.

5.2.7. A Criterion for Single-Molecule Detection

Interpretation of force–distance curves can be difficult. It is seldom the case that only single molecules are located between the tip and the sample. Usually many filaments are stretched quasi-simultaneously and detach from either the sample or the tip shortly after the other. How can we decide whether an individual molecule or a bundle of several filaments has been extended?

All the models have one thing in common: $F(x)$ is a function of x/L . It seems reasonable that the elasticity of a linear polymer scales with its contour length. Longer polymers are softer than shorter ones. Two polymer filaments of different length exhibit different extension curves. The intrinsic properties, however, such as l_K , κ , Φ , and l_p are independent of the polymer length. Thus, polymers of the same molecule but different length can be described by a common force–extension curve $F(x/L)$. Figure 19 displays the force F versus the normalized displacement x/L . This graph enables one to compare force–distance curves of different filaments. The agreement between normalized curves is strong evidence for single molecules.

This approach requires the determination of the contour length L for every single filament, that is, one has to fit the parameters of the corresponding polymer model to the data. But there is an easier way to normalize force–distance curves: a given force–extension curve of filament “a”, $F_a(x)$, has to be transformed into $F_a(x/x_a^*)$ under the condition:

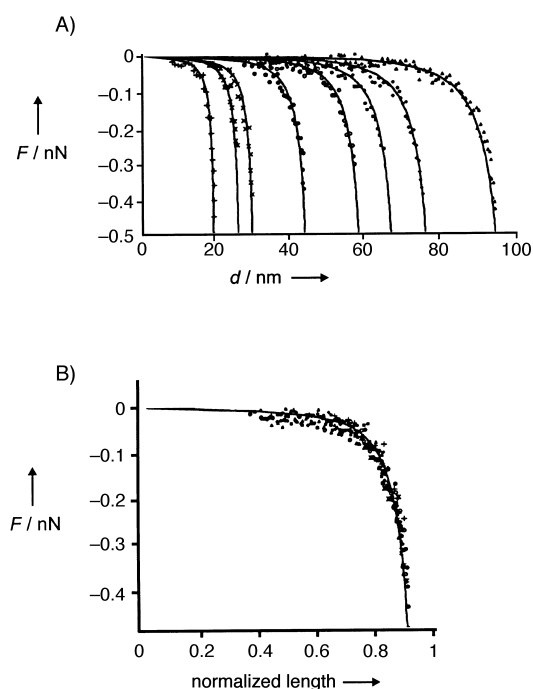


Figure 19. A) Force–distance curves of polymethacrylate filaments of different contour lengths together with curve fits according to the WLC model. B) By normalizing the force curves it becomes clear that single molecules of one class are indeed observed. Loss of contact occurs if the molecules are stretched to 90% of the contour length.^[116]

$F_a(x_a^*) = F^*$. In principle, the force F^* can be chosen arbitrarily. However, it is wise to choose a rather large value for F^* (if there is one) in the range of a conformational transition. From the experimental data x_a^* merely needs to be determined. This method can be applied to filament “b”, in which the force F^* is the same for all filaments ($F^* = F_a(x_a^*) = F_b(x_b^*) = \dots$). If all normalized curves coincide ($F_a(x/x_a^*)$, $F_b(x/x_b^*)$, ...), all the filaments belong to the same class. The filaments are then either supramolecular structures or single polymer molecules. That means that normalizing the data is independent of the polymer model, with the only requirement

that the force needs to be dependent on the displacement x/L .^[142]

The determination of molecular forces on the level of single molecules is accompanied by several difficulties. Besides problems concerned with instrumentation, such as the determination of spring constants and limited pulling velocities, the surface chemistry can play a pivotal role if controversial results are obtained. In some cases different binding strengths for the same complex were obtained dependent on the surface chemistry. Background adhesion and different tip radii can influence the data. Particularly, the determination of the binding strength of DNA has apparently led to controversial results. Contaminations might be a problem if elastic properties of filaments are described.

5.3 Polysaccharides

The mechanical properties of many polysaccharides can be described by the extended FJC model. A summary of the experimental data obtained from different types of polysaccharides is given in Table 6.

Experiments with dextran, which is composed of $\alpha 1 \rightarrow 6$ -linked glucose monomers, demonstrate that force spectroscopy gives valuable information about the molecular mechanics of filamentous molecules. The occurrence of a shoulder in the force–extension curve for the extension of dextran is typical.^[45] The shoulder arises from a reversible structural change accompanied by a stiffening of the molecule. Amylose, a polysaccharide consisting of $\alpha 1 \rightarrow 4$ -connected pyranose monomers, exhibits a similar behavior. The force–extension curve below the conformational change can be described by means of the extended FJC model (Table 6). Monte Carlo simulations match with the observed force–extension curves and reveal the equilibrium constant K and in turn the energy barrier for the conformational transition: $E_2 - E_1 = 13.2 k_B T = k_B T \ln K$.^[109]

Table 6. Molecular parameters of different polysaccharides in aqueous solution. $E_2 - E_1$: energy barrier of two conformations; Δx_u , Δx_t : potential width, v : pulling velocity.

Polymer	Kuhn length [$\text{\AA}$]	Transition at F^* ($v \approx 1 \mu\text{m s}^{-1}$)	Segment elasticity [N m^{-1}]
dextran ^[45, 109] (native)	–	700–850 pN (reversible) $E_2 - E_1 = 13.2 k_B T$ ($C_5 - C_6$ flip) $\Delta x_u = \Delta x_t = 0.32 \text{ \AA}$	8 ± 1 ($F < F^*$) 40 ± 10 ($F > F^*$)
dextran ^[110] (native)	4.4 ($F < F^*$) 5.7 ($F > F^*$)	850 $\pm$ 140 pN (reversible) $E_2 - E_1 = 18.6 k_B T$ (chair $\rightarrow$ boat) $\Delta x_u + \Delta x_t = 1.284 \text{ \AA}$	14.6 ± 2.7 ($F < F^*$)
dextran ^[45] (carboxymethylated)	6 ± 0.5 ($F < F^*$)	250–350 pN (reversible)	6.7 ($F < F^*$) 17 ($F > F^*$)
dextran ^[110] (periodate oxidation)	2 ± 0.26	–	34.2 ± 8.3
amylose ^[110, 152] (native)	4.5 ($F < F^*$) 5.4 ($F > F^*$)	275 $\pm$ 45 pN (reversible) $E_2 - E_1 = 7.7 k_B T$ (chair $\rightarrow$ boat) $\Delta x_u + \Delta x_t = 0.44 \text{ \AA}$	5.6 ± 0.8 ($F < F^*$)
amylose ^[110] (periodate oxidation)	1.8 ± 0.25	–	34 ± 7.3
pullulan ^[110] (native)	4.5	–	10.2 ± 0.93
pullulan ^[110] (periodate oxidation)	2 ± 0.15	–	47.8 ± 5
xanthan ^[99] (native)	–	400 pN (irreversible)	–
xanthan ^[99] (denaturated)	–	–	5 ± 0.5
cellulose ^[99] (carboxymethylated.)	–	–	5 ± 0.5

But what happens during the conformational change on the molecular level? Molecular dynamics simulations indicate that deformation of the O-glycosidic bond occurs. Every glucose monomer experiences a lengthening of 0.6 Å, that is by about 10%. This would explain the plateau of the force–extension curve (Figure 20). The deformation of the bond is fully reversible: the bond angles snap back to their original position upon relaxation.

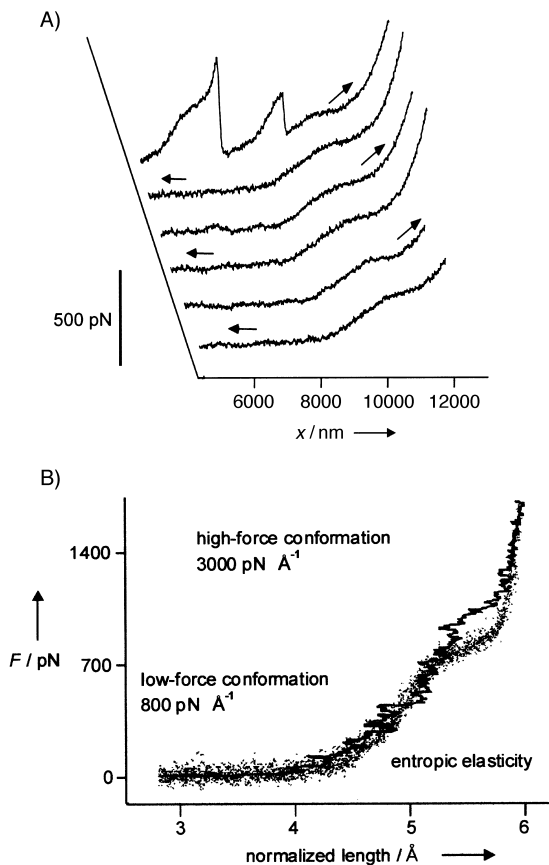


Figure 20. A) Subsequent force measurements of carboxymethylated dextran. In the first curve two short-chain molecules are torn apart. The remaining molecule can be stretched and relaxed several times without loss of contact. The characteristic “shoulder” observed at a force of approximately 300 pN results from a conformational transition of the polysaccharide chain. Curves that are monitored one after the other coincide. This result demonstrates that the structural change occurs in thermodynamic equilibrium (pulling velocity was ca. $1 \mu\text{m s}^{-1}$). B) In a normalized presentation the single molecule character of the native dextran molecules can be observed readily; all curves show the same course (x/L was multiplied by the length of one dextran molecule). The structural transition occurs at approximately 700 pN. The dark curve is the result of molecular dynamic simulations.^[6, 45, 110]

Two different conformational changes of the pyranose monomer are conceivable, the *trans-gauche* transition of the O-glycosidic bond of the $\alpha 1 \rightarrow 6$ -glycosidic dextran and a chair–twisted-boat transition of amylose with $\alpha 1 \rightarrow 4$ -glycosidic bonds.^[143] Both changes account for the stretching of the filament. Ab-initio calculations reveal that the external force can cause a transition from the chair to the energetically unfavorable twisted-boat conformation. The energy barrier is: $E_2 - E_1 = 18.6 k_B T$.^[110] This value is comparable to the result obtained from Monte Carlo simulations (see above).

The oxidation of pyranose with periodate abolishes the conformational transition and the shoulder in the force–extension curve vanishes.^[110] Dextran, amylose, and pullulan display almost the same behavior upon extension (Table 6). The opening of the sugar ring elicits a stiffening of the polysaccharides, which emphasizes the influence of the ring structure on the mechanical properties of the material.

An example of a polysaccharide exhibiting an irreversible conformational change is xanthan^[99] (Figure 21). In its native state xanthan exhibits a helical conformation that is stabilized

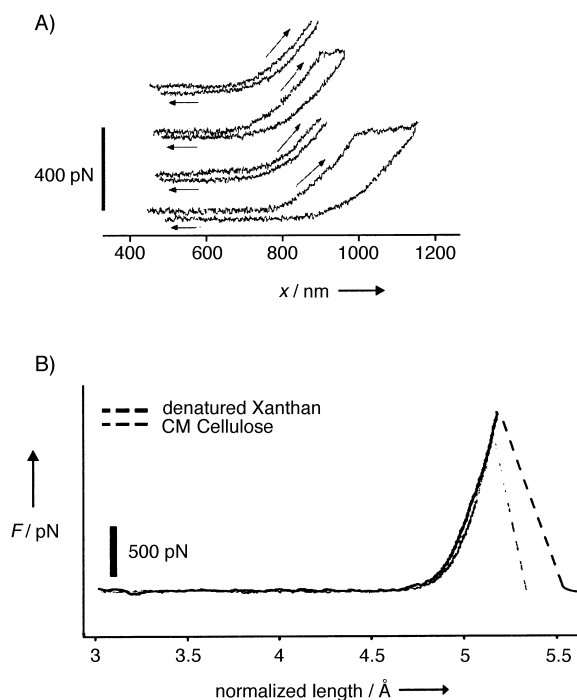


Figure 21. A) Several force–distance curves of native xanthan directly monitored one after the other without loss of the molecular contact.^[99] The extension of the polymer was subsequently increased until a plateau occurred at a force of approximately 400 pN. A hysteresis between the back and forward direction was observed; on the timescale of the experiment, the conformational transition occurs in a thermodynamic nonequilibrium. B) Denatured xanthan and carboxymethyl cellulose exhibit practically identical features. The normalized distance is multiplied by the length of a glucose monomer.^[99]

by noncovalent interactions. The unfolding of the secondary structure is accomplished by applying an external force. Denatured xanthan, which exhibits no helical conformation, shows no conformational transition within the force–extension curve.^[99] The mechanical properties of denatured xanthan resembles those of carboxymethylcellulose, which has the same $\beta 1 \rightarrow 4$ -Glc-backbone.^[99]

5.4. Polynucleotides

The mechanical properties of DNA are of great interest as a consequence of its biological significance. Its elasticity has been extensively studied by means of optical tweezers—the molecule is trapped by a laser beam—and other methods of micromanipulation.^[144] The number of investigations per-

formed with the force microscope is also increasing.^[24, 85, 87] The advantage of force spectroscopy over optical tweezers is the possibility of exerting forces in the nanonewton range. Most studies with common techniques are limited to forces below 100 pN. Remarkable structural changes already occur in this range, however.^[85, 86, 88, 89, 108] This section provides a survey dealing with the mechanics of DNA as probed by the scanning force microscope.

The mechanical properties of DNA are not only of academic interest. In the active cell the active gene must become accessible in order for replication, regulation, and transcription to occur, although DNA is packaged by proteins. Force spectroscopy measurements on polyinosine revealed that single-stranded DNA (ssDNA) behaves like an entropic (extensible) chain.

The elastic properties of double-stranded DNA (dsDNA), however, are more complex. A comprehensive review on the mechanical properties of DNA is given by Austin et al.^[102] As a consequence of its double-helix structure dsDNA is very stiff and less flexible than ssDNA. Many experiments employ λ -phage DNA. Conformational changes and smaller forces occur at smaller forces than in polysaccharides (Section 5.3), which makes a sensitive method of detection necessary.

Below 60 pN the extended WLC model is most suited for describing the mechanical behavior of dsDNA. However, exerting higher forces results in significant structural changes. Native dsDNA adopts the so-called *B* conformation and changes to the *S* conformation (*S*: stretched) at 65 pN. The transition occurs fast within a force range of approximately 5 pN, which is indicative of a process of high cooperativity. At this point, the molecule can be stretched to almost twice its contour length (Figure 22).^[85] Notably, before the structure of DNA was unraveled, there were speculations in the fifties about these kind of elastic properties.

The transition from the *B* to the *S* conformation is probably accompanied by a decoiling of DNA. Léger et al. showed that the force where the *B*–*S* transition occurs depends on the

number of twists. Different DNA structures were characterized in this way.^[145] The opened *S*-DNA conformation facilitates access to the base pairs compared to the *B* conformation. This situation might be relevant for DNA transcription. The structure and reason for the high cooperativity remain to be elucidated. Molecular dynamics simulations indicate that two different structures are conceivable in the stretched conformation.^[146] It is difficult to distinguish between them on the basis of force measurements since the deformation energies for both structures are almost identical. It is important for the reaction pathway on which strand the force is exerted and whether the molecule can rotate freely during extension. In the case of free rotation, the helix can be entirely uncoiled. However, if rotation is suppressed the direction of the base pairs changes. Pulling causes a bending of the base pairs with respect to the longitudinal axis, which is accompanied by a reduction in the diameter of the helix. There are some indications that the flat ribbonlike structure of DNA has some significance under physiological conditions. It is conceivable that this conformation is stabilized by DNA-binding proteins.

The melting of the DNA occurs at higher forces of around 150 pN. Both the melting of DNA and the *B* to *S* transition at low forces are sequence dependent. Duplex-poly(dA-dT)- and duplex-poly(dG-dC)-DNA exhibit a self-complementary base sequence, which can result in the formation of hairpin loops after relaxation. Rief et al. studied the reversibility of melting and the conformational change of the *B* to the *S* form.^[85] They found that the *B*–*S* transition occurs much faster than the withdrawal of the cantilever (the pulling velocity is in the range of $\mu\text{m s}^{-1}$) and is therefore fully reversible. Moreover, the threshold force is not dependent on the pulling velocity either. The melting of DNA, however, depends on the pulling velocity and displays a typical hysteresis between the approach and retraction curves, which indicates an irreversible process.

5.5. Polypeptides and Proteins

Many proteins are designed to cope with mechanical load: actin and myosin^[147] are responsible for muscle contraction, whereas the stability of connective tissue and cartilage are based on extracellular matrix proteins such as laminin, collagen, vimentin, fibronectin, and proteoglycans.^[148] Individual cells may migrate on surfaces because of elastic anchor proteins. Leukocytes are known to roll along the endothelium of blood vessels if pushed ahead by the bloodstream. The contact between endothelial cells and the white corpuscles is established by proteins, which are stretched during rolling. The elasticity of these molecules is based on their particular structure and intramolecular interactions—mostly noncovalent interactions and disulfide bridges. Besides hydrogen bonding, electrostatic interactions and hydrophobic effects are responsible for the stability of proteins. It has not been possible to distinguish between the different kinds of interactions by force spectroscopy. However, collective forces that determine the shape of the protein domains can be measured accurately.

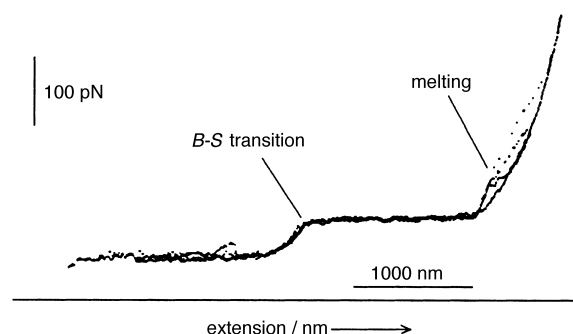


Figure 22. Superposition of different force–extension curves of dsDNA from the λ -phage obtained at different pulling velocities ($0.15\text{--}3\ \mu\text{m s}^{-1}$). The contour length of the molecule is approximately $2\ \mu\text{m}$. A conformational change from the *B* to the *S* conformation occurs at 65 pN. At this point the DNA can be stretched beyond $3\ \mu\text{m}$ without any detectable increase in the restoring force. The nucleotide strands separate (melt) at approximately 150 pN. The force at which this event takes place depends on the pulling velocity. The process is therefore irreversible. Despite the partial melting of dsDNA (hysteresis) the *B* state is formed upon relaxation. This can be deduced from the overlap of the back and forward z scan immediately before the *B*–*S* transition occurs. Even after reaching the actual melting transition this can occur, however, it is not inevitable.^[85]

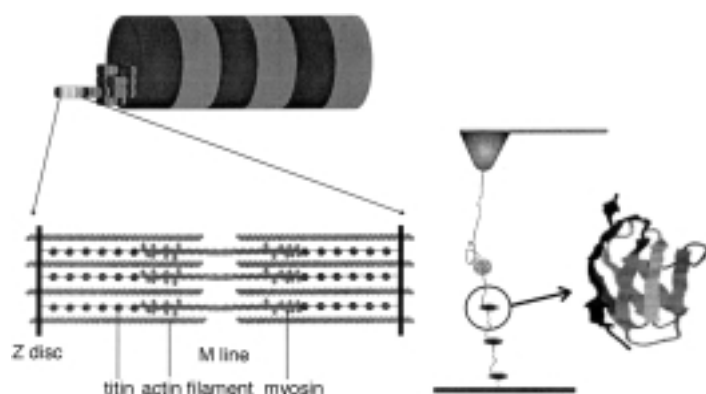


Figure 23. Titin, the most well-known elastic filament, stems from the sarcomere of striped muscles and is responsible for elastic anchoring and central orientation of the myosin filaments. Titin is a 3000 kDa protein and is one of the largest proteins known. It is composed of tandem fibronectin III (FN3) and structurally related immunoglobulin (Ig) domains (constant region). FN3 domains are composed of 98–102 amino acids and possess seven β -sheet peptide chains that form a β -barrel. Ig domains exhibit essentially the same structure. The elastic stretching of the I band, which can be four times larger than the original length, can only be explained by a reversible folding and unfolding of the FN3 and Ig domains.

Until now, linear muscle and adhesion proteins, such as titin (Figure 23) and tenascin, which consist of globular subunits have been preferred for investigation. Stretching such a modular protein results in the subsequent unfolding of the globular domains: the sequence is determined by the stability of the subunits: the less-stable units unfold before the more-stable ones. Each unfolding event is accompanied by a sudden lengthening of the filament (Figure 24B). The unfolding of a single domain results in a fast relaxation of the bent cantilever. This process is repeated for each subunit upon extension of the filament and results in a saw-tooth profile of the force–extension curve (Figure 24A). Such a curve was observed for native and recombinant proteins such as titin, tenascin, and spectrin.

The change from the folded to the unfolded state of each domain is highly cooperative without the occurrence of intermediate species. Therefore, the process can be described by means of a two-state model, which has turned out to be useful in interpreting the rupture of ligand–receptor couples (Figure 18) or the force–induced conformational changes of dextran (Figure 20). The mechanism is always identical: Pulling the cantilever reduces the energy barrier separating the folded and unfolded states until thermal fluctuations bring about the structural change. The refolding of a molecule such as titin is unlikely because of the high asymmetry of the potential $x_f \ll x_u$. The folding rate is negligibly small under external force. The stretched polypeptide chain has to contract itself against the external force over a length of more than 100 Å. Therefore, the conformational change does not occur in thermal equilibrium. This change is in contrast to the conformational change of dextran, which displays only an extension of 0.32 Å at pulling velocities of a few $\mu\text{m s}^{-1}$, and is therefore fully thermodynamically reversible. The measurement of forces of systems that are not performed in thermal equilibrium have the following consequences:

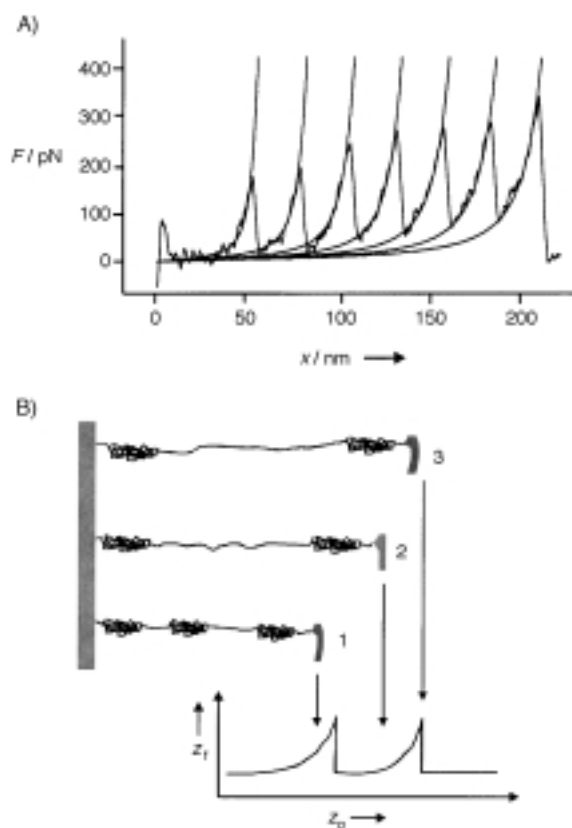


Figure 24. A) Force-induced conformational change of a recombinant titin fragment. Seven immunoglobulin domains unfold subsequently without removal of the molecule from the tip or detachment of the molecules from the substrate. The partial curves can be described by the WLC model ($l_p = 4$ Å) in which the contour length increases each time by 280 Å.^[152] B) Schematic representation of the ascribed process. The polypeptide strand that is folded in several domains is unfolded one domain after the other, with the weakest domains unfolding first: the tip takes up the molecule and stretches it (1). If the domain unfolds (2) the tip–sample distance suddenly increases. The free polypeptide strand is stretched (2–3) until the pulling force reaches a threshold value and the domain unfolds.^[152]

1) The refolding of a stretched molecule is slow compared to the timescale of the experiment. Since equilibrium does not occur immediately, the kinetics of the reaction has to be considered. Thus, the force at which unfolding occurs depends on the pulling velocity (Figure 25A).

2) The force–extension curve is characterized by a hysteresis of the approach and retraction curve since the structural change of the molecule is irreversible within the timescale of the experiment (Figure 25B).

Cross-linked polylysine at pH 7 exhibits a reversible extension (Figure 26).^[68] It was possible to extend and stretch the molecule within one minute without any detectable changes in the force–extension curve. At the end of force curve 72 an irreversible overstretching occurred and resulted in a smaller persistence length. Computer simulations of the reaction kinetics provide information about parameters (Table 7) such as the potential width of the conformational transition and the unfolding rate. If, however, the transition occurs within thermal equilibrium, as is the case for dextran, the rate constants cannot be determined separately.

Since force spectroscopy is capable of detecting small variations in the number of amino acids, it is possible

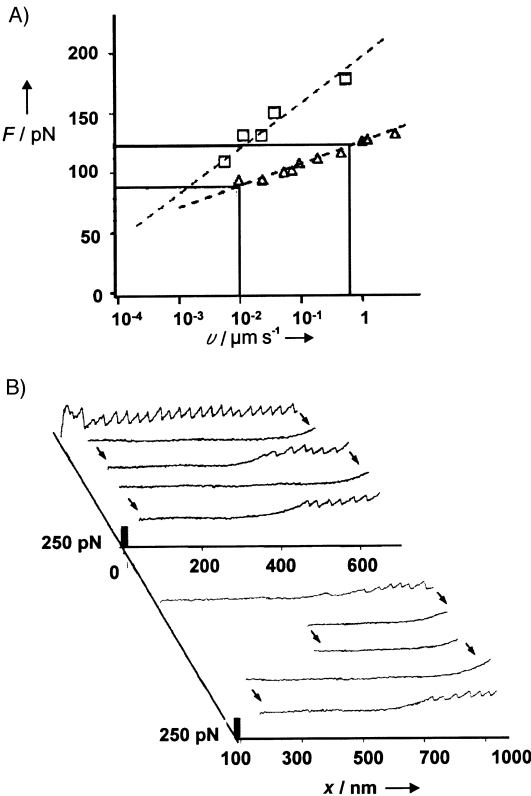


Figure 25. A) Unfolding force as a function of pulling velocity (semi-logarithmic plot). Values for native titin ($\square$) and tenascin–fibronectin ($\triangle$), a recombinant fragment of similar structure, are shown. The dashed lines are the results of computer simulations.^[136] B) The subsequent force measurements of titin reveal a hysteresis; on the timescale of the experiment the conformational transition is irreversible. Since the refolding of the molecule is very slow, the first stretching curve exhibits more unfolding events than the following ones. Only when the time between the extension and relaxation were longer could the saw-tooth profile be fully regained. The applied force prevents refolding of the genuine molecular structure. Only when the protein is relaxed can the saw-tooth pattern be observed again.^[152]

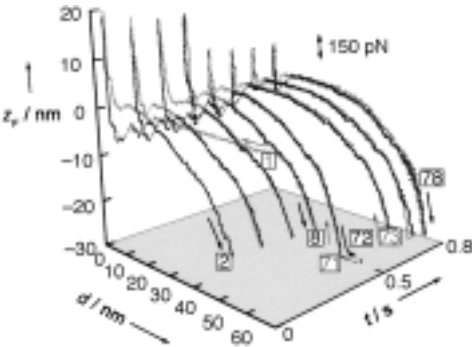


Figure 26. Reversible unfolding–folding cycles of polylysine (gold-covered Si_3N_4 tip acts on a gold substrate covered with thiolated poly-L-lysine (pH 7, pulling velocity 100 nm s^{-1}). The numbers indicate the sequence of measurements. The overstretching of the molecules after 72 force–distance curves results in a decrease in the chain stiffness. Polylysine was derivatized using iminothiolane in order to ensure attachment of the molecules on the gold.^[68]

to explore basic differences in the primary structure of the protein. This can be demonstrated by a comparison between spectrin and titin. Spectrin consists of α -helix bundles, while titin subunits exhibit a β -barrel structure. Both molecules

Table 7. Molecular parameters of different proteins in aqueous solution. Immunoglobulins (Ig), the repeating units of titin, and fibronectin-III domains (Fn-III), subunits of tenascin and titin, have a similar structure (β -barrel). Spectrin, however, consists of α -helical subunits. Spectrin stabilizes the cytoskeleton of erythrocytes. Tenascin originates from the extracellular matrix, while titin is found in muscles. Only few data are available from the nonlinear α_2 -macroglobulin. α_u , α_f [s]: unfolding and folding rate, respectively, in the absence of an external force, Δx_u , Δx_f [Å]: width of the energy barrier of unfolding and folding, respectively, v : pulling velocity.

Polypeptide	Persistence length [Å]	Transition (domain folding) $v = 0.01 - 0.5 \mu\text{m s}^{-1}$	Lengthening [Å]
Ig peptide ^[137, 138, 152] (recombinant titin fragment), titin ^[109, 152] (native)	8 (< 50 pN) 4 (> 50 pN)	130 pN/190 pN $\alpha_u = 0.3 \times 10^{-4}$ $\alpha_f = 2$ $\Delta x_u = 3$ $\Delta x_f > 150$	266 280
Fn-III peptide ^[153] (recombinant tenascin fragment), tenascin ^[153] (native)	4.2 ± 2.2 (> 50 pN)	$\sim 90 \text{ pN} / \sim 150 \text{ pN}$ $\alpha_u = 4.6 \times 10^{-4}$ $\alpha_f = 3$ $\Delta x_u = 3$, $\Delta x_f = 10$	285 ± 40
Fn-III peptide ^[137] (recombinant tenascin fragment)	8 (< 50 pN) 4 (> 50 pN)	ca. 90 pN / $\sim 160 \text{ pN}$ $\alpha_u = 0.3 \times 10^{-4}$ ($l_p = 4 \text{ Å}$) $\Delta x_u = 5.5$	286
spectrin ^[138] (native)	8 (< 50 pN)	< 25 pN/ca. 30 pN $\alpha_u = 0.3 \times 10^{-4}$ $\Delta x_u = 17 \pm 5$	317 ± 3
α_2 -macroglobulin ^[29]	stiffness: ca. 15 mN m^{-1} , ca. 65 mN m^{-1}		

denature at almost the same temperature; thus, the activation energy of unfolding is also similar. However, the forces which are necessary to unfold spectrin are much lower than those needed to unfold titin. The reason for this can be found in the course of the folding–unfolding potential. Although the height of the energy barrier of spectrin is similar to that of titin its width is six times larger (Table 7). Consequently, the unfolding state is very shallow and more extended than that of titin, that is, the measured unfolding forces of spectrin domains are lower by nearly the same factor.

In contrast to the β -barrel structures, the tertiary structure of spectrin is not stabilized by hydrogen bonds. Hydrogen bonds only stabilize the helix itself, and not the entire bundle. Thus, the tertiary structure is mainly maintained by weak hydrophobic interactions that lead to smaller unfolding forces. It remains to be elucidated whether the different mechanical stabilities of the two proteins are of any biological significance.

6. Summary and Outlook

*Wo rohe Kräfte sinnlos walten,
da kann sich kein Gebild gestalten.“*

Friedrich von Schiller (1759–1805), *Lied von der Glocke*

With regard to force measurements this quotation from the German dramatist Schiller should read instead: “Wo rohe Kräfte sinnlos walten, da kann sich kein Gebilde *fallen*.” since high forces and pulling velocities can rupture bonds and unfold proteins but render binding and folding highly improbable. The simple model of Bell^[80] can describe most

of the results obtained from force measurements. It should be emphasized that reversible conformational changes are not excluded, but depend on the topology of the potential and the pulling velocity of the cantilever. Examples of a reversible transition between two conformations upon exerting an external force are the change from the chair to the twisted-boat conformation of dextran as well as the change from the *B* to *S* conformation of the DNA duplex. Irreversible processes are characterized by a hysteresis between the approach and retraction of the cantilever, a dependence of the force at which the transition occurs on the pulling velocity, and an asymmetric potential. For example, the extension of modular proteins such as titin, a filamentous muscle protein, spectrin from erythrocytes, and tenascin, an extracellular protein, shows that kinetic analysis according to a classic two-state model in conjunction with generic polymer models permits qualitative as well as quantitative data to be derived on the mechanical properties of polymers.

Force microscopy has developed into a powerful tool for probing the mechanical properties of single molecules such as conformational transition states, bending modules, binding strength, rate constants, and the topology of the potential. Future prospects have been intimated by Müller, who reported the first experiments on the folding and unfolding cycles of bacteriorhodopsin in its native environment in conjunction with high resolution imaging.^[149] It was shown that single helices could be removed from the bundle assembly. Besides experiments with isolated systems, the first force experiments with whole cells have been conducted on the quantification of cell adhesion.^[150]

Scanning force spectroscopy combines high-resolution imaging with manipulation of single molecules and provides the possibility of obtaining insights into the mechanical behavior and structural details of synthetic polymers and biomolecules on a molecular level.

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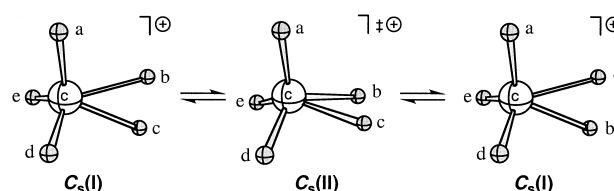
Does CH₅⁺ Have (a) “Structure?” A Tough Test for Experiment and Theory**

Peter R. Schreiner*

In a very naive view, the notion of structure seems like a fundamental law of physics: atoms bond together to form molecules in regular, predictable ways. This quasi-classic framework, however, is not supported in the sense of strict quantum mechanics, and is only possible if one assumes the separation of nuclear and electronic motion (Born–Oppenheimer approximation) which forms the necessary hierarchy to describe the “structure” of a system within classic observables.^[1] Although many molecules exist in isomeric forms, chemists mostly refer to the structure of a compound as the geometrical isomer lowest in energy. But what happens if other isomers (or stationary structures) are energetically very close and the barriers for their interconversion are small so that there is practically no preference, even at the lowest achievable temperatures? This situation arises for the methonium ion, CH₅⁺, whose three lowest lying stationary structures are nearly degenerate and equilibration of the minima is facile (barriers < 1 kcal mol^{−1}), even at 0 K. The recently reported high-resolution infrared spectrum of this enigmatic species by Oka et al.^[2] seems to confirm theoretical predictions that this molecule is highly fluxional and that the term “structure” may have to be refined for CH₅⁺.^[3–5]

The methonium ion is the smallest protonated alkane and was suggested by Olah et al. as the prototype for the entire group of nonclassical carbonium ions, which play important roles as intermediates in acid-catalyzed transformations of hydrocarbons and in many electrophilic reactions.^[6] Hence, the significance of the structure of this ion is that it is the cornerstone for mechanistic proposals for these and related transformations.^[7, 8] The methonium ion is also of astrochemical interest as it may serve as a spectroscopically sensitive indicator for the abundance of methane in cold interstellar clouds.

Although CH₅⁺ was discovered as early as 1952 by Tal’roze and Lyubimova in mass spectrometric studies,^[9] where it is still used to date as an agent for chemical ionization, its geometry has never been determined experimentally. As a matter of fact, the five hydrogen atoms around CH₅⁺ were considered equivalent^[10] before the advent of the first insightful theoretical predictions in the late 1960s and early 1970s by the groups of Kutzelnigg, Pople, and others.^[11–15] These studies suggested that CH₅⁺ consists of a pyramidalized CH₃⁺ unit strongly bound ($D_0 = 40–45$ kcal mol^{−1})^[16, 17] to an H₂ molecule, resulting in a formal three-center, two-electron bonding arrangement which is at the heart of Olah’s electrophilic carbonium ion chemistry.^[8] One of the two resulting C_s-symmetric structures is the global minimum, C_s(I), whereas the other, C_s(II), is a transition structure for the rotation of the H₂ moiety (Scheme 1) which is associated with an extremely low barrier at all levels of theory (around 0.1 kcal mol^{−1}). There are also C_{4v} and D_{3h} forms which are higher order stationary points meaning that they are chemically not very meaningful.^[18]



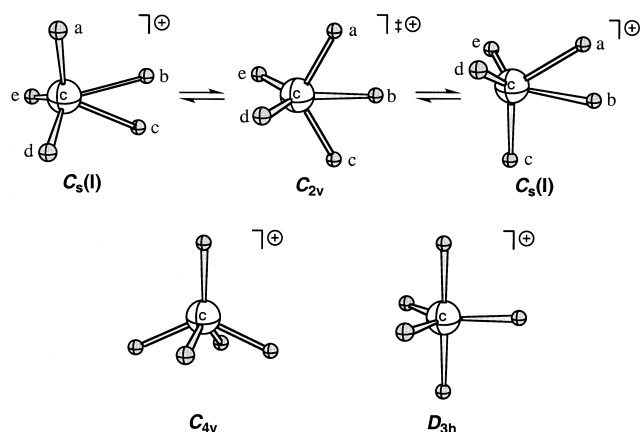
Scheme 1. The computed global CH₅⁺ minimum, C_s(I), and the transition structure for the H₂-rotation, C_s(II).

A second, C_{2v}-symmetric transition state, which does not display a recognizable H₂ subunit,^[13] is responsible for exchanging the protons of the H₂ moiety with those of the CH₃⁺ tripod (Scheme 2). Hence, the relative energy of the C_{2v} structure determines whether CH₅⁺ is indeed the nonclassical carbonium ion prototype, that is, whether the barrier is sufficiently high or whether all C–H bonds are statistically averaged in the case of a vanishingly low energy of this transition structure. This is when the situation becomes challenging for experiment and theory, as it is increasingly difficult to resolve the spectral data and to determine the relative energies of a molecule with decreasing barriers for interconversion of low-lying minima.

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Scheme 2. The exchange (via transition structure C_{2v}) of equivalent protons in CH_5^+ ($C_s(I)$) and the higher order (i.e., nonminima) stationary structures with C_{4v} and D_{3h} symmetry.

The C_{2v} barrier is very sensitive to electron correlation treatment and the size of the basis set.^[19] Improvement in the sophistication of the theoretical treatment, for instance, by using the highly correlated coupled-cluster approach^[20] in conjunction with very large basis sets lowers the C_{2v} barrier to less than 1 kcal mol^{-1} .^[3, 5] As the zero-point vibrational energy (ZPVE) correction is of similar magnitude, it appears that proton scrambling occurs essentially freely, even at 0 K. In practical chemical terms, this was expressed in calling the methonium ion *unique* rather than serving as the nonclassical carbonium ion prototype.^[3, 4] Kutzelnigg et al., however, pointed out that the use of harmonic ZPVE corrections may not be entirely correct for energetically very close-lying stationary points.^[5]

This classical quantum-mechanical analysis was both supported and criticized by highly elaborate density functional calculations which take into account the dynamics of the system in the sense of quantum-statistical mechanics.^[21] Here it was found that CH_5^+ undergoes large-amplitude pseudorotations, which result in hydrogen scrambling and statistically equivalent protons. Nevertheless, there is a high probability of finding structures akin to the classical $C_s(I)$ quantum ground state. Parrinello referred to this situation as an “intramolecular liquid” where the protons move, but in a highly correlated way that preserves local structure.^[22] Such a situation can then only be defined in a statistical sense using distribution functions rather than by assigning atomic coordinates. Clearly, this is an uncomfortable situation for chemists who require that a stable molecule, which CH_5^+ obviously is with respect to dissociation and deprotonation, must have (a) structure.^[23] A possible solution of this dilemma may be offered by Marx and Savin who demonstrated that a topological analysis of the electron localization function (ELF) of representative configurations including tunneling and zero-point vibrational effects is useful in extracting structure even from highly fluxional molecules.^[24] Such an analysis also suggests that CH_5^+ is indeed very floppy but shows considerable statistical preference for $C_s(I)$ -like structures.

The recently reported experimental IR spectrum of CH_5^+ by Oka et al. (Figure 1),^[2] a major achievement in high-

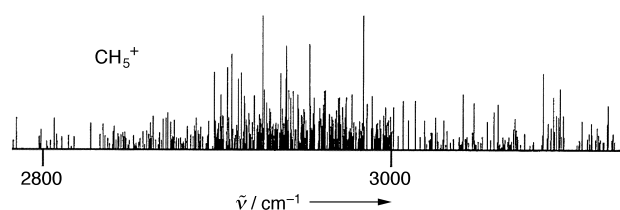


Figure 1. High-resolution IR spectrum of CH_5^+ . (Reproduced with permission from T. Oka).

resolution infrared spectroscopy, allows a first experimental analysis of the theoretical predictions. Although it is relatively easy to generate CH_5^+ by means of electron impact ionization of methane [Eq. (1)] or by proton transfer [Eqs. (2) and (3)],



the experimental setup to record a resolved IR spectrum is rather demanding because the resolution is highly dependent on the dimensions of the discharge tube, the energy of the laser system, the gas-mixing ratios of H_2 and CH_4 (50:1 in the experiment reported by Oka et al.), and the total pressure. Despite extensive efforts by the top practitioners, the spectrum in question had never been resolved due to the apparent fluctuonality of the methonium ion.^[25]

The groups of Hiraoka^[26] and Lee^[27] used a limited number of “solvent” molecules [CH_4 and $(\text{H}_2)_n$; $n = 1-6$] to slow down the rate of hydrogen scrambling. In spite of the stabilizing effect when attaching just one H_2 to CH_5^+ , the IR spectrum remains unresolved due to strong anharmonic couplings among vibrational modes.^[27, 28] With three hydrogen molecules attached, the CH_5^+ core becomes semirigid and allows some interpretation of the corresponding IR spectrum. However, as these approaches strongly influence the energetics and structures of the target molecule, none of them can claim to have resolved the vibrational bands of unperturbed CH_5^+ .

Oka’s spectrum consists of about 900 spectral lines in the narrow region of $3150-2770 \text{ cm}^{-1}$ (Figure 1), but appears to be significantly different from other close-lying absorptions due to other species and possible impurities. As the experimental spectrum is extremely complicated and does not show regularity or symmetry, it supports the collective theoretical evidence on the highly fluxional nature of CH_5^+ . The answer to the crucial question whether a preference for the $C_s(I)$ quantum ground state exists,^[29] must await the assignment of the experimental absorptions. This will probably require that the IR spectrum be recorded at very low temperatures and that the spectra of isotopomers be used as additional sources of information. From a theoretical point of view, accurate full ab initio potentials and variational calculations are needed but will require much more powerful computers than available today. The theoretically carefully elaborated rotational spectrum of CH_5^+ also still awaits its experimental realization.^[30-32] This fascinating story continues.^[33]

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Organometallic Transformations Demonstrate That Fluorocarbons Are Reactive Molecules

Thomas G. Richmond*

Fluorine forms the strongest single bond to carbon. The great strength of this bond, along with the small size and high electronegativity of fluorine, conspire to give rise to the unusual properties associated with highly fluorinated organic molecules.^[1] Indeed, the most characteristic chemical property of fluorocarbons is their *lack* of reactivity. The stability imparted by the C–F bond leads to the long atmospheric lifetimes of chlorofluorocarbons (CFCs); saturated perfluorocarbons (PFCs) are even more stable, with CF₄ having an estimated stratospheric lifetime of more than 10 000 years.^[2]

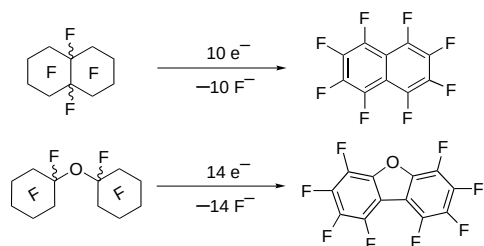
The unique properties of fluorinated groups have been exploited by organometallic chemists to stabilize unusual molecules and provide a benchmark for comparisons with analogous hydrocarbon ligands.^[3,4] Current activity has focused on the preparation of highly fluorinated ligands that contain “fluorous ponytails”. These “ponytails” impart solubility in PFC solvents, a property which is necessary for

application in “fluorous” biphasic chemistry.^[5] This technique exploits the unusual solubility properties of PFCs to address the technologically important problem of the separation of PFC-containing catalysts or reagents from hydrocarbon-soluble products. Clearly the success of this strategy requires that the PFC phase remains inert under the reaction conditions. This article will trace recent developments in the reaction chemistry of saturated PFCs and PFC ligands in organometallic complexes with an emphasis on how organometallic reagents enable transformations to be performed under remarkably mild conditions.^[6]

The advances in the reaction chemistry of totally saturated PFCs and CFCs with special focus on the defluorination chemistry were summarized by Saunders in 1996.^[7] Strong reducing agents mineralize PFCs and CFCs but partial and selective C–F activation has been achieved under homogeneous conditions with the sodium benzophenone radical anion or with cobaltocene in the presence of lithium trifluoromethanesulfonate.^[8] Metal–organic electron donors have been applied to the synthesis of organofluorine compounds (Scheme 1).^[9]

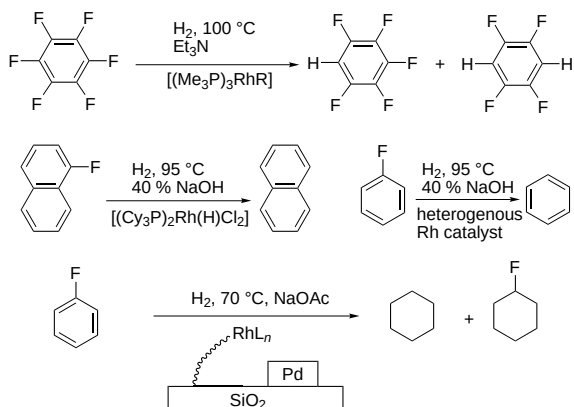
Catalytic defluorination chemistry has been achieved utilizing early transition metallocenes ([Cp₂TiF₂], [Cp₂ZrCl₂])

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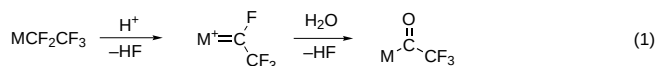
Scheme 1. Examples of reductive defluorination chemistry applied to the synthesis of perfluoroaromatics.

and amalgamated aluminum or magnesium as the final reductant.^[10] Crabtree and co-workers developed a photochemical defluorination system based on dcamethylferrocene and pioneered PFC functionalization chemistry utilizing gas-phase Hg-sensitized photolysis in the presence of ammonia.^[11] All of these reactions (with the exception of a $[\text{Cp}_2\text{ZrCl}_2]/\text{Mg}/\text{Hg}$ system,^[10] which reduces perfluorocyclohexane to 1,2,4,5-tetrafluorobenzene) exploit the enhanced reactivity of tertiary C–F bonds, which has been termed the “Achilles Heel” of perfluorocarbons, to initiate reductive defluorination.^[12] The common theme in these defluorination transformations is electron transfer in the presence of a Lewis acidic metal cation. Simple hydrogenation of a PFC (either free or as part of a ligand) under mild conditions was unknown until the report by Hughes and Smith (ref. [16]) discussed below. In contrast, significant progress has been made in the hydrogenolysis of aromatic C–F bonds in both homogeneous and heterogeneous systems, including some examples of catalytic hydrogenation (Scheme 2).^[13]

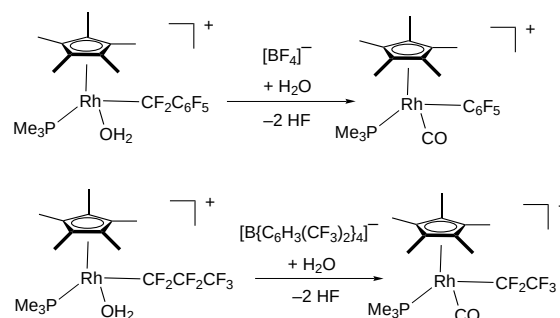


Scheme 2. Hydrogenolysis of aromatic C–F bonds with H_2 .

An important exception to the inert nature of perfluorocarbon ligands is the Lewis or proton acid enhanced hydrolysis of α -fluorine atoms made thermodynamically feasible by the formation of strong C–O and H–F bonds and kinetically accessible by the stabilization of a carbene intermediate by the transition metal [Eq. (1)].^[14]



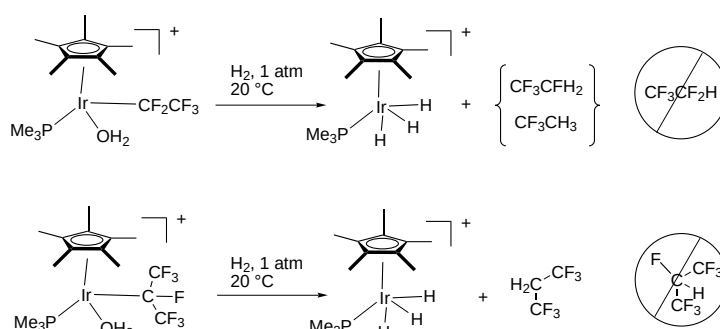
Hughes and co-workers recently illustrated the subtle nuances of this reaction in a series of cationic rhodium(III) complexes in which coordinated water is demonstrated to be sufficiently acidic to hydrolyze the α -fluorines of a perfluorobenzyl group into HF and coordinated CO (Scheme 3).^[15]



Scheme 3. Hydrolysis of α -carbon–fluorine bonds activated by a rhodium(III) center.

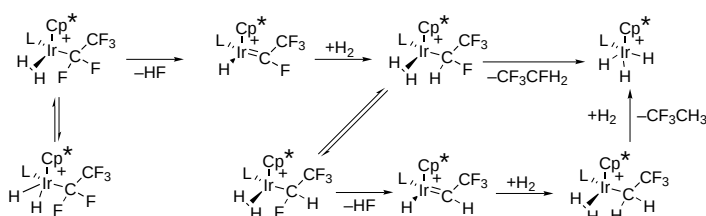
The enhanced activity of coordinated water is demonstrated by the absence of reactivity in acetone, which displaces the water ligand. To effect the same transformation in the less reactive perfluoropropyl system, the non-hydrogen-bonding ion $[\text{B}(\text{C}_6\text{H}_3(\text{CF}_3)_2)_4]^-$ is required to enhance the activity of the bound water. In addition to the unusually mild conditions for C–F hydrolysis, this work provides evidence that coordination to a metal center enhances the reactivity of water and this activity can be influenced by the hydrogen-bonding properties of the counterion.

Treatment of the analogous iridium(III) complexes with hydrogen (1 atm) at room temperature results in a facile hydrogenolysis of the α -CF₂ group and the production of hydrofluorocarbons (CF_3CFH_2 and CF_3CH_3 in an approximate 1:1 mixture), and the known iridium trihydride as illustrated for the pentafluoroethyl compound (Scheme 4).^[16]



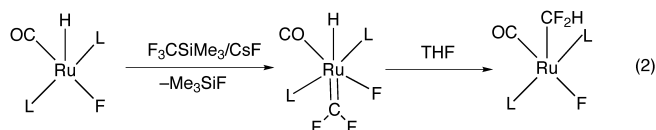
Scheme 4. Production of HCFCs by hydrogenolysis of with H_2 at an iridium(III) center.

Interestingly, the reaction is selective for H_2 and no hydrolysis products are detected despite the presence of the aqua ligand in the iridium complex. A series of reactions consistent with the absence of mono-hydrogen containing products is given in Scheme 5.



Scheme 5. Mechanistic scheme for the hydrogenolysis of perfluoroalkyl groups at an iridium(III) center ($\text{Cp}^* = \text{C}_5\text{Me}_5$).

The substitution of water by H_2 generates an η^2 -dihydrogen complex; the $\eta^2\text{-H}_2$ ligand is known to be more acidic than coordinated water and this increase in acidity is sufficient to cause HF elimination and the generation of a carbene intermediate. Reductive elimination, which might be anticipated from the classical dihydride complex, is not favored because of the stability of the iridium–perfluoroalkyl bond. This hypothesis was confirmed by the independent generation of the complex $[\text{Cp}^*\text{Ir}(\text{PMe})_3(\text{H})(\text{CF}_2\text{CF}_2\text{CF}_3)]$ which proved to be stable to reductive elimination up to 150°C . Importantly, protonation of this complex in the presence of H_2 affords hydrofluorocarbon species that are analogous to those in Scheme 4. Production of CF_3CH_3 suggests that hydrogenolysis of the second C–F bond competes with the reductive elimination process. An alternative mechanism which involves α -fluoro migration to the iridium center and leads to the loss of HF from the metal cannot be eliminated. Caulton and Huang have demonstrated that there is an equilibrium between $\text{Ru}-\text{CF}_3$ and $\text{Ru}(\text{F})(\text{CF}_2)$ bonding modes and that the latter species provides an example of the hydrogenolysis of a C–F bond [Eq. (2)].^[17]



Note that this transformation involves the net replacement of F by H in a trifluoromethyl group. A related α -fluoro migration is important in nickel(0)-mediated transformations of perfluoroalkyl iodides.^[18]

Organometallic complexes provide a versatile basis for the discovery of new reaction chemistry of fluorocarbons.^[6] The work of Hughes and co-workers is particularly significant because it demonstrates how σ -coordination ($\text{M}-\text{CF}_2\text{R}_\text{F}$) of a fluorocarbon to a metal center can not only activate α -C–F bonds but also the relatively meek reagents (water and hydrogen). These reagents participate in the C–F bond activation that results in the synthesis of hydrofluorocarbons. Significant progress has been noted in the chemistry of highly fluorinated aromatics; this includes recent reports that range from the oligomerization of hexafluorobenzene initiated by the thermolysis of $[\text{Cp}_2\text{Zr}(\text{C}_6\text{F}_5)_2]$ to the $\text{Ni}^{(0)}$ -assisted defluorination of 2,4,6-trifluoropyrimidine to prepare new pyrimidine and pyrimidinone derivatives.^[19] The use of electron-rich metal centers (as well as strong organic electron donors) to mediate reductive defluorination of PFCs is now

complemented by the possibility of the mild and selective reduction of PFCs by molecular hydrogen. Significant challenges remain on the way to the true catalytic functionalization (in this instance partial hydrogenation) of PFCs. Activation of PFCs by C–F oxidative addition to a transition metal as a means of entry into the hydrogenation manifold as described above has yet to be achieved. The exceptionally poor coordinating ability of PFCs ($\text{M} \leftarrow \text{FCR}_n$) mitigates against C–F activation.^[20] Both technologies should prove applicable in the synthesis of fluorine-containing molecules (including hydrofluorocarbons) to replace CFCs and to aid the understanding of the scope and limitations of catalytic chemistry in the fluorous phase.

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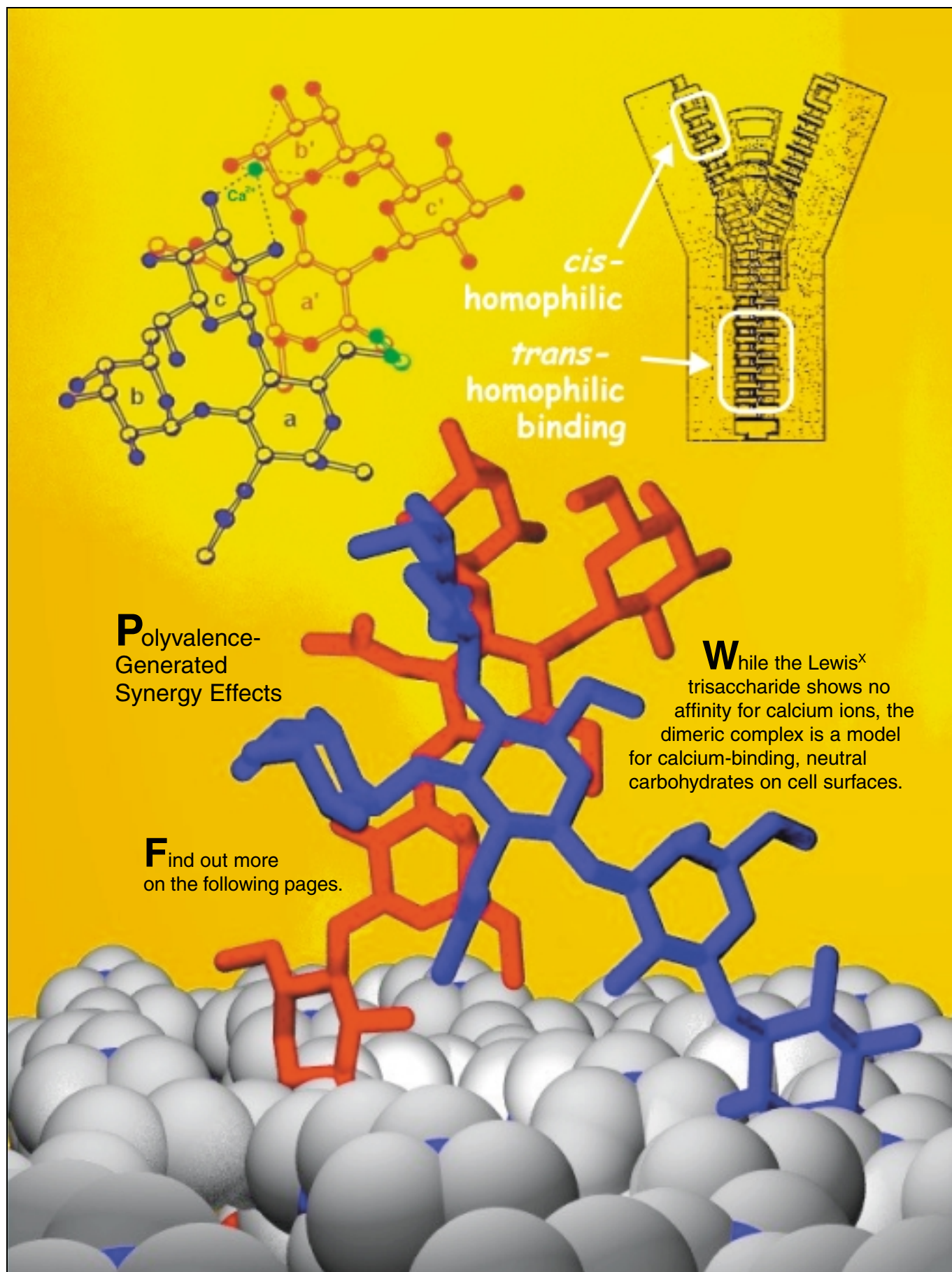
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Calcium-Dependent Carbohydrate – Carbohydrate Recognition between Lewis^X Blood Group Antigens**

Armin Geyer,* Christian Gege, and Richard R. Schmidt*

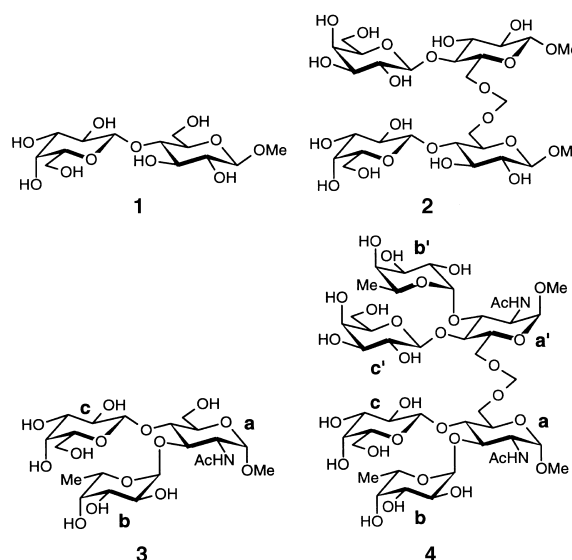
Dedicated to Professor Horst Kessler on the occasion of his 60th birthday

Lewis^X (Le^X) is the terminal trisaccharide moiety of numerous cell-surface glycolipids and glycoproteins which are involved in selectin-mediated cell–cell adhesion and recognition processes.^[1] In addition to protein–carbohydrate binding, there are adhesive forces between cells which are based on direct carbohydrate–carbohydrate interactions, for instance on homophilic Le^X–Le^X binding.^[2] Molecular details of the homophilic recognition between neutral carbohydrate moieties are incompletely understood because of chemical complexity of the cell membrane. Ligand–receptor complexes with weak affinity constants between singular entities (K_a values in the mM^{-1} range) multiply to polyvalent intercellular adhesive forces of high affinity.^[3] The experimental characterization of the synergistic multiplication of weak carbohydrate–carbohydrate affinities to considerable intercellular forces is an analytical challenge. Protein-mediated binding and charge effects must be excluded; separation of membrane components can even be induced by their differing phase transition properties.^[4]

The cation-dependent clustering of membrane-anchored sialylated,^[5] and also neutral,^[6] Lewis glycolipids has been described. However, in isotropic solution, no self-assembly of Le^X glycoconjugates or significant metal complexation was detectable.^[7] The very weak calcium affinity of the neutral Le^X oligosaccharides obviously requires their tethering to a lipid bilayer in order to make clustering detectable.^[8] Yet, conformational details of the Le^X clusters could not be determined until now.

We describe here how we have mimicked the preorientation of Le^X moieties with a flexible spacer between two Le^X trisaccharides. Six rotatable bonds permit several relative orientations of the Le^X moieties. In the presence of calcium, a single mode of self-assembly was observed by NMR spectroscopy and for the first time conformational details of a Le^X cluster were quantified. The cooperative calcium complexation of a simple Le^X cluster contrasts the ion-binding behaviour of other carbohydrate clusters.

Mono- and oligosaccharides, such as the methyl lactoside **1** (Scheme 1), have a weak calcium affinity. The lactose moieties of **2** are kept in close vicinity by the methylene spacer but no sterical restrictions force the disaccharide moieties of **2** into a prearranged conformation. The two



Scheme 1. Two methyl lactosides **1** were covalently tethered in the bis-disaccharide **2**. Similarly, two Le^X trisaccharides **3** lead to the bistrisaccharide **4**. The methylene spacers in **2** and **4** keep the homotopic moieties in close vicinity without restraining their relative orientations. **a** = *N*-acetylglucosamine, **b** = fucose, **c** = galactose.

covalently tethered lactoses **2** have an affinity for calcium ions with a complex formation constant K_a of 8M^{-1} .^[9] The K_a value is 7M^{-1} for the monomeric lactoside **1**. The affinity of the bis-disaccharide **2** for calcium equals to the affinity of disaccharide **1** for calcium ions; therefore, the average affinity per lactose is divided by approximately two (Figure 1). Lactose lacks any cooperativity for calcium binding and the affinity of the bivalent receptor **2** is much lower than the multiplied monomeric affinities ($K_{bi} \ll K_{mono} K_{mono}$; $\Delta G_{bi} > \Delta G_{mono} + \Delta G_{mono}$), thereby avoiding the accumulation of cationic charges. Although crystal structures with selective coordination of lactose to calcium salts are known,^[10] lactose binds calcium only weakly in isotropic solution.

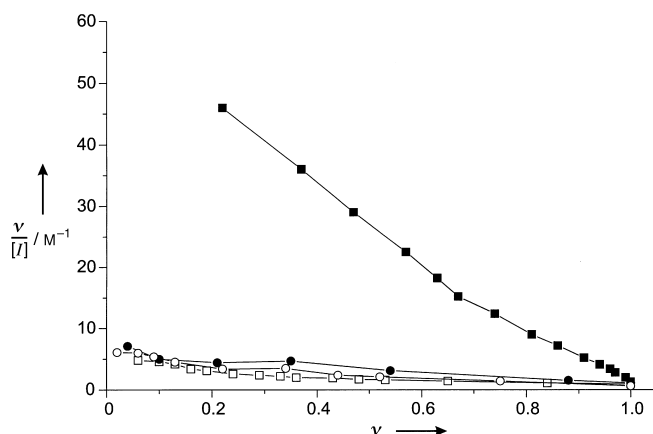


Figure 1. Scatchard plots^[20] of **1** (○), **2** (●), **3** (□), and **4** (■). The fraction of complexed carbohydrate (v) is plotted against the ratio of v/I (I = concentration of uncomplexed calcium ions). The affinity constant K_a [M^{-1}] is obtained from the y axis by linear extrapolation to $v = 0$. Only compound **4** yields a straight line, with an affinity constant of $K_a = 55\text{M}^{-1}$. Secondary binding sites are populated at high concentrations of calcium ions and a reduced slope is observed at $v > 0.7$. Weak affinities below $K_a = 10\text{M}^{-1}$ are slightly overestimated because full complexation ($v = 1$) is not reached without secondary effects playing a dominant role.

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The Le^X trisaccharide **3** behaves differently. The calcium affinity of **3**, which is weak in methanol ($K_a = 6 \text{ M}^{-1}$) and not detectable in water, multiplies to one of the highest affinities measured for a neutral oligosaccharide^[11] when two Le^X trisaccharides are covalently linked through O6 of *N*-acetylglucosamine (**4**, $K_a = 55 \text{ M}^{-1}$, Figure 2). The affinity of **4** as a receptor for calcium ions is even detectable in water ($K_a = 5\text{--}10 \text{ M}^{-1}$). The Le^X trisaccharide moieties cooperatively assemble and form a new receptor site with a significantly enhanced

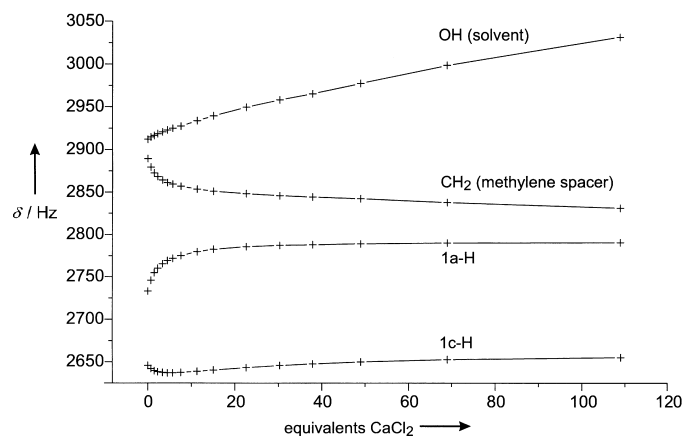


Figure 2. The chemical shifts (¹H NMR spectroscopy) of **4** in [D₄]methanol are plotted against the amount of calcium chloride added. A strong chemical shift dependence of the proton resonances is observed below ten equivalents of calcium chloride. The complexation of calcium freezes the conformational equilibrium of the trisaccharide moieties of **4**, and all chemical shifts show calcium dependence. The conformational effect of the calcium complexation causes a highfield shift for 1c-H which is inverted at higher calcium levels due to the nonselective binding of calcium to **4**. All curves, except for the solvent resonance, show a double exponential dependence on the calcium concentration.

affinity for calcium ions ($K_{bi} \geq K_{mono} K_{mono}$). The two Le^X trisaccharides show this cooperativity of calcium binding in isotropic solution and this allows proof to be obtained spectroscopically for the existence of homophilic carbohydrate–carbohydrate recognition.^[12]

The solution structure of **4** was investigated by NMR spectroscopy.^[13] As expected for a flexible spacer, no NOE contacts are visible between the homotopic trisaccharide moieties of **4** in the absence of calcium ions. The trisaccharide moieties are separated by six single bonds and the NOE cross-signal intensities resemble the intensities of **3**. These six single bonds become constrained in the calcium complex of **4**. The ROESY^[14] spectrum of the calcium complex of **4** exhibits new NOE contacts (Figure 3). The additional NOE contacts prove the self-assembly of the homotopic trisaccharide moieties into a defined receptor conformation. The high on- and off-rates of calcium-ion complexation^[15] average the expected hexasaccharide signal set to a trisaccharide signal set which contains the additional NOE results. The NOE contacts are caused by average proton–proton distances of less than four ångströms and indicate hydrophobic contacts between the two trisaccharide units of **4**. They were quantified according to the two-spin approximation:^[16] 2a-H–2c-H (approximately 3.0 Å), 1a-H–6b-CH₃ (approximately 3.2 Å), and 6b-CH₃–2a-COCH₃ (approximately 3.5 Å). The new cross signals identify

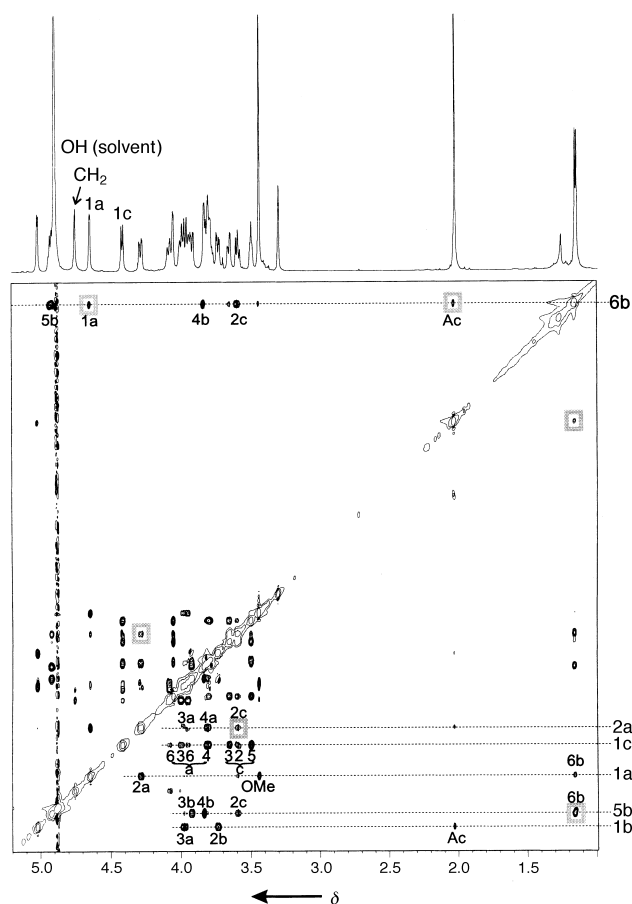


Figure 3. Compensated ROESY spectrum (600 MHz, 4 kHz spin lock, 200 ms mixing time, CD₃OD, 300 K) of **4** in the presence of 15 equivalents of calcium chloride. Rotating frame NOE interactions, which are not visible in the absence of calcium chloride, are indicated by gray boxes. Other cross signals than those indicated do not change their intensities relative to the ROESY spectrum of **4** without calcium chloride. The cross-signal intensities correspond to average interproton distances between 1.8 and 4 Å.

a cross-shaped, approximately 90°, relative orientation of the trisaccharide units of **4** (Figure 4).^[17] The hydrophobic contact stabilizes a neighbouring polar cation-binding site. This only polar contact site between the trisaccharide moieties is a hydrophilic cavity which is flanked by the five hydroxyl groups of rings c, b', and c' (Figure 4). The calcium ion fits into the NOE-derived structure with Ca–O distances of approximately 2.5 Å.^[18]

Le^X glycosphingolipids are oriented at the outer layer of cellular membranes. *cis*-Homophilic contacts between Le^X glycosphingolipids stabilize glycolipid microdomains within one membrane. *trans*-Homophilic contacts mediate the cell–cell adhesion between the outer membrane layers of different cells. Parallel^[19] or antiparallel^[8] molecular recognition between individual Lewis glycolipids was proposed to explain the two processes. The cross-shaped orientation of the covalently tethered Le^X trisaccharide moieties in **4** allows for *cis*- and *trans*-homophilic contacts between Le^X glycoconjugates without resorting to different recognition phenomena. Thus, pairs of dimers from adjacent cell membranes can stack above each other and stabilize cell–cell contacts (Figure 5).

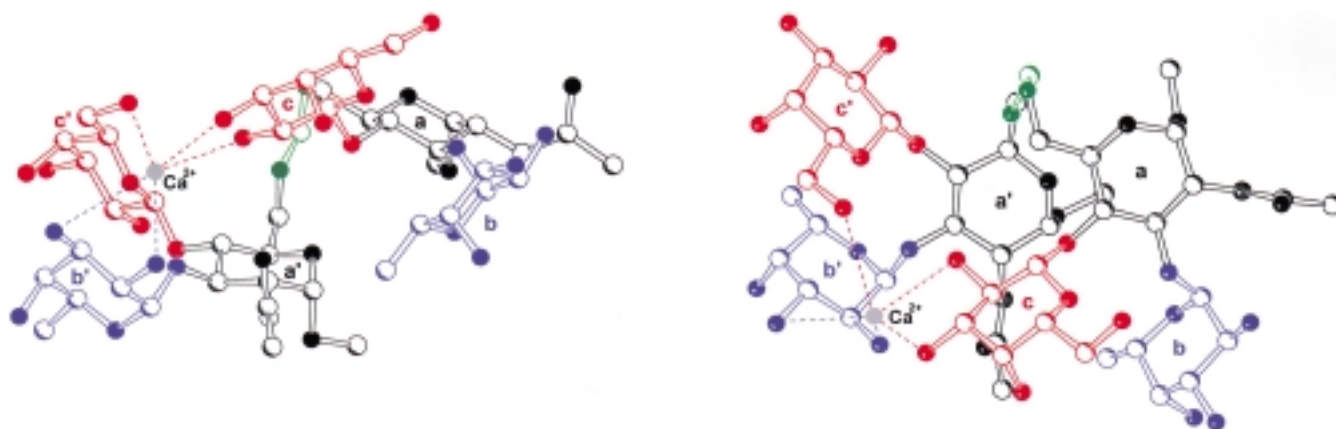


Figure 4. Solution conformation of the calcium complex of **4**. The Le^x trisaccharide populates one well-defined conformational minimum, as known from previous experimental studies.^[21] Torsional restraints kept the glycosidic torsions of fucose (b) and galactose (c) at this minimum in a molecular dynamics simulation of **4**. The rotational mobility about the covalent tether was not restricted and the experimental NOE values were included as weak additional restraints. The exocyclic torsion of *N*-acetylglucosamine (a) populates the *gauche*–*trans* rotamer in pyranose a and the *gauche*–*gauche* rotamer in ring a'. The methylene spacer linking the *N*-acetylglucosamines is shown in green. Rings a and a' are coplanar (left) and assume a cross-shaped relative orientation, which is more easily visible from a different direction (right). The position of the calcium ion is tentatively assigned at the hydrophilic contact site of the trisaccharide moieties of **4**.

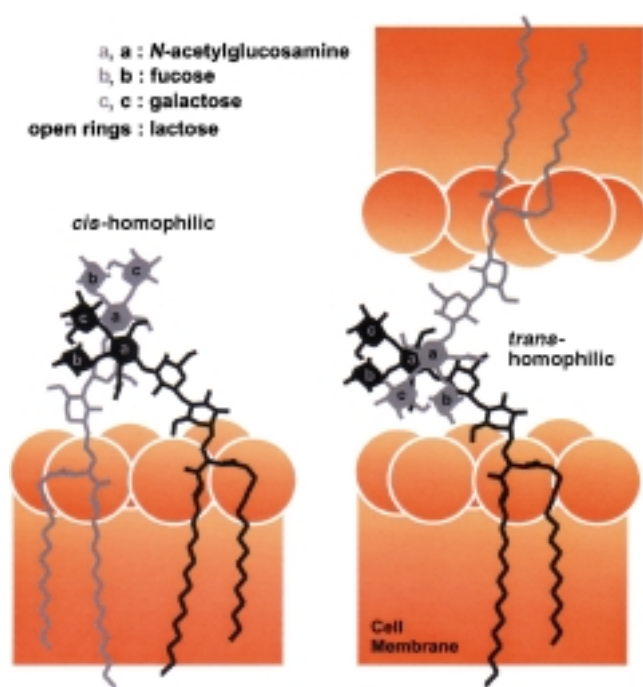
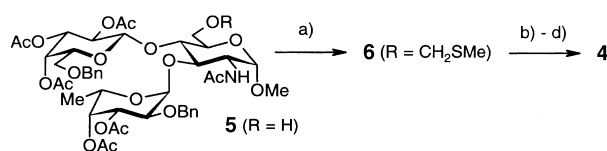


Figure 5. Two Le^x pentasaccharides (lactoses: open pyranose rings, Le^x trisaccharides: filled pyranose rings) are sketched with the relative orientation of the Le^x trisaccharide headgroups as determined from the calcium complex of **4**. The structures show the proposed Le^x–Le^x interaction between glycolipids which are anchored to the same membrane (*cis*-homophilic contact) or which are anchored to different membranes (*trans*-homophilic contact).

Experimental Section

Synthesis of **4**: The Le^x trisaccharide **5** (Scheme 2) was assembled from the monosaccharide building blocks using the trichloroacetimidate method.^[5b] This selectively deprotected trisaccharide was transformed into the thioacetal **6**. An iodonium-mediated reaction with **5** gave the dimer which was deprotected to **4**.



Scheme 2. a) Dimethyl sulfide, dibenzoylperoxide, 2,6-lutidine (89%); b) **5**, *N*-iodosuccinamide, trifluoromethanesulfonic acid, 4 Å molecular sieves, CH₂Cl₂ (58%); c) Pd/C, H₂; d) NaOMe, MeOH (90% over two steps).

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Solid-Phase Synthesis of Doubly Labeled Peptide Nucleic Acids as Probes for the Real-Time Detection of Hybridization**

Oliver Seitz*

The mutual recognition of two complementary nucleic acid strands is the molecular basis for most of the current approaches in oligonucleotide-based diagnostics and therapy. Much work has therefore been devoted to the development of methods that allow for detection of the hybridization event. The hybridization assays that are most commonly used employ a solid phase in order to facilitate the separation of bound from unbound analytes.^[1] In contrast, assays that proceed in homogeneous solution avoid problems such as nonspecific adsorption and the tedious washing protocols associated with heterogeneous assays.^[2] In addition, homogeneous assays can provide a means to perform real-time measurements in vitro and even in vivo.^[3] Conventional DNA-based probes, however, are susceptible to nucleolytic degradation which complicates their use in living cells. Herein, a method for real-time DNA measurements is presented that takes advantage of the enzyme-stable DNA-analogue peptide nucleic acid.^[4] It is demonstrated that the application of a highly orthogonal protecting-group strategy, in combination with chemoselective conjugation reactions, allows for the rapid and automatable solid-phase synthesis of doubly labeled PNA probes suitable for homogeneous DNA detection.

Peptide nucleic acids (PNAs) bind with a remarkably high affinity and selectivity to complementary nucleic acids.^[5] The structure of the resulting duplexes is similar to that of the corresponding DNA·DNA or DNA·RNA duplex molecules. However, there are striking differences between the structure of DNA and PNA single strands. For single-stranded PNA oligomers the analysis of the temperature-dependent UV absorbance reveals a sigmoidal melting curve.^[6] The observed hypochromicity can reach values up to 30%, which indicates that, in PNA single strands, base stacking is a favourable process. We reckoned that, due to this inter- or intramolecular association, a fluorescence-donor and a fluorescence-quencher group appended to the unhybridized PNA molecule **1** would be positioned in close proximity (Figure 1).^[7] In analogy to the DNA-based molecular beacons **2**, which were introduced by the pioneering work of Kramer and Tyagi, collisional quenching and the distance-dependent fluorescence resonance energy transfer (FRET) would diminish the fluorescence of the donor label.^[8a] Hybridization with complementary nucleic acids should induce a structural reorgan-

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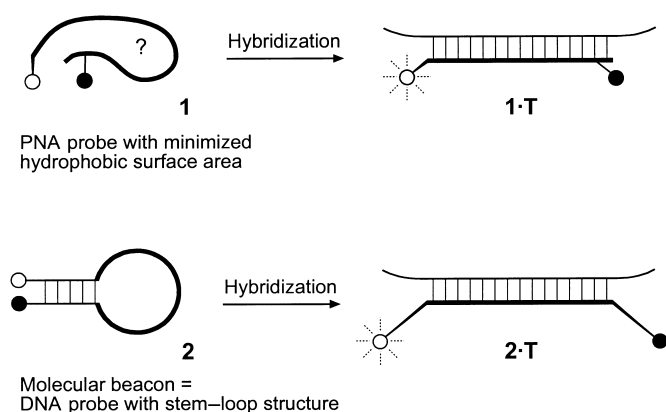


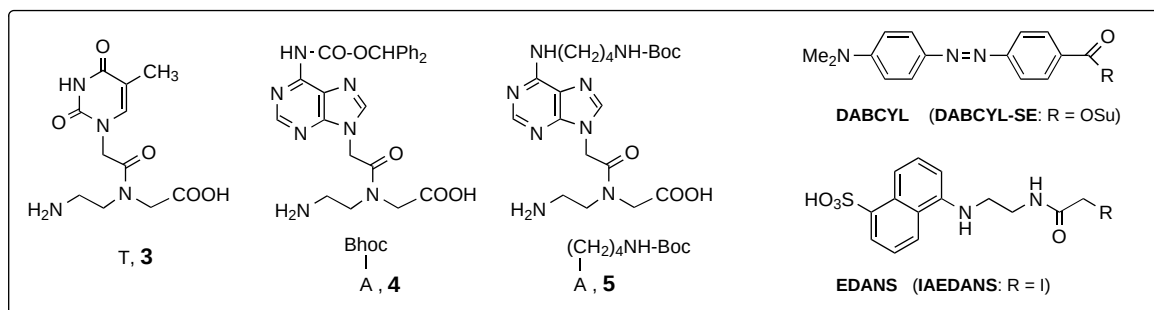
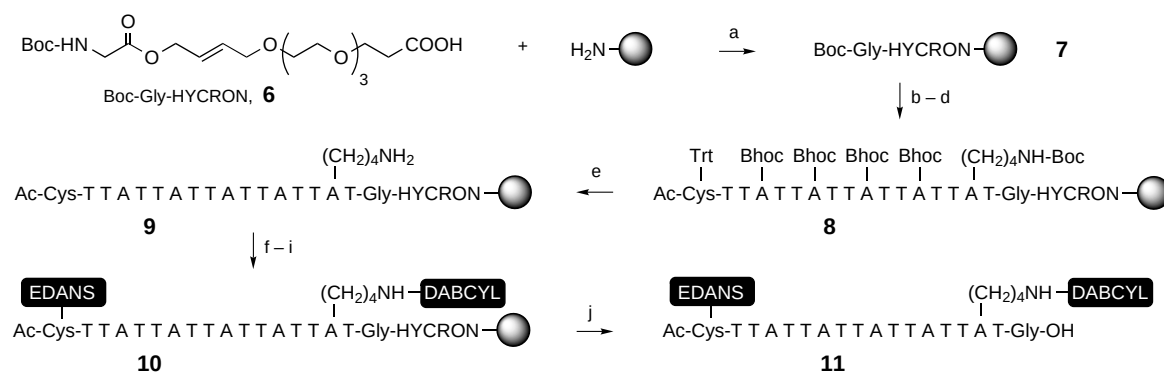
Figure 1. Unhybridized PNA forms intra- or intermolecular associates of unknown structure. The representation of the doubly labeled PNA probe **1** is intended to illustrate that appropriately appended fluorescence donor and fluorescence quencher groups could be located in close proximity. In analogy to the molecular beacons **2**, which are designed to form a stem-loop structure, the fluorescence is quenched due to collisional quenching and fluorescence resonance energy transfer (FRET). When the probe sequence anneals to the target sequence **T**, a structural reorganization increases the donor-quencher distance within the duplexes **1·T** and **2·T** and fluorescence can occur.

isation. In the double helical arrangement of duplexes **1·T** and **2·T** the distance between the donor and the quencher is increased and quenching processes become less likely to occur. As a result the doubly labeled probes become fluorescent upon hybridization.^[8b,c] A remarkable feature of

the PNA-based approach is that there is no need to incorporate secondary structure forming, but target-unrelated sequences.^[9, 10]

For the synthesis of doubly labeled PNA conjugates, a highly flexible and automatable strategy was desired, which would enable all reactions, including the labeling steps, to be performed on the solid phase. Given the unknown structure of unhybridized PNA, it was unclear how the hybridization-induced change of the distance between the donor and the quencher group could be maximized. A strategy which combines terminal and internal labeling offers a high degree of flexibility. By shifting the internal conjugation site throughout the sequence, the donor-quencher distance can be varied without changing the length of the oligomer. Since access to the C terminus can be hindered on solid phases, the preferred strategy was to attach one label to the N terminus and the second to an internal conjugation site that would be provided by the *N*⁶-aminoalkyl modified adenine building block **5**.^[11] The HYCRON anchor was chosen as a linker.^[12] Both acid- and base-labile protecting groups can be removed without detriment to the allylic ester linkage.^[13]

The solid-phase synthesis of the PNA conjugates **11** and **12** proceeded as outlined in Scheme 1. *N*-Fluoren-9-ylmethoxycarbonyl (Fmoc) protected monomers were used with the benzhydryloxycarbonyl (Bhoc) and the *tert*-butyloxycarbonyl (Boc) groups employed for permanent protection of the exocyclic amino groups of the adenine **4** and the aminoalkyl tether of the *N*⁶-modified adenine building block **5**, respec-



Scheme 1. a) 1. HBTU, HOBt, DIPEA, DMF; 2. Ac₂O:Pyr (1:10); b) 1. TFA; 2. DIPEA:DMF (1:9); c) iterative cycles of: 1. piperidine/DMF (1:4); 2. Fmoc-B^{Bhoc}-OH, HATU, *i*Pr₂NEt, Pyr, DMF; 3. Ac₂O, Pyr, DMF; d) 1. piperidine/DMF (1:4); 2. Ac₂O, Pyr, DMF; e) TFA:ethanedithiol:H₂O (95:2.5:2.5); f) DABCYL-SE (10 equiv), DMF:Pyr:NMM (7:1:1); g) piperidine/DMF (1:4); h) DTT (10 equiv), DMF:H₂O:NMM (9:3:1); i) IAEDANS (10 equiv), DMF:H₂O:NMM (9:3:1); j) [Pd(PPh₃)₄], morpholine, DMSO, DMF, 10% (based on **8**). DIPEA = *N*-Ethyl-*N,N*-diisopropylamine, DTT = dithiothreitol, HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazole[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate, HBTU = *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxy-benzotriazole, NMM = *N*-methylmorpholine.

tively.^[14] After completion of the iterative building-block coupling, the on-resin labeling was initiated. The *S*-trityl, the *N*-Boc, and all the Bhoc protecting groups were removed by treating the fully protected PNA **8** with a trifluoroacetic acid (TFA)/ethanedithiol/water mixture. The unprotected PNA oligomer remained attached to the HYCRON support.

The resin-bound PNA **9** was treated with the 4-(4'-dimethylaminophenylazo)benzoic acid hydroxysuccinimide ester (DABCYL-SE) in order to attach the DABCYL group to the primary amino group of the *N*⁶-aminoalkyl modified adenine. Any thioester that would have been formed by a concomitant acylation of the cysteine thiol groups was cleaved by subsequent treatment with DMF/piperidine. Unprotected thiol groups, however, readily form disulfides. Hence, a dithiothreitol reduction was performed in order to liberate the thiol groups, to which the 5-(2-aminoethylamino) 1-naphthalene sulfonic acid (EDANS) group was attached by a selective alkylation with 5-(2'-iodoacetamidoethyl)amino-naphthalene sulfonic acid (IAEDANS).

The final detachment was accomplished by subjecting the resin-bound PNA conjugate **10** to a Pd⁰-catalysed allyl transfer using the allyl scavenger morpholine. The subsequent reverse-phase HPLC purification furnished the PNA conjugate **11** and the positional isomer **12** in high purity. UV/Vis spectroscopy and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) and nanospray mass analyses confirmed the identity of the doubly labeled PNA probes **11** and **12**.^[15] It should be noted that, as the attachment of the EDANS and the DABCYL moieties conferred an increased hydrophobicity, the purification of the PNA conjugates was greatly facilitated.

It is known that the introduction of fluorescent labels affects the duplex stability, particularly when attached to internal nucleobases.^[16] However, the PNA backbone confers a substantial stabilization which might be able to compensate for the perturbation introduced by the labeling. Indeed, analysis of the temperature-dependent UV absorbance revealed melting temperatures of 30.5 °C for the **11**·**13** duplex and 28.5 °C for the **12**·**13** duplex, which compare well with the temperature of 30.4 °C for the unmodified DNA·DNA duplex.^[17]

Both conjugates, the doubly labeled PNA probes **11** and **12**, were used in hybridization experiments targeting the complementary oligonucleotide **13** and the sequence-unrelated oligonucleotide **14**. Figure 2 shows that the single-stranded PNA conjugates exhibited a quenched EDANS fluorescence. Addition of an excess of the mismatched oligonucleotide **14** led only to a minor change of the fluorescence spectrum (Figures 2 and 3). However, when a fourfold excess of the complementary oligonucleotide **13** was added at 25 °C, the fluorescence spectra changed dramatically. The emission of PNA probes **11** and **12** was enhanced by factors of 4.6 and 3, respectively. The intensification of the fluorescence was even more pronounced when the hybridizations were carried out at 20 °C, with enhancements by factors of 6.4 and 4.3, respectively. A spectral shift that could be indicative of a hydrophobicity-induced increase of the fluorescence was not detectable. It is thus likely that the mechanism by which the fluorescence increased followed the dequenching process that

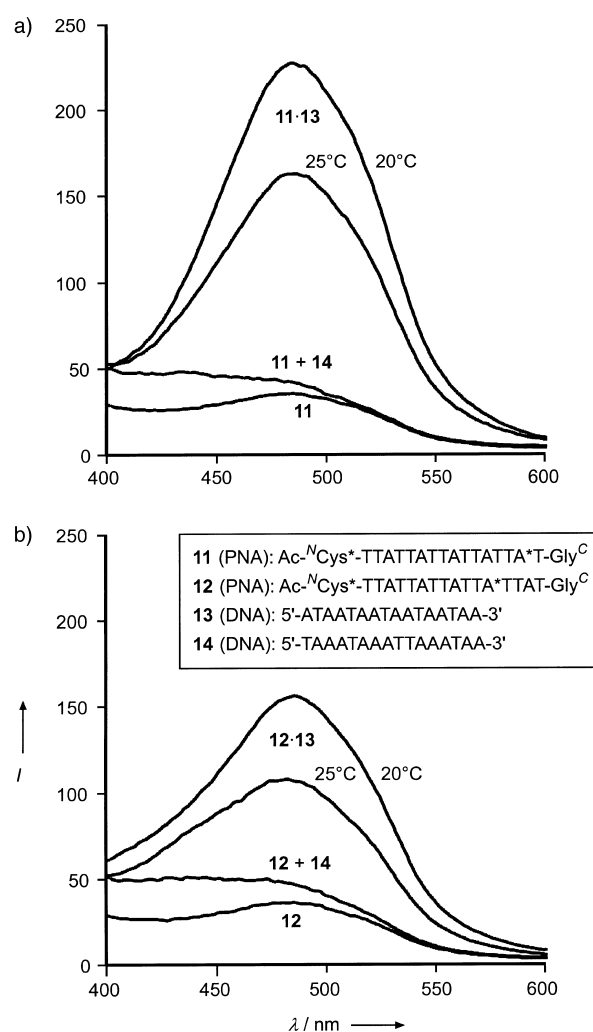


Figure 2. Normalized fluorescence spectra (arbitrary units) of a) the PNA probe **11** and b) the PNA probe **12**. The inset shows the oligomers used (Cys* = Cys(EDANS), A* = A(*N*⁶(CH₂)₄NH-DABCYL)). Measurement conditions: 100 mM NaCl, 10 mM NaH₂PO₄, 0.1 mM EDTA, pH 7, 298 K, 1 μM **11** or **12**, 4 μM **13** or **14** when added, excitation at 335 nm. The uppermost curves were measured at 1.5 μM probe concentration and 293 K. EDTA = ethylenediaminetetraacetate.

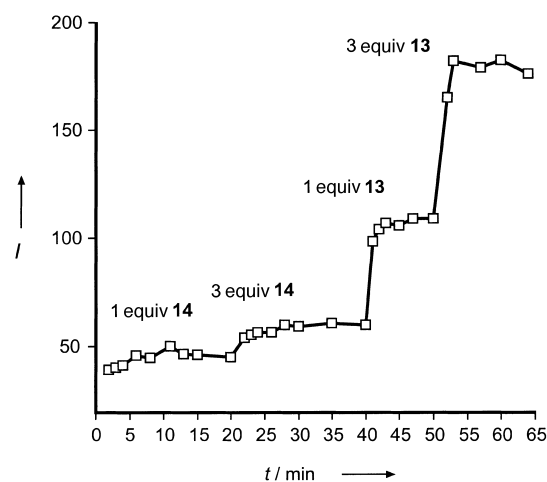


Figure 3. Fluorescence emission of probe **11** after addition of **14** (1 equiv, 0 min), **14** (3 equiv, 21 min), **13** (1 equiv, 40 min), and **13** (3 equiv, 51 min). Measurement conditions: 100 mM NaCl, 10 mM NaH₂PO₄, 0.1 mM EDTA, pH 7, 298 K, 1 μM of **11**, excitation at 335 nm.

is observed for equally labeled molecular beacons. Analogous to molecular beacons, preparatory denaturation or renaturation procedures were not required and the probes began to fluoresce almost immediately after addition of the target oligonucleotide. As illustrated in Figure 3 the fluorescence of PNA probe **11** reached a plateau five minutes thereafter.

It was shown that the combined use of orthogonal protecting-group techniques and chemoselective conjugations allows for the rapid solid-phase synthesis of doubly labeled PNA probes. The PNA conjugates were demonstrated to be only weakly fluorescing in the single-stranded state. Hybridization of the weakly fluorescing PNA probes to a complementary oligonucleotide conferred a vivid fluorescence enhancement providing a means for homogeneous DNA detection. It has to be emphasized that target-unrelated arm sequences were not required for maintaining the structural integrity of the probes. The observed fluorescence quenching, however, might depend on both the sequence and the site of labeling. Future studies will reveal whether the fluorescence increase can be generalized to any sequence. Applications such as real-time polymerase chain reaction monitoring and real-time RNA detection in living cells could be feasible and benefit from the increased biostability of the PNA-based hybridization probes.

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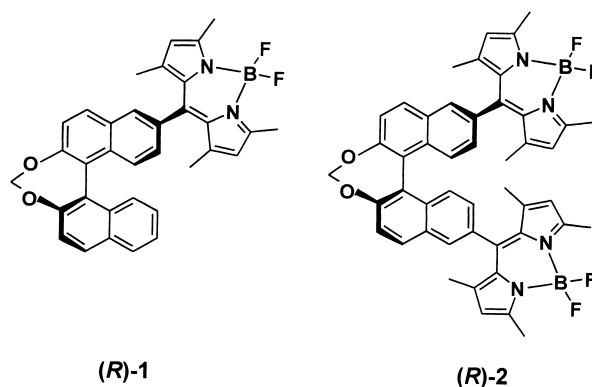
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Redox Switches with Chiroptical Signal Expression Based on Binaphthyl Boron Dipyrromethene Conjugates**

Gerhard Beer, Christoph Niederalt, Stefan Grimme,* and Jörg Daub*

The interest in molecular building blocks with chiral and optoelectronic properties is driven on one hand by the higher specificity in molecular recognition and on the other by the polarization of electromagnetic radiation or light. Herein we report the synthesis and properties of optically active binaphthyl boron dipyrromethene (BDP) conjugates. Because of their favorable absorption and emission properties BDP dyes are frequently used as fluorescent probes for proton or metal ion detection,^[1] in light-harvesting complexes within artificial photosynthetic arrays,^[2] or as laser dyes.^[3] It is also known that BDP dyes with appropriate functionalization can be reversibly oxidized and reduced.^[1a, 4] Herein we present the optically active BDP derivatives (*R*)-**1** and (*R*)-**2** which were obtained by derivatization of a chiral 1,1'-



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binaphthyl system. The compounds were investigated with regard to their circular dichroism, their emission properties, their electrochemical behavior, and their potential use as chiroptic redox switches.

The compounds (*R*)-1 and (*R*)-2 were synthesized from enantiomerically pure 1,1'-binaphthyl carbaldehyde precursors and 2,4-dimethylpyrrole.^[4b, 5]

The UV/Vis absorption spectra of (*R*)-1 and (*R*)-2 show the characteristic intense absorption bands of the BDP chromophore ((*R*)-1: $\lambda = 497$ nm, $\epsilon = 65900$ L mol⁻¹ cm⁻¹; (*R*)-2: $\lambda = 497$ nm, $\epsilon = 132700$ L mol⁻¹ cm⁻¹) in the long-wavelength region, whereas in the short-wavelength region transitions of the binaphthyl chromophore dominate. The emission spectra of (*R*)-1 and (*R*)-2 are characterized by BDP fluorescence with maxima at $\lambda = 507$ nm ($\Phi_f = 0.79$) and $\lambda = 509$ nm ($\Phi_f = 0.69$), respectively.^[6] Investigation of the electrochemically generated luminescence (ECL)^[7] of (*R*)-2 shows a bathochromically shifted emission maximum at 546 nm. The circular dichroism (CD; Figure 1) for the long-wavelength absorption of the BDP chromophore in the

phore, are calculated at $\lambda = 420$ nm and $\lambda = 418$ nm (0.45 eV blue-shifted with respect to the experimental bands) with large rotatory strengths (*R*) of 976×10^{-40} and -886×10^{-40} cgs, respectively (Figure 2). This is in agreement with

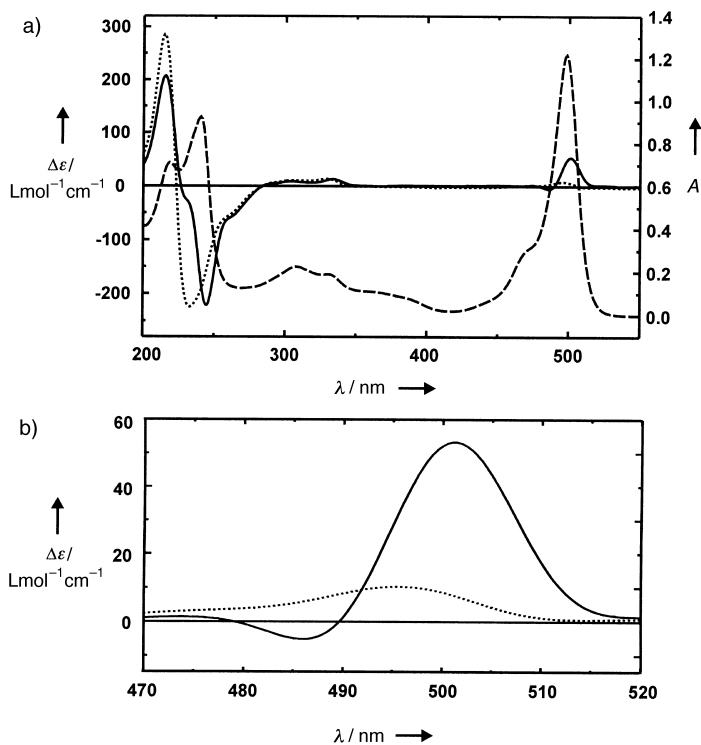


Figure 1. a) CD spectra of (*R*)-1 (dotted line) and (*R*)-2 (solid line), UV/Vis absorption spectrum of (*R*)-2 (dashed line) in acetonitrile; b) the BDP absorption region of the CD spectra of (*R*)-1 (dotted line) and (*R*)-2 (solid line).

monosubstituted-BDP compound (*R*)-1 shows a positive CD effect ($\lambda = 495$ nm, $\Delta\epsilon_{\max} = +10$ L mol⁻¹ cm⁻¹) and in the disubstituted-BDP compound ((*R*)-2) a positive, strong asymmetric couplet ($\lambda = 501$ nm, $\Delta\epsilon_{\max} = 53$ L mol⁻¹ cm⁻¹; $\lambda = 485$ nm, $\Delta\epsilon_{\max} = -5$ L mol⁻¹ cm⁻¹). The first excited states of (*R*)-1, (*R*)-2, and of several model systems were investigated by means of quantum-chemical calculations.^[8] The first transitions of the di-BDP-substituted compound (*R*)-2, which correspond to the symmetrical and antisymmetrical combination of the long-wavelength transition of the BDP chromo-

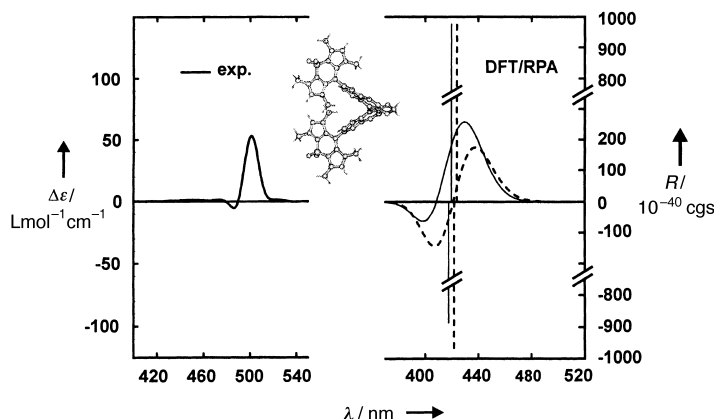


Figure 2. Comparison of the calculated absorption wavelengths, and the rotatory strengths *R* for the first transitions of (*R*)-2 (solid line, right) with results for the model system B (dashed line) and the experimental bands (left). The simulated CD spectra were obtained by the addition of Gaussian curves with weighted calculated rotatory strengths and for which a half width of 0.25 eV was employed. In model B the two BDP chromophores are considered to be in the same arrangement as in (*R*)-2 but without the binaphthyl skeleton.

an exciton-coupled CD signal^[9] which can be expected because the large electrical transition-dipole-moment of the BDP chromophore is polarized along the long molecular axis.^[10] However, because of the large distance between the two BDP chromophores the two transitions of the couplet are only slightly split (0.014 eV); thus the rotatory strength of the two transitions cancel each other out to a large extent. The measurable Cotton effects are therefore relatively small and a weak, strongly asymmetric, rather atypical exciton couplet results.^[11] In model system B the binaphthyl skeleton was removed from the structure of (*R*)-2, and the resulting free valences were saturated with H atoms at a standard distance (1.09 Å). For this model system approximately equal rotatory strengths were obtained for the first two transitions ($R = -966 \times 10^{-40}$, $R = 986 \times 10^{-40}$ cgs). Thus, the asymmetry of the long-wavelength couplet of (*R*)-2 is predominantly caused by interactions with the binaphthyl skeleton. For the mono-BDP-substituted compound (*R*)-1 the rotatory strength of the first transition, which is localized at the BDP chromophore, is calculated to be $+29 \times 10^{-40}$ cgs (Figure 3). To examine the origin of the Cotton effect of this transition, calculations were carried out for two model systems. In model system M^I the OCH₂O bridge and the naphthyl ring, which is not bound directly to the BDP chromophore (ring II), were removed from the calculated structure of (*R*)-1, while in model system M^{II} the OCH₂O bridge and the naphthyl ring bound directly to the BDP chromophore (ring I) were removed. The resulting free valences were saturated with H atoms at a standard distance (1.09 Å). For M^I a rotatory strength of only $R = 2 \times 10^{-40}$ cgs was obtained for the first transition, whereas for M^{II} a rotatory strength of 15×10^{-40} cgs was obtained. The Cotton effect of the first transition of (*R*)-1 thus seems to be caused

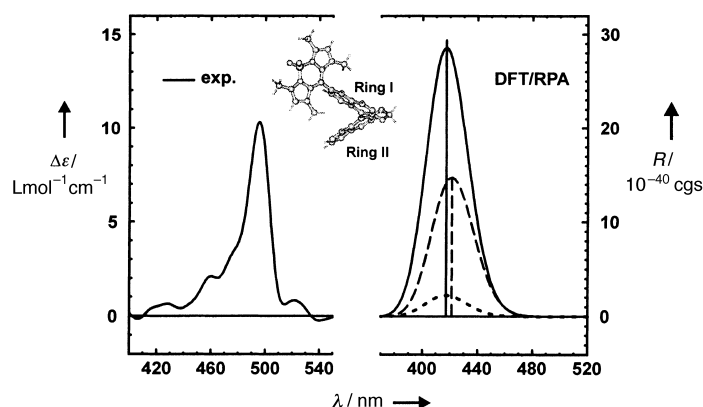


Figure 3. Comparison of the calculated absorption wavelengths and the rotatory strengths R for the first transition of (R) -**1** (solid line, right) with results for the model systems M^I (dotted line) and M^{II} (dashed line) and the experimental band (left). In the model systems M^I and M^{II} the BDP chromophore is, in each case, considered together with only one naphthyl ring of the binaphthyl skeleton (model M^I contains ring I, model M^{II} contains ring II).

by the interaction between the electric transition-dipole-moment of the long-wavelength transition of the BDP chromophore and electric dipole moment in ring II induced by this dipole. These results are also in agreement with a sector rule, which was derived from consideration of the interaction between the electric transition-dipole-moment of a chromophore with neighboring groups.^[12] The intense long-wavelength transition allows the potential of the optically active BDP compounds as electrochemically controlled chiroptical switches to be tested. The redox behavior of (R) -**1** and (R) -**2** is most strongly influenced by the BDP chromophore, because the binaphthyl skeleton is electrochemically inactive. Oxidation of the monosubstituted compound (R) -**1** occurs at a half-wave potential of $E_{1/2} = 785$ mV (versus the ferrocenium ion/ferrocene couple) and is quasi-reversible. The reversible reduction cycle at $E_{1/2} = -1515$ mV is stable over several cycles in a thin-layer experiment. Analogous behavior is found for the disubstituted compound (R) -**2** with a quasi-reversible oxidation at $E_{1/2} = +795$ mV and a reversible reduction at $E_{1/2} = -1510$ mV. Thus, one electron is transferred per chromophore, which means overall two electrons are transferred to (R) -**2**. Through CD spectroelectrochemistry^[13] and taking (R) -**2** as an example we have shown the application of these compounds as molecular “chiroelectro” switches^[14]. UV/Vis-spectroelectrochemistry reveals that the absorption band at 495 nm disappears upon reduction of the neutral compound to the bisradical dianion and a weak radical absorption band with a maximum at 561 nm appears instead (Figure 4). In analogy to these results CD-spectroelectrochemical measurements show a decrease in the intensity of the Cotton effect at 501 nm with continuous increase of the reductive potential, and finally its total disappearance after the solution is completely electrolyzed (Figure 5). Detection of the radical-anion band in the CD spectrum is not possible. The shape of the initial CD spectrum is restored completely after reoxidation: thus the CD signal at 501 nm can be switched on and off electrochemically (Figure 5). In analogy to these results identical behavior can be

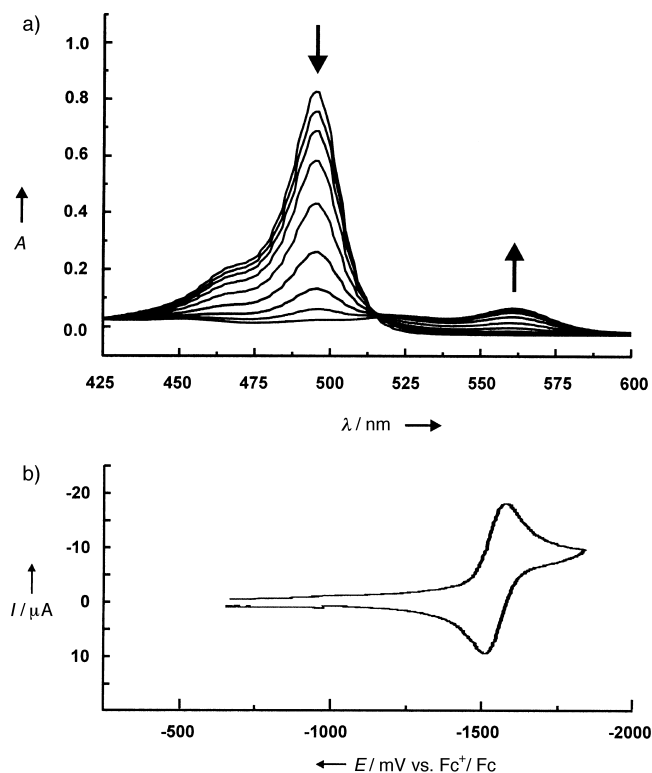


Figure 4. a) UV/Vis-spectroelectrochemical investigation of the reversible reduction (R) -**2** $\rightleftharpoons$ (R) -**2**²⁻ in acetonitrile; b) cyclic voltammogram of the reduction.

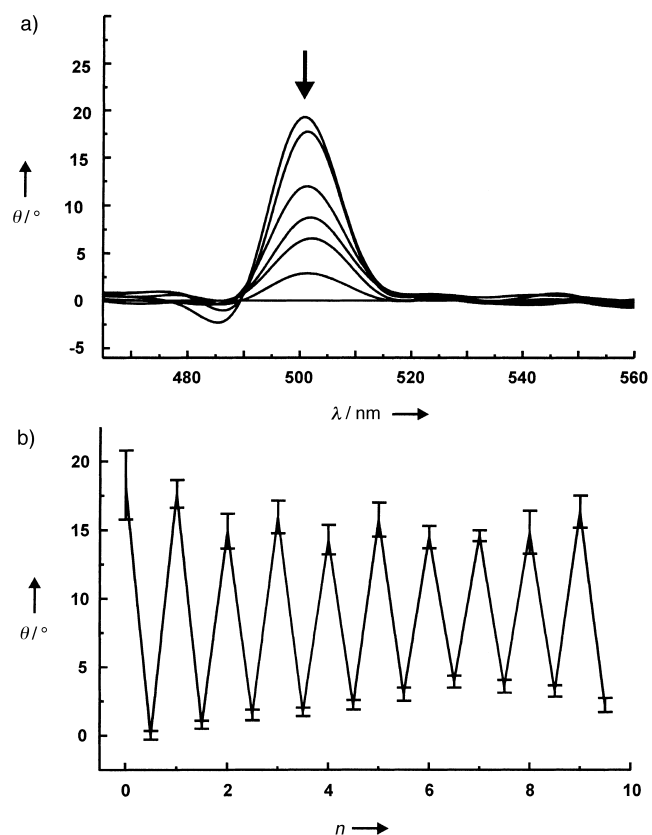


Figure 5. a) CD-spectroelectrochemical investigation of the reversible reduction (R) -**2** $\rightleftharpoons$ (R) -**2**²⁻; b) variation of the CD signal intensity at $\lambda = 501$ nm with repeated switching between the neutral compound and the reduced form over ten cycles (n = number of cycles).

observed for the monosubstituted compound (*R*)-**1**; however, in the CD spectrum the intensity of the signal is less than that of (*R*)-**2**. Thus, the presented compounds combine optoelectronic properties and chirality. These results are of relevance in several applications: a) chiral fluorophores within sensor systems,^[15] b) circular-polarized photoluminescence^[16] and also electrochemiluminescence, c) organic light-emitting diodes (OLED) with circular polarized luminescence,^[17] and d) light-emitting diodes (LED) powered by spin-polarized carriers.^[18]

Experimental Section

Cyclic voltammetry: solvent acetonitrile; potentials [mV] versus ferrocenium/ferrocene (Fc⁺/Fc) as internal standard; reversible half wave potential $E_{1/2}$. Measurement conditions: room temperature, scan speed 250 mV s⁻¹, working electrode: platinum-disc electrode, quasi reference electrode: Ag/AgCl, counter electrode: platinum electrode, supporting electrolyte: 0.1 M tetrabutylammonium hexafluorophosphate.

UV/Vis and CD spectroelectrochemistry: solvent acetonitrile, transmission cell with minigrid gold working electrode.

Optical spectra: concentration 10⁻⁵ to 10⁻⁶ M in acetonitrile.

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Allosteric Regulation of Artificial Phosphoesterase Activity by Metal Ions**

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In enzyme catalysis allosteric regulation is the control of enzyme activity by noncovalent modifiers (molecules or ions) which bind to the enzyme at a site other than the active site but alter the conformation of the active site. Often, metal ions are involved in allosteric regulation in enzymes. In the active site of *E. coli* alkaline phosphatase a phosphate monoester substrate is hydrolyzed at a dizinc(II) site, while a “structure-stabilizing” Mg²⁺ ion located about 6 Å away from the zinc ions is a strong allosteric activator.^[1] Replacement of Mg²⁺ by

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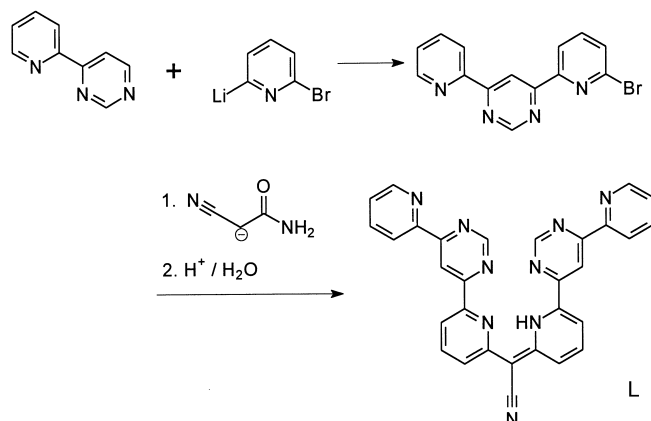
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other divalent metal centers alters or even inhibits the catalytic activity of natural alkaline phosphatases^[2] or their mutants.^[3]

For artificial systems allosteric tuning of the receptor properties by metal ions or organic molecules has been widely explored in supramolecular host–guest chemistry.^[4] However, reports on allosteric regulation of synthetic molecular catalysts are very rare. On the basis of kinetic data allostery was proposed for cyclodextrin-catalyzed ester and alkyl nitrite hydrolysis which is enhanced by the addition of certain organic molecules.^[5]

Herein we describe allosteric regulation of a synthetic metal-based catalyst for phosphodiester cleavage. In our system two functional metal ions (denoted M_f) are exposed for direct interaction with the substrate while reactivity is modulated by a third “structural” metal (M_s).

The novel polypyridyl ligand **L** which has one tetradentate and two bidentate metal binding sites was prepared in two steps from 4-(2-pyridyl)pyrimidine (Scheme 1).^[6] The carbon–carbon coupling with 2-bromo-6-lithiopyridine gave 4-(6-bromo-2-pyridyl)-6-(2-pyridyl)pyrimidine. By using



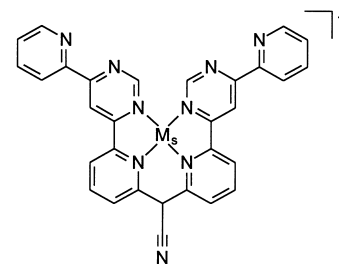
Scheme 1. Synthesis of **L**.

a method for the preparation of macrocycles from bromopyridines and cyanacetamide^[7] **L** was obtained as a dark red crystalline solid. The single-crystal X-ray data^[8] shows that **L** exists in a fully conjugated form in which a proton is localized at the nitrogen atom of an internal pyridine unit.

Reaction of M^{2+} salts with **L** gave 1:1 complexes $[(L-H)Cu]NO_3$, $[(L-H)Ni]NO_3$, and $[(L-H)Pd]ClO_4$ as purple microcrystalline solids which were identified by $[(L-H)M]^+$ patterns in matrix-assisted laser desorption ionization (MALDI) mass spectra and $C_8H_8N_8$ analyses ($L-H$ (L “minus” H) corresponds to the deprotonated form of the ligand). The EPR spectrum of $[(L-H)Cu]^+$ in a $CHCl_3$ /methanol glass at 77 K indicates a planar N_4 coordination of the Cu center ($g_{||} = 2.207$, $A_{||} = 196$ G). The ^1H NMR spectrum of the square-

planar Pd complex suggests C_2 symmetry. In the ^1H NMR spectrum of $[(L-H)Ni]^+$ in $[D_6]DMSO$ paramagnetic shifts ($\Delta\delta = 6–90$) relative to the signals of the free ligand and extensive line broadening for five ^1H signals (coordinated pyridine (py) and pyrimidine (pym)) are observed, while four signals (uncoordinated py) are shifted $\Delta\delta < 2$ ppm. Clearly, octahedral Ni^{II} , with axial solvent coordination, is favored in solution over a diamagnetic square-planar structure.

The high stability of the mononuclear complexes in solution is demonstrated by photometry: strong chelating ligands like ethylenediamine and ethylenediaminetetraacetate (EDTA) in 20-fold excess (at pH 8) do not extract the metal ions from the complexes. The formation of trinuclear complexes $[(L-H)M_sCu_2]^{5+}$ was studied by the photometric titration of $[(L-H)-M_s]^+$ with copper(II) nitrate solution. We carried out the titrations under the same conditions as the kinetic experiments (see below), but 2-(hydroxypropyl)-*p*-nitrophenylphosphate (HPNP) was replaced by more stable dimethylphosphate. The coordination of Cu^{II} to the bidentate sites of **L** is monitored by a decrease of the absorbance at 500 nm and an increase of the absorbance at 635 nm (Figure 1) for which sharp isosbestic



$[(L-H)M_s]^+$ $M_s = Cu^{II}, Ni^{II}, Pd^{II}$

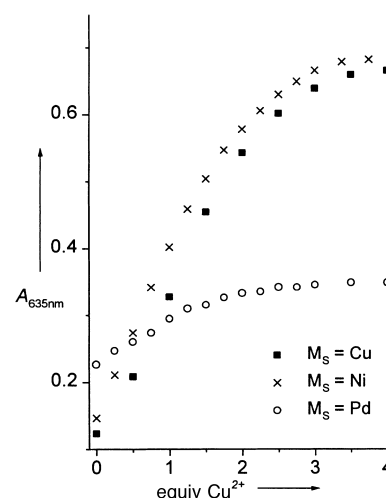


Figure 1. Increase of the absorbance at 635 nm on photometric titration of $[(L-H)M_s]^+$ ($10^{-4} M$) with copper(II) nitrate solution. Water:DMSO 3:1, pH 7.0, $5 \times 10^{-4} M$ sodium dimethylphosphate, buffer 5 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), $T = 20^\circ C$.

points are seen. The color of the solutions changes from purple to dark blue, for $M_s = Cu, Ni$, or to bluish-purple for $M_s = Pd$. The bathochromic shift of λ_{max} for this Pd complex and therefore the increase of the absorbance at 635 nm (Figure 1) are less significant than for the other complexes.

From their intensities ($\epsilon = 6000–9000 M^{-1} cm^{-1}$) we conclude that optical bands of the complexes are ligand-centered

$\pi-\pi^*$ transitions, while metal d-d transitions make only a minor contribution to the band intensity. Less efficient complexation of Cu by the bidentate sites of L is readily evident since excess Cu is required for the quantitative formation of $[(L-H)M_sCu_2]^{5+}$ (ca. 4 equiv). Under electrospray MS ionization conditions labile Cu ions dissociate from the complex and intense $[(L-H)M_s]^+$ peaks are observed.

However, minor patterns for polynuclear species $[(L-H)Cu_2(CH_3CO_2)]^{2+}$ and $[(L-H)Cu_3(CH_3CO_2)_3]^{2+}$ are seen in the spectrum of an aqueous solution containing $[(L-H)Cu]NO_3$ and 10 equivalents copper(II) acetate.

Reaction of $[(L-H)Cu]NO_3$ with two equivalents of copper(II) perchlorate and KH_2PO_4 gave a dark blue microcrystalline complex with the correct elemental analysis for $[(L-H)Cu_3(PO_4)](ClO_4)_2$.

We have explored the possibility that in complexes $[(L-H)NiCu_2]^{5+}$ and $[(L-H)PdCu_2]^{5+}$ Cu may substitute Ni and Pd in the tetradentate site. Solutions containing $[(L-H)M_s]^+$ ($M_s = Ni, Pd$) and four equivalents of Cu^{II} (for conditions see caption to Figure 1) were kept at 20 °C for 15 min and then subjected to MALDI mass spectrometry. Since we found strong patterns corresponding to $[(L-H)Ni]^+$ and $[(L-H)Pd]^+$ but only trace (<1%) $[(L-H)Cu]^+$ patterns, metal exchange at the tetradentate site is negligible for short reaction times. Control experiments with mixtures of freshly prepared solutions of $[(L-H)M_s]^+$ ($M = Ni, Pd$) + 4 Cu and $[(L-H)Cu]^+$ + 4 Cu gave patterns corresponding to $[(L-H)M_s]^+$ and $[(L-H)Cu]^+$ in the expected ratio.

Reactivity studies were performed with the RNA analogue HPNP which is widely used to investigate the phosphoesterase activity of metal complexes. The intramolecular cyclization of this phosphodiester (Scheme 2) can be easily monitored photometrically by the increase in the absorbance at 400 nm, which corresponds to released nitrophenolate. Kinetic studies were performed in a buffered water:DMSO (3:1) mixture (pH 7.0, complex 10^{-4} M, HPNP 5×10^{-4} M). Cleavage activity of the mononuclear complexes is very low ($k_{obs} < 2 \times 10^{-6} s^{-1}$) because the Pd complex is coordinatively saturated and interaction of the substrate with the axial coordination

sites (for Ni and possibly even Cu complexes) provides, at best, modest activation.

The cleavage rate increases dramatically when Cu^{2+} ions are added to the $[(L-H)M_s]^+$ solution (Figure 2). In agreement^[9] with photometric results (Figure 1) more than two equivalents of Cu are required for quantitative formation of

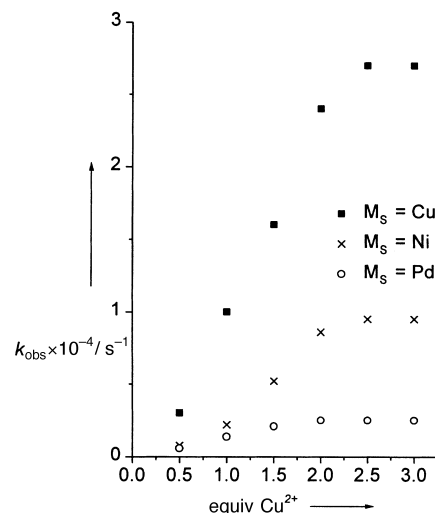
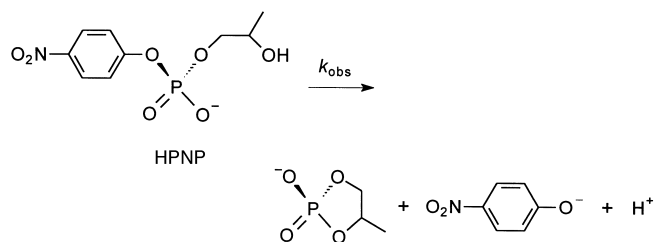


Figure 2. Values of k_{obs} for the cleavage of HPNP (5×10^{-4} M) by $[(L-H)M_s]^+$ (10^{-4} M) at varying copper(II) nitrate concentrations derived from initial reaction rates for reaction times of < 15 min. Water:DMSO 3:1, pH 7.0, buffer 5 MOPS, $T = 20^\circ C$. Average values of three kinetic runs, that were reproducible to within 15%.

$[(L-H)M_sCu_2]^{5+}$ and for the maximum level of activity to be achieved. Many examples for the efficient cleavage of phosphate esters by the cooperation of two metal ions in phosphoesterase model complexes have been reported.^[10] In its reactivity toward HPNP our system with two functional Cu ions is comparable to other artificial dicopper(II) phosphoesterases.^[11]

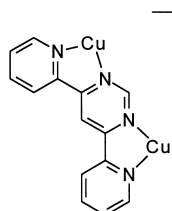
The kinetics of in situ prepared $[(L-H)Cu_3]^{5+}$ solutions were explored in more detail. The pH rate profile has a maximum at pH 7, the reaction is first order in complex concentration (range $0.5-3 \times 10^{-4}$ M) and first order in HPNP (substrate) concentration (range $10^{-4}-10^{-3}$ M). At least two catalytic turnovers without loss of activity were detected for a HPNP concentration of 2.5 mM and a complex concentration of 10^{-4} M. Addition of one equivalent of PO_4^{3-} per complex completely inhibits HPNP cleavage, whereas the addition of five equivalents of $NaClO_4$ or $NaNO_3$ has no effect on k_{obs} .

Remarkably, the reactivity of $[(L-H)M_sCu_2]^{5+}$ complexes toward HPNP depends strongly on the structural metal M_s . The complex $[(L-H)NiCu_2]^{5+}$ is three times more reactive and $[(L-H)Cu_3]^{5+}$ is ten times more reactive than $[(L-H)PdCu_2]^{5+}$. In our view this must be a consequence of differences in catalyst conformation and thus in the preorganization of functional copper ions, depending on the identity of the structural metal ions M_s . Even subtle differences in the ionic radius of M_s and in its tendency to distort the N_4M_s coordination plane are expected to have substantial effects on both the M_f-M_f distance and the ligand conformation. A



Scheme 2. Intramolecular cleavage of the phosphodiester 2-(hydroxypropyl)-*p*-nitrophenylphosphate (HPNP).

direct contribution of the structural metal M_s to Cu-mediated catalysis, and even hydrogen bonding of the phosphate oxygen atoms of a Cu-coordinated phosphate with axial M_s-H_2O , appear sterically impossible from a ball-and-stick model. Also, efficient cooperation of two metals across the bridging pyrimidine is ruled out by experiment: with the dicopper(II) complex of 4,6-di(2-pyridyl)pyrimidine, $[(dppm)Cu_2]^{4+}$, k_{obs} for HPNP cleavage is only $8 \times 10^{-6} s^{-1}$



$[(dppm)Cu_2]^{4+}$

(for conditions see caption from Figure 2).

Our system may be considered as a prototype of a synthetic allosteric catalyst having well defined catalytic and allosteric subunits, although it does not mimic the typical case of enzyme regulation in which a metal ion reversibly binds to the allosteric site as an external modifier. We see, however, certain parallels between our system and alkaline phosphatase enzymes in which the replacement of a structural Mg^{2+} ion by other divalent metal ions strongly influences the catalytic activity mediated by two functional Zn^{2+} ions.^[2,3] Another remarkable aspect is the possibility of fine-tuning the preorganization of the two functional metal ions M_i by variation of the structural metal M_s . This allows the systematic investigation of metal-metal cooperation in various reactions for a range of M_i-M_i separations and relative orientations of the M_i coordination polyhedra. Other approaches to this problem have been described but require laborious syntheses of a series of binucleating ligands with different spacers.^[12]

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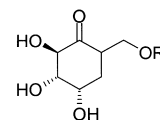
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Hexacyclinic acid, a Polyketide from *Streptomyces* with a Novel Carbon Skeleton**

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As shown in former investigations, *Streptomyces cellulosa* subsp. *griseorubiginosus* (strain S1013) only produces the carbasugars gabosine D (**1**) and E (**2**) when cultivated in Erlenmeyer flasks with a rolled oats medium.^[1] Applying the OSMAC approach (OSMAC = one strain/many compounds)^[2] in combination with the well established chemical screening method,^[3] we intended to examine whether the production of new metabolites can be induced in this strain.



1: R = H
2: R = COCH₃

The OSMAC approach is based on the observation that individual strains are able to produce more metabolites than normally detected in a routine screening program. So it is easy to discover new natural products even when cultivating well-examined strains within the group of actinomycetes or fungi, with the aim being to induce or promote the biosynthesis of metabolites by variation of the cultivation parameters. According to this, one can discuss whether the detection of a new metabolite depends only on an increased production rate of a former, not previously provable, substance or if the natural product is formed for the first time. Within the OSMAC approach, the cultivation medium and culture vessel can be varied effectively, besides the temperature, aeration, pH value, and/or light intensity. C and N sources, as well as the addition of inorganic salts, enzyme inhibitors, or adsorbent materials, play an important role as part of the media. The intention of the OSMAC approach is the generation of

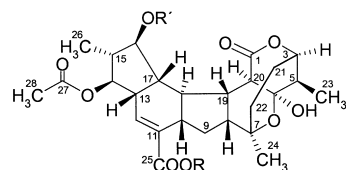
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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

variability concerning the metabolite formation and chemical diversity with regard to the carbon skeleton of the substances. Under varied conditions, some of our talented strains formed up to 50 secondary metabolites, whereby the number of new compounds was comparatively high.^[2] The OSMAC approach proves to be very efficient while searching for new metabolites.^[4] It is based on experience and finds its rationality in the fact that the genome of actinomycetes (approximately 8 Mb; 1 Mb = 10⁶ bases), as well as that of talented fungi (approximately 30 Mb), is much larger than corresponds to the basic requirements of these organisms.^[5] Thus, the silent biosynthetic genes, which can be activated, constitute the potential of this approach.

Following the OSMAC approach, our strain S1013 has been cultivated in different culture vessels, using a rolled oats medium. In the culture filtrate extract from a 10L fermentor we detected, by thin layer chromatography, an additional metabolite besides **1** and **2**. It gave an intensive blue color with anisaldehyde/H₂SO₄ as the staining reagent (*R*_f = 0.30, silica gel, chloroform/methanol 9/1), but its yield was not high enough for structure elucidation. Fermentation optimization by addition of different alkali halides resulted in strikingly increased yields (by a factor of 10–20) of this metabolite in the presence of 1 g L⁻¹ of sodium bromide. This effect did not occur with lower or higher concentrations of the salt or by adding other salts. In different culture vessels the yield was up to 13 mg L⁻¹; with additional variation of the C source it rose to as much as 56 mg L⁻¹. Column chromatography on silica gel (chloroform/methanol 7/3 and 9/1) led to the isolation, in a



- 3**: R = H, R' = H
4: R = CH₃, R' = H
5: R = CH₃, R' = (S)-MTPA
6: R = CH₃, R' = (R)-MTPA

3 shows the molecular ion at *m/z* 508 [*M*+NH₄]⁺. The molecular formula C₂₆H₃₄O₉ results from a high resolution (HR) EI mass spectrum (*m/z* 490). The ¹H NMR spectrum of **3** is characterized by a complex aliphatic region which can be differentiated with [D₅]pyridine to a large extent. The ¹³C NMR spectrum exhibits 26 signals, two of them characterize a double bond and three can be attributed to carbonyl groups. The calculated double-bond equivalents derive from the molecular formula in combination with the ¹³C NMR data and require cyclizations leading to a hexacyclic core structure of **3**. With the aid of several two-dimensional NMR spectra (COSY, HSQC, HMBC) the structures of two fragments could be established: the left five-membered ring with the acetoxy group and the right tricyclic ring system. The fragments could not be connected unequivocally because of overlapping proton signals in this section of the molecule and because of HMBC correlations whose ²*J*_{CH} character could not be clearly defined. Also, the use of calculation programs like COCON leads to more than one plausible proposal for

a reasonable quantity, of the new metabolite, which was named hexacyclenic acid after its structure, **3**.^[6]

Compound **3** crystallizes from methanol/water and is readily soluble in organic solvents. The direct chemical ionization (DCI) mass spectrum of

the structure of **3**.^[7] Finally, elucidation of the structure including the relative configuration was done by X-ray analysis of **3** (Figure 1).^[8] NOESY NMR spectra, in connection with the other 2D NMR data, enabled the unambiguous assignment of all ¹H and ¹³C NMR signals.

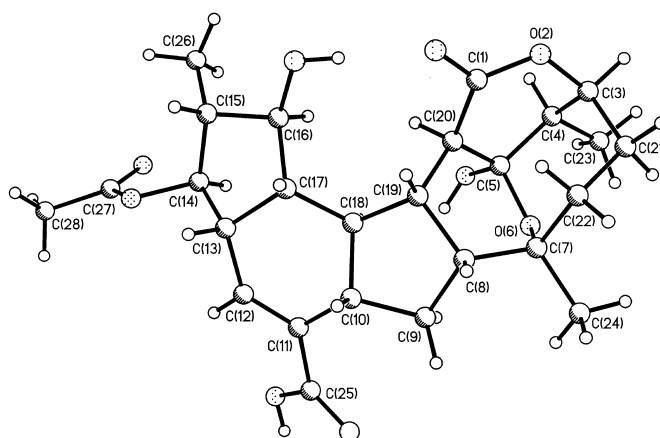


Figure 1. Crystal structure of hexacyclenic acid (**3**) with crystallographic numbering pattern. Only the relative configuration resulted from the X-ray analysis. The given absolute configuration was determined using Mosher's ester methodology.

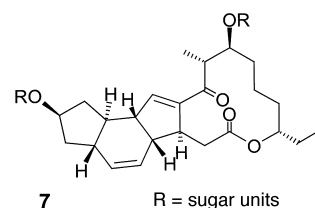
The absolute configuration of **3** was evaluated by performing the advanced Mosher's ester methodology. Therefore, we transformed the methyl ester of hexacyclenic acid **4**, which results from a methylation of **3** with diazomethane, into the diastereomeric Mosher's esters **5** and **6**.^[9] Table 1 shows selected ¹H NMR data for **4**–**6**. From these data, the position of the Mosher's ester can be determined as C-16 and the absolute configuration of C-16 in **4** is derived as *S*, which results in the absolute configuration of **3** being: 3*S*, 4*R*, 5*R*, 7*S*, 8*R*, 10*S*, 13*R*, 14*R*, 15*R*, 16*S*, 17*R*, 18*S*, 19*S*, 20*R*.

Table 1. Selected ¹H NMR data (125.7 MHz, [D₅]pyridine) of **4** and the Mosher's esters **5** and **6**.

H Atom	4	5	6	δ(5) – δ(6)
26-H ₃	1.18	1.51	1.47	+ 0.04
15-H	2.36	2.55	2.39	+ 0.16
16-H	4.15	6.03	6.04	<i>S</i> ^[a]
17-H	2.49	2.61	2.73	– 0.12
18-H	2.49	2.54	2.59	– 0.05
19-H	3.21	3.12	3.20	– 0.08

[a] Resulting chirality.

Compound **3** has a novel skeleton with a 5/6/5 ring system annulated to a bridged tricycle, which is characterized by a δ-lactone and a cyclic hemiketal. The left tricyclic section of **3** can be found similarly in the spinosynes **7**,^[10] insecticidal metabolites from streptomycetes, and in ikarugamycin.^[11] In the given combination of structure elements, the hexacyclic skeleton of **3** with its 14 chiral



centers and different functionalities is not comparable with any known natural product.

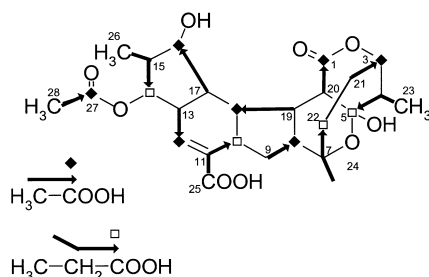
Compound **3** shows weak cytotoxicity for three tested cell lines (HM02, HEP G2, and MCF7) with GI_{50} values up to $14.0 \mu\text{mol L}^{-1}$. Further biological tests are in progress.

Polycyclic nonaromatic structures are characteristic for terpenoids but occur only rarely within actinomycetes. In order to get an idea of the biosynthesis of **3**, first feeding experiments were carried out. Feeding of $[1-^{13}\text{C}]$ acetate to growing cultures of the strain resulted in signal enhancements of C-1, C-3, C-8, C-16, C-18, and C-27 (Table 2, Scheme 1). Values for specific incorporations of C-5 and C-12 are increased but not unambiguously. The lower incorporation in C-12 can be explained by the difficulty of detection of its comparatively broad ^{13}C NMR signal. Another feeding experiment with $[1-^{13}\text{C}]$ propionate led to strong enrichments of C-5, C-10, C-14, and C-22 (Table 2, Scheme 1).

Table 2. ^{13}C NMR data (125.7 MHz, $[\text{D}_5]\text{pyridine}$) of hexacyclenic acid (**3**) and specific ^{13}C incorporation (relative to the natural intensity of the C-28 signal) after feeding experiments with $[1-^{13}\text{C}]$ acetate (A) and $[1-^{13}\text{C}]$ propionate (B).

	δ	A	B
C-1	177.0	2.1	0.3
C-3	78.9	3.3	0.3
C-4	45.0	-0.2	0.1
C-5	95.3	0.6	75.9
C-7	72.9	0.2	-0.1
C-8	46.1	3.1	0.2
C-9	31.9	-0.2	0.1
C-10	42.3	-0.4	54.5
C-11	136.4	-0.6	0.0
C-12	139.0	0.6	1.5
C-13	45.7	-0.2	-0.1
C-14	80.0	-0.2	37.6
C-15	47.8	-0.1	0.0
C-16	80.4	3.2	0.0
C-17	50.2	-0.3	0.0
C-18	53.2	1.7	-0.1
C-19	42.8	-0.2	0.4
C-20	48.3	-0.1	-0.3
C-21	23.6	-0.2	0.1
C-22	35.5	0.2	68.0
C-23	8.8	-0.1	0.2
C-24	27.2	-0.1	0.3
C-25	169.0	n.d. ^[a]	1.2
C-26	14.7	-0.1	0.2
C-27	170.8	5.9	0.0
C-28	20.9	0.0	0.0

[a] n.d. = not determined.



Scheme 1. Labeling pattern of hexacyclenic acid (**3**). Results of feeding experiments with $[1-^{13}\text{C}]$ acetate and $[1-^{13}\text{C}]$ propionate are shown, as well as the proposed direction of incorporation of the precursors.

These experiments reveal that hexacyclenic acid (**3**) is a polyketide built by seven acetate and four propionate units (Scheme 1). This however must be confirmed by further experiments, just like the determination of the starter unit. If one assumes only one polyketide chain, built by a modular type I polyketide synthase, multiple unusual cyclisation steps are necessary to generate **3**. We suppose an intramolecular Diels–Alder reaction, aldol reaction, lactonisation, and ketalisation which would provide all the functionalities needed in the intermediates. Further experiments to investigate the biosynthesis of hexacyclenic acid **3**, a novel type I polyketide, are in progress.

Experimental Section

Cultivation: Strain S1013 was grown on agar slopes (10 g L^{-1} soluble starch, 2 g L^{-1} $(\text{NH}_4)_2\text{SO}_4$, 2 g L^{-1} CaCO_3 , 1 g L^{-1} K_2HPO_4 , 1 g L^{-1} NaCl , 1 g L^{-1} MgSO_4 , 20 g L^{-1} agar, pH 7.0 prior to sterilization) at 28°C until sporulation, and then stored at 4°C . One eighth of these agar slopes was used to inoculate 100 mL of a soybean/mannitol medium (20 g L^{-1} D-mannitol, 20 g L^{-1} soybean meal (degreased), pH 7.2 prior to sterilization) in 300 mL Erlenmeyer flasks. These cultures were incubated on a rotary shaker (180 rpm) at 30°C for 72 h. A 900 mL portion of this preculture was used to inoculate a 10 L fermentor (Braun, Biostat E, medium: 20 g L^{-1} rolled oats (cooked for 20 min and filtered), 1 g L^{-1} NaBr , 2.5 mL trace element solution (3 g L^{-1} $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1 g L^{-1} $\text{Fe}^{\text{III}}/\text{citrate}$, 0.2 g L^{-1} MnSO_4 , 0.1 g L^{-1} ZnCl_2 , 0.025 g L^{-1} $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.03 g L^{-1} $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, 0.004 g L^{-1} CoCl_2 , 0.01 g L^{-1} $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$), pH 7.0 prior to sterilization). The culture was incubated at 30°C , 10 L min^{-1} aeration and harvested after 120 h.

Isolation of **3:** The culture broth was separated from the mycelium by centrifugation and the filtrate was concentrated by lyophilization. The crude residue was extracted with methanol and the combined organic layers were evaporated. The residue was purified twice by column chromatography on silica gel (first column: $4 \times 27 \text{ cm}$, chloroform/methanol 7/3; second column: $2.5 \times 32 \text{ cm}$, chloroform/methanol 9/1) to yield 130 mg of **3** as a solid.

Labeled precursors and feeding experiments: Sodium $[1-^{13}\text{C}]$ acetate and sodium $[1-^{13}\text{C}]$ propionate (each 99% ^{13}C) were obtained from ISOTEC Inc. The main culture of the feeding experiments was carried out in ten 300 mL Erlenmeyer flasks, which contained 100 mL medium, at 30°C and 180 rpm for 120 h. The cultures were inoculated with the described 72 h preculture. The precursors (1 g each) were added as sterile aqueous solutions, adjusted to pH 7.0, following the pulse feeding method in 1 mL portions at 80, 86, 94, 100, and 106 h after incubation. Isolation and purification followed the description above to yield 1.5 mg of **3** ($[1-^{13}\text{C}]$ -acetate) or 56 mg of **3** ($[1-^{13}\text{C}]$ propionate).

Hexacyclenic acid (3**):** $\text{C}_{26}\text{H}_{34}\text{O}_9$ ($M_r = 490.55$); m.p. 162°C ; $[\alpha]_{\text{D}}^{20} = +69^\circ$ ($c = 0.11$ in MeOH); DCI-MS (200 eV): m/z : 508 ($[\text{M}+\text{NH}_4]^+$, 100%); HR EI MS (70 eV): 490.2202, found as calculated for $\text{C}_{26}\text{H}_{34}\text{O}_9$; IR (KBr): $\tilde{\nu}_{\text{max}} = 3428, 2926, 1734, 1719, 1702, 1658, 1637, 1381, 1250, 1090, 1033 \text{ cm}^{-1}$; UV (MeOH): $\lambda_{\text{max}} (\epsilon) = 202 \text{ nm}$ (11 514); ^1H NMR (500 MHz, $[\text{D}_5]\text{pyridine}$): $\delta = 1.07$ (s, 3H, 24- H_3), 1.14 (d, $J = 6.9 \text{ Hz}$, 3H, 23- H_3), 1.19 (d, $J = 6.5 \text{ Hz}$, 3H, 26- H_3), 1.55 (dm, $J = 15.5 \text{ Hz}$, 1H, 22- H_b), 1.65 (m, 1H, 22- H_b), 1.72 (m, 1H, 21- H_a), 1.79 (ddd, $J = 12.0, 12.0, 12.0 \text{ Hz}$, 1H, 9- H_a), 1.92 (m, 1H, 21- H_b), 2.05 (s, 3H, 28- H_3), 2.11 (ddd, $J = 11.5, 11.5, 6.6 \text{ Hz}$, 1H, 8- H), 2.21 (m, 1H, 10- H), 2.26 (dq, $J = 6.9, 3.5 \text{ Hz}$, 1H, 4- H), 2.36 (ddq, $J = 10.0, 10.0, 6.5 \text{ Hz}$, 1H, 15- H), 2.55 (m, 2H, 18- H , 17- H), 2.79 (ddd, $J = 11.5, 6.5, 6.5 \text{ Hz}$, 1H, 9- H_b), 3.11 (dddd, $J \approx 10, \approx 10, \approx 2, \approx 2 \text{ Hz}$, 1H, 13- H), 3.22 (dd, $J = 11.5, 10.5 \text{ Hz}$, 1H, 19- H), 3.95 (br.s, 1H, 20- H), 4.17 (dd, $J = 10.0, 5.9 \text{ Hz}$, 1H, 16- H), 4.42 (ddd, $J = 3.5, 3.5, 3.5 \text{ Hz}$, 1H, 3- H), 5.00 (dd, $J = 10.0, 10.0 \text{ Hz}$, 1H, 14- H), 7.26 (dd, $J = 2.5, 2.5 \text{ Hz}$, 1H, 12- H); ^{13}C NMR: see Table 2.

Data for compounds **4–6**: see the Supporting Information.

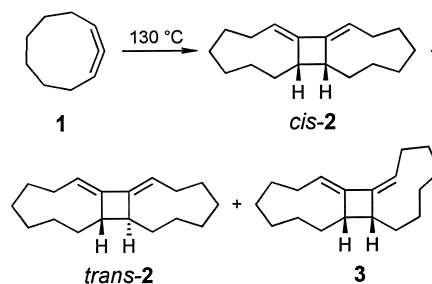
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The Dimerization of Chiral Allenes: Pairs of Enantiomers and Pairs of Homomers Furnish Different Diastereomers**

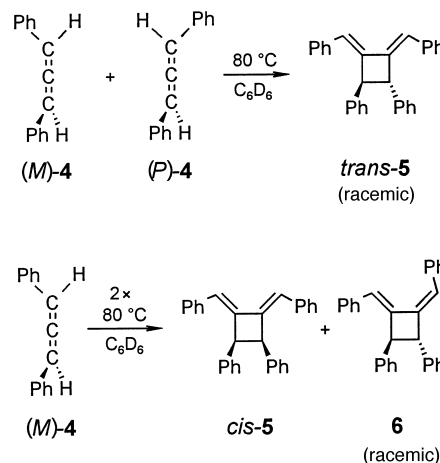
Manfred Christl,* Stefan Groetsch, and Kurt Günther

At room temperature, cyclonona-1,2-diene (**1**) can be stored virtually unlimited. Proceeding at 130°C , its dimerization furnishes the 1,2-bis(methylene)cyclobutane derivatives *cis*-**2**, *trans*-**2**, and **3** in the ratio of 5:10:1 in the case of the



racemic mixture, whereas a nearly pure enantiomer affords almost only *cis*-**2**.^[1] These findings suggest the interpretation that the combination of a pair of homomers in racemic **1** gives rise to *cis*-**2** and that of a pair of enantiomers provides preferentially *trans*-**2** and additionally **3**.

1,3-Diphenylallene (**4**) had been studied as a second example for the dimerization of chiral nonracemic allenes. By using a sample with an optical purity of 35 %, the optical rotation did decrease to zero, however, the ratio of products (*cis*-**5**, *trans*-**5**, and **6**) was unchanged compared to that from the dimerization of racemic **4**.^[2] Assuming that the enantiomeric excess could have been too small for the observation of



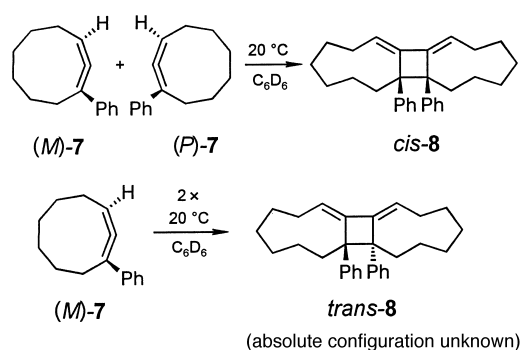
a deviation from the behavior of racemic **4**, we repeated the experiments. On heating in C₆D₆ at 80°C for five days, racemic **4** was consumed to the extent of 93 % and yielded *cis*-**5**, *trans*-**5**, and **6** in the ratio of 44:28:28. The pure enantiomers of **4** were obtained by HPLC on Chiralcel OD^[3] and dimerized to give predominantly *cis*-**5** and **6** and only a small proportion of *trans*-**5** (*cis*-**5**:*trans*-**5**:**6** = 46:4:50). Therefore, we conclude that in racemic **4** pairs of enantiomers prefer to form *trans*-**5**, while pairs of homomers produce mainly *cis*-**5** or **6**.

Recently, we reported on the dimerization of racemic 1-phenylcyclonona-1,2-diene (**7**) in CDCl₃, which occurs already at 20°C with formation of *cis*- and *trans*-**8** in the ratio of about 1:2.^[4] The reaction has now been conducted in C₆D₆ and the product ratio was found to be very close to 50:50. The pure enantiomers of **7** were obtained by HPLC of the racemic mixture on Chiralcel OD with hexane as eluant. Their dimerization in C₆D₆ (several weeks at 20°C) gave *trans*-**8** with high preference (*cis*-**8**:*trans*-**8** = 8:92). These results

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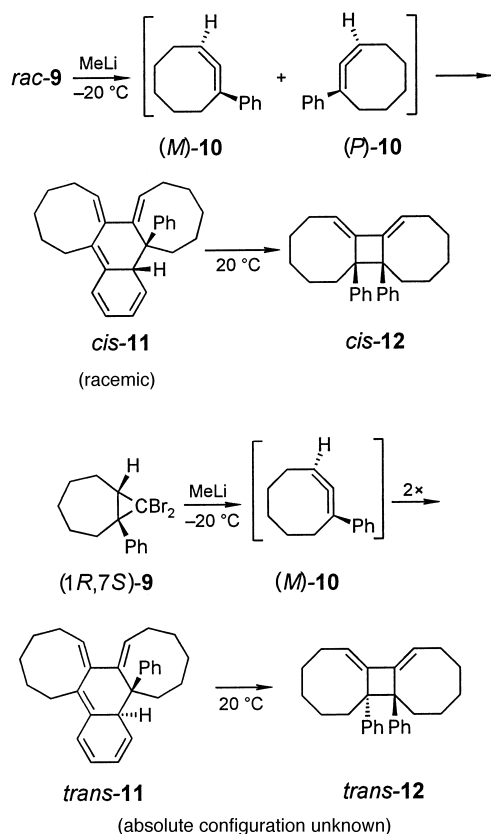
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[**] Cycloallenes, Part 14. This work was supported by the Deutsche Forschungsgemeinschaft, by the Fonds der Chemischen Industrie, and by CHEMETALL GmbH. Part 13: M. Christl, S. Groetsch, *Eur. J. Org. Chem.* **2000**, 1871–1874.



suggest the highly selective production of *cis*-**8** from pairs of enantiomers in racemic **7**, whereas pairs of homomers furnish *trans*-**8**; the ratio of the rate constants is 2:1. In comparison with the situation in **1**, the stereochemical course is reversed, as the *meso*-form (*cis*-**2** and *cis*-**8**, respectively) originates from pairs of homomers there and from pairs of enantiomers here. The optical activity of the *trans*-**8** products, obtained from (+)-**7** and (–)-**7**, does not support the formation of racemic mixtures.^[5]

The above results with **7** aroused our curiosity about 1-phenylcycloocta-1,2-diene (**10**). Some time ago we had generated this cycloallene as a racemic mixture (*rac*-**10**) and obtained its unusual dimer *cis*-**11**, which probably emerges from the diradical intermediate **21** in a dead-end equilibrium and rearranges slowly to *cis*-**12** at 20 °C. Since *cis*-**12** is the only known *cis* dimer of a cycloallene with an eight-membered or a smaller ring, it has also to be considered as unusual.^[6] Dimerizing rapidly even at –40 °C, **10** cannot be observed directly,^[6] which is why only its precursor **9** could be employed

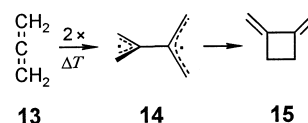


in the form of pure enantiomers. Racemic **9** (*rac*-**9**) was resolved by HPLC on Chiralpak AD with hexane as eluant. Optically active samples of **4** and **1** can in fact be prepared via nonracemic *trans*-2,3-diphenylcyclopropylidene^[7] and from nonracemic 9,9-dibromo-*trans*-bicyclo[6.1.0]nonane^[8] by the Doering–Moore–Skattebøl reaction, but the stereochemical course of the conversion of (1*R*,7*S*)- and (1*S*,7*R*)-**9** into **10** was unclear because of the absence of *C*₂ symmetry in these substrates. The analysis of the ring opening of cyclopropylidene to allene by quantum-chemical calculations reveals a quite complex process.^[9]

After treatment of the pure enantiomers of **9** with methylolithium in diethyl ether we indeed observed at best a very small quantity of *cis*-**11** and *cis*-**12** (error limit 6 %). The only identified product was *trans*-**12**, which we had observed previously on heating of *cis*-**12** to 160 °C.^[6] For a short time after each of the experiments had been carried out, another product could be detected by ¹H NMR spectroscopy. This product rearranged completely to *trans*-**12** within one day at room temperature. Owing to the similarity of the data to those of *cis*-**11**, we assign structure *trans*-**11** to it. The optical activity as well as HPLC on Chiralpak AD with isohexane as eluant demonstrate the nonracemic nature of both *trans*-**12** samples.

These experiments prove the highly selective conversion of (1*R*,7*S*)- and (1*S*,7*R*)-**9** into one enantiomer of **10** each (presumably (*M*)-**10** is formed from (1*R*,7*S*)-**9**). Giving rise to the dimers *trans*-**12**, the pure enantiomers **10** exhibit, at variance with the racemic mixture, a behavior typical for small-ring cycloallenes. Careful analysis of the crude product from *rac*-**9** indicated the presence of a small amount of *trans*-**12**; the ratio of (*cis*-**11** + *cis*-**12**):*trans*-**12** was 95:5. Thus, *rac*-**10** is a remarkable example for molecular recognition with significant selectivity; pairs of enantiomers react 38 times faster than pairs of homomers.

To rationalize these phenomena, we first consider the energetics of the dimerization of the parent allene (**13**). Roth and Schaffers^[10] determined $\Delta H^\ddagger$ and $\Delta S^\ddagger$ with high precision to be 26.9 kcal mol^{–1} and 25.9 cal mol^{–1} K^{–1}, respectively, and located the intermediate tetramethyleneethane diradical **14** about 32 and about 8 kcal mol^{–1}, respectively, below the

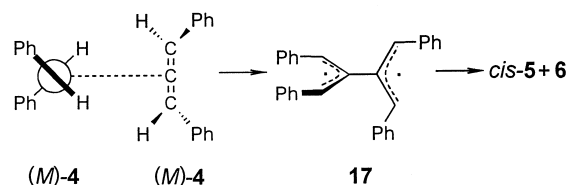
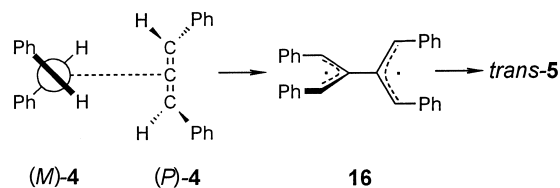


transition state of its generation from two molecules of **13** and the transition state en route to the final product, that is 1,2-(bismethylene)cyclobutane (**15**). Quantum-chemical calculations attribute to the singlet state of **14** the conformation with the allyl-radical subunits arranged perpendicularly to each other.^[11]

Previously, we had proposed such diradicals as intermediates for the dimerization of *rac*-**7** and *rac*-**10**, in the course of which the ring closures should occur as least-motion processes, corresponding to a conrotation. Accordingly, it must be assumed that stereoisomeric diradicals are generated selectively from pairs of enantiomers and homomers, respectively,

and are transformed to the products without extensive isomerization.

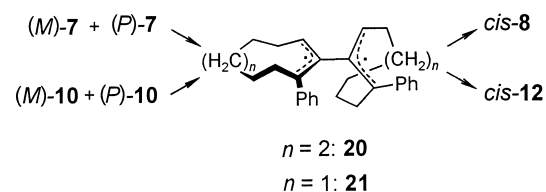
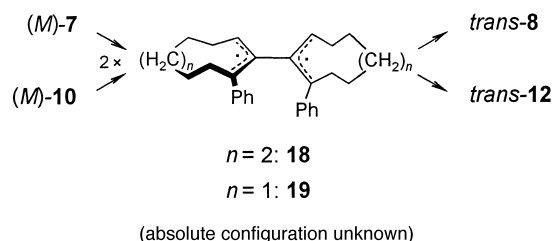
En route to *trans*-**5**, the most likely diradical is **16**, which should be formed on the approach of (*M*)-**4** and (*P*)-**4** with their sterically unhindered faces in the crossed arrangement



along their C_2 axes. The corresponding transition state shows equal interactions between the hydrogen atoms of one allene subunit and the phenyl groups of the other with the effect that the phenyl groups of **16** are as far away as possible from the other half of the molecule.

The diradical **17** should be the precursor of *cis*-**5** and **6**. In the corresponding transition state from two homomers of **4**, the hydrogen atoms of the allene subunits approach each other in pairs rather closely. Forced by the steric crowding, one hydrogen atom moves out of the way such that an allyl radical moiety with an *E,Z* configuration is generated. With regard to the formation of **5** and **6**, the diradicals **16** and **17** as well as their four diastereomers had been discussed in detail previously, but without a definite conclusion.^[2, 12]

Transition states from two allene molecules in the crossed arrangement have to be assumed also for the dimerization of **7** and **10**. However, because of the variety of the possibilities and of the steric interactions—even methylene groups not directly linked to the allene moiety can impair the close contact with the other molecule—the situation is not easily comprehensible. Within the discussed mechanistic concept though, there is no doubt that pairs of homomers of **7** and **10** give rise to the diradicals **18** and **19**, respectively, which



eventually collapse to give *trans*-**8** and *trans*-**12**, whereas pairs of enantiomers of **7** and **10** generate the diradicals **20** and **21**, respectively, from which *cis*-**8** and *cis*-**12** result. The non-racemic nature of the *trans* products supports a considerable conformational stability of **18** and **19**, and the only small admixtures of *cis*-**8** and *cis*-**12** confirm their configurative persistence as well, that is only insignificant proportions of **18** and **19** isomerize to **20** and **21**, respectively.

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Five-Coordinate Carbides in Ti-Al-C Complexes**

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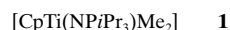
Fifty years after the discovery of catalytic olefin polymerization by Ziegler and Natta, polyolefin products are commodity chemicals. The production of these materials is a significant driving force in our economy; one that continues to inspire research. Over the last twenty years much effort has been directed towards tunable “single-site catalysts”.^[1–6] The

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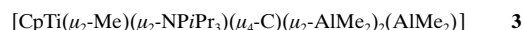
vast majority of systems developed and certainly those in commercial practice require the use of Al-based activators. Despite the importance of such alkylaluminum reagents few well-defined products derived from the stoichiometric reactions of early transition metal compounds and Al reagents are known. While Al reagents may act simply as alkylating agents for early transition metal species, there are several examples in which C–H bond activation processes provide novel products. The most familiar example is the Tebbe reagent,^[7, 8] $[\text{Cp}_2\text{Ti}(\mu\text{-CH}_2)(\mu\text{-Cl})\text{AlMe}_2]$ derived from the reaction of $[\text{Cp}_2\text{TiCl}_2]$ and AlMe_3 . Roesky et al. have recently described the formation of the very interesting Zr–Al and Hf–Al clusters $[(\text{Cp}^*\text{MMe})(\mu\text{-F})_2\text{AlMe}_2]_2$ and $[(\text{Cp}^*\text{M})_3\text{Al}_6\text{Me}_8(\mu_3\text{-CH}_2)_2(\mu_4\text{-CH})_4(\mu_3\text{-CH})]$.^[9, 10] In these cases, it is the attraction of the Al centers to the fluoride ligands that initiates the reactions of $[\text{Cp}^*\text{MF}_3]$ with AlMe_3 . Similarly we have recently shown that the interaction of AlMe_3 with the S and/or N ligands of $[\text{CpTi}(\text{NPR}_3)(\text{SR})_2]$ results in the formation of a Ti-thiolate–Al–carbide species.^[11] Herein, we demonstrate that the remarkable process of triple C–H bond activation proceeds directly in the reactions of the dialkyl complexes $[\text{CpTi}(\text{NPR}_3)\text{Me}_2]$ with AlMe_3 . Moreover, the resulting Ti–Al–carbide complexes establish equilibria with excess AlMe_3 to provide an unusually facile interconversion between carbide species with distorted tetrahedral and five-coordinate, pseudo trigonal bipyramidal geometries.

The reaction of **1** with more than four equivalents of AlMe_3 in toluene or hexane proceeds over 16 h to produce a red



crystalline product **2** in 75 % yield. In the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of compound **2** there is a single resonance at $\delta = 50.9$. The ^1H and ^{13}C (APT) NMR spectra (ATP = attached proton test) are consistent with the presence of cyclopentadienyl and phosphinimide ligands. Additionally the ^1H NMR spectrum revealed four broad resonance signals near $\delta = 0$ that integrated to approximately 30 hydrogen atoms; this suggests a 4:1 combination of Al and Ti in **2**. Recrystallization of **2** from benzene afforded crystals of the slightly different species **3**. The $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of **3** shows a single resonance signal at $\delta = 49.8$ and the ^1H and ^{13}C (APT) NMR spectra are slightly different than those of **2**. In addition, the ^1H NMR spectrum of **3** shows three broad resonances that integrate to 21 hydrogen atoms (near $\delta = 0$) and are attributed to Al-bound methyl groups. The $^{27}\text{Al}\{^1\text{H}\}$ NMR spectra show three broad resonances at $\delta = 149.0$, 114.0, and 38.0. These data suggest a 3:1 combination of Al and Ti in **3**.

The structure of **3**^[12] was confirmed by X-ray crystallography (Figure 1).



The pseudo “three-legged piano stool” coordination sphere of the Ti center in **3** comprises a cyclopentadienyl ring, a methyl group, a phosphinimide nitrogen atom, and a carbide carbon atom; three aluminum atoms complete the bonding

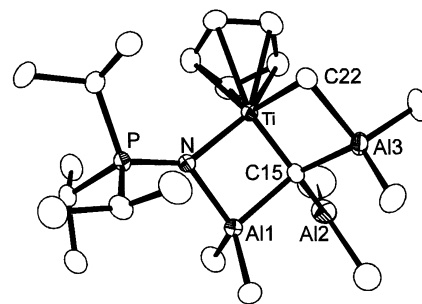
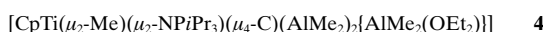


Figure 1. Structure of **3**, (ORTEP drawing 20% thermal ellipsoids are shown). Hydrogen atoms are omitted for clarity.

sphere of the carbide unit. Two of the AlMe_2 moieties bridge, respectively, the carbide center and the Ti-bound methyl group, and the carbide center and the phosphinimide nitrogen atom bound to the Ti center. The third AlMe_2 fragment occupies a terminal position on the carbide center. The most interesting feature is the geometry about the carbide carbon atom. The titanium–carbide (Ti–C15) bond in **3** (1.874(4) Å) is significantly shorter than the terminal Ti–CH₃ bond (2.123(5) Å) in $[\text{Cp}(t\text{Bu}_3\text{PN})\text{TiMe}(\mu_2\text{-CH}_3\text{B}(\text{C}_6\text{F}_5)_3)]$.^[13] This relatively short Ti–C15 bond, along with the Ti–C22 bond of 2.301(4) Å, infers that the Ti–C15 bond has some degree of multiple-bond character. This view is supported by the flattened geometry of the carbide carbon as evidenced by the Ti–C–Al2 and Al1–C–Al3 angles of 145.4(2) and 122.8(2)°, respectively, in **3**.

Resonance signals attributed to the carbide carbon atoms of **2** and **3** were not observed in the initial $^{13}\text{C}\{^1\text{H}\}$ NMR data. This is presumably because of the cumulative effects of the long relaxation time of the quaternary carbon atom, the quadrupolar nature of Al atoms bonded to the carbide, and the low natural abundance of the ^{13}C isotope. However, preparation of **1** employing ^{13}C -labeled $[\text{MeMgBr}]$ and subsequent reaction with AlMe_3 in toluene results in the formation of labeled **2**. Methyl exchange between **1** and AlMe_3 results in a statistical distribution of ^{13}C atoms in the carbide and AlMe sites in **2** and **3**. The resonance signals observed in the $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of ^{13}C -labeled **2** and **3** at $\delta = 298.1$ and 304.7, respectively, are attributed to the carbides of **2** and **3**.

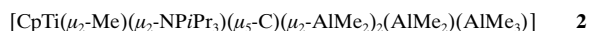
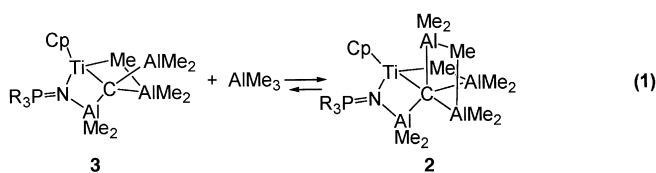
Compound **3** remains Lewis acidic primarily as a result of the presence of the three-coordinate Al center (Al2). This is demonstrated by the coordination of diethyl ether to the Al2 center of **2**, affording **4**. In $[\text{D}_6]$ benzene the $^{13}\text{C}\{^1\text{H}\}$ NMR resonance for the carbide carbon atom in **4** is at $\delta = 312.8$; this



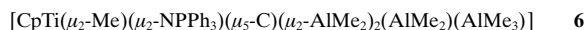
reflects a slight change in the coordination environment of the carbide carbon center. It is noteworthy that the ^1H NMR spectrum of **4** is much sharper in the methyl region than those of **2** or **3**, presumably as coordination of diethylether to Al2 precludes methyl exchange processes between the adjacent AlMe_2 sites.

Interestingly, the reaction of **2** with excess AlMe_3 in benzene indicates that it participates in an already

existing equilibrium with **3** [Eq. (1)]. This infers the given formulation of **2**.



Variable-temperature NMR studies established the equilibrium constant for Equation (1) to be 129 M^{-1} at 260 K. The corresponding activation parameters are $\Delta G^\ddagger(260\text{ K}) = -10.5\text{ kJ mol}^{-1}$, $\Delta H^\ddagger = -57.9\text{ kJ mol}^{-1}$ and $\Delta S^\ddagger = -0.183\text{ kJ mol}^{-1}\text{ K}^{-1}$. When ^{13}C -labeled **2** was employed, the carbide resonance was observed at $\delta = 241.2$ in $[\text{D}_6]\text{benzene}$ at 260 K. This shift is considerably upfield of those of **3** or **4** and indicates a dramatic change in the environment of the carbide carbon center in **2**. An interaction of the carbide carbon with the additional equivalent of AlMe_3 is proposed. This proposal is further supported by the isolation of compound **6** in 88 % yield from the reaction of $[\text{CpTi}(\text{NPPH}_3)\text{Me}_2]$ (**5**) with an excess of AlMe_3 . The $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of compound **6** shows a single resonance signal at $\delta = 25.4$ and the ^1H NMR data are consistent with the given formulation for **6**.



Crystallographic studies of **6**^[12] confirmed this structure and established the presence of a five-coordinate carbide center (Figures 2a and b). The gross structural features of **6** are similar to those described for **2**, with an additional equivalent of AlMe_3 bonded by the Al4 center to the carbide carbon atom (C24) and by a methyl bridge to the Al3 center. The geometry about the carbide carbon atom in **6** is distorted trigonal bipyramidal. The Ti-C24-Al3 vector is essentially linear with an angle of $175.77(17)^\circ$. The angle of these pseudo-axial metal centers to those in the pseudo-equatorial plane range from $78.21(11)$ – $104.82(14)^\circ$, while the angles in the trigonal plane vary from $114.45(14)$ to $130.77(15)^\circ$. These distortions are consistent with the presence of strain that arises from the two fused four-membered Ti-C-Al-C and Ti-C-Al-N rings in these complexes.

Hypercoordinate carbide species such as **2** and **6** are rare. Schmidbaur and co-workers^[14] have described a gold carbocation $[(\text{Ph}_3\text{PAu})_5\text{C}]^+$. This species exhibits a C_{3v} -symmetric trigonal-bipyramidal geometry at the carbon center is similar to that predicted by computational studies for the cations $[\text{CH}_3]^+$ and $[\text{CLi}_5]^+$.^[15] In recent work, Akiba et al.^[16] have described the hypervalent organic carbocation $[(\text{MeO})_2\text{C}\{(\text{MeO})_2\text{C}_{14}\text{H}_7\}]^+$. Compounds **2** and **6** are not to be considered as hypervalent; they are electron-deficient, hypercoordinate species. As such, these are the first neutral compounds in which four- and five-coordinate carbon atoms interconvert in this unprecedented and facile manner.

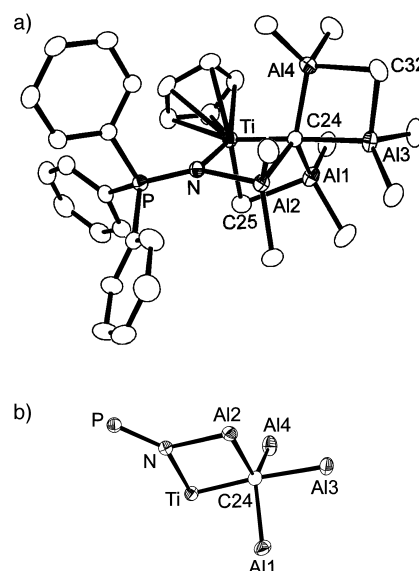


Figure 2. a) Structure of **6** (ORTEP drawing 20 % thermal ellipsoids are shown). Hydrogen atoms are omitted for clarity. b) The carbide coordination sphere of **6** (ORTEP drawing). Selected distances [$\text{\AA}$] and angles [$^\circ$]: Ti-C24 1.975(3), Al4-C24 2.085(3), Al1-C24 2.096(3), Al2-C24 2.150(3), Al3-C24 2.118(3); Ti-C24-Al4 $104.82(14)$, Ti-C24-Al1 $83.98(12)$, Al4-C24-Al1 $130.77(15)$, Ti-C24-Al3 $175.77(17)$, Al4-C24-Al3 $78.21(11)$, Al1-C24-Al3 $91.80(12)$, Ti1-C24-Al2 $83.60(11)$, Al4-C24-Al2 $114.45(14)$, Al1-C24-Al2 $114.65(14)$, Al3-C24-Al2 $97.90(12)$.

Experimental Section

2: A solution of AlMe_3 (2.0 M, 2.52 mmol) in hexane (1.26 mL) was added to a solution of complex **1** (0.200 g, 0.630 mmol) in hexane (10 mL). Over 16 h at room temperature a light orange solid precipitated. The solid was isolated by filtration and washed with hexane ($1 \times 5\text{ mL}$). ^1H NMR (C_6D_6): $\delta = 6.11$ (br s, 5H; Cp), 1.78 (sept, $^3J_{\text{HH}} = 7.1\text{ Hz}$, 3H; $\text{PCH}(\text{CH}_3)_2$), 0.73 (dd, $^3J_{\text{PH}} = 14.3\text{ Hz}$, $^3J_{\text{HH}} = 7.1\text{ Hz}$, 9H; $\text{PCH}(\text{CH}_3)_2$), 0.66 (dd, $^3J_{\text{PH}} = 14.8\text{ Hz}$, $^3J_{\text{HH}} = 7.1\text{ Hz}$, 9H; $\text{PCH}(\text{CH}_3)_2$), 0.09 (br s, 3H; AlCH_3); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): $\delta = 50.9$ (br s); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): $\delta = 298.2$ (s; Ti(C)Al₃), 109.8 (s; Cp), 26.8 (d, $^1J_{\text{PC}} = 58.8\text{ Hz}$; PCHMe_2), 16.5 (d, $^2J_{\text{PC}} = 44.1\text{ Hz}$; $\text{PCH}_2(\text{CH}_3)_2$), 9.9 (s; Ti-CH₃), -0.1 , -4.8 , -6.1 (br s; Al-CH₃).

3: Successive recrystallizations of **2** from benzene afforded dark red crystalline **3** in 80 % yield. ^1H NMR (C_6D_6): $\delta = 6.19$ (br s, 5H; Cp), 1.84 (sept, $^3J_{\text{HH}} = 7.1\text{ Hz}$, 3H; $\text{PCH}(\text{CH}_3)_2$), 0.78 (dd, $^3J_{\text{PH}} = 14.8\text{ Hz}$, $^3J_{\text{HH}} = 7.1\text{ Hz}$, 9H; $\text{PCH}(\text{CH}_3)_2$), 0.72 (dd, $^3J_{\text{PH}} = 14.0\text{ Hz}$, $^3J_{\text{HH}} = 7.1\text{ Hz}$, 9H; $\text{PCH}(\text{CH}_3)_2$), 0.12 (br s, 3H; AlCH_3), -0.10 (br s, 9H; AlCH_3), -0.24 (br s, 9H; AlCH_3); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): $\delta = 49.8$; $^{27}\text{Al}\{^1\text{H}\}$ NMR (C_6D_6): $\delta = 149.00$ (br s), 114.00 (br s), 38.00 (br s); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): $\delta = 304.7$ (s; Ti(C)Al₃), 109.9 (s; Cp), 27.0 (d, $^1J_{\text{PC}} = 56.6\text{ Hz}$; $\text{PCH}(\text{CH}_3)_2$), 16.8 (d, $^2J_{\text{PC}} = 42.4\text{ Hz}$; $\text{PCH}(\text{CH}_3)_2$), 10.2 (s; Ti-CH₃), -0.6 , -4.5 (s; AlCH_3); elemental analysis calcd (%) for $\text{C}_{22}\text{H}_{47}\text{Al}_3\text{NP}_2\text{Ti}$: C 54.43, H 9.76, N 2.89; found: C 54.20, H 9.39, N 2.75.

4: Recrystallization of complex **3** from benzene with the addition of several equivalents of diethyl ether afforded complex **4**. Yield > 95 % (by ^1H NMR); ^1H NMR (C_6D_6): $\delta = 6.30$ (s, 5H; Cp), 3.48 (quartet, > 4H; OCH_2CH_3), 1.95 (sept, $^3J_{\text{HH}} = 6.8\text{ Hz}$, 3H; $\text{PCH}(\text{CH}_3)_2$), 0.84 (t, > 6H; OCH_2CH_3), 0.83 ($2 \times$ dd, 18H; $\text{PCH}(\text{CH}_3)_2$), 0.18 (s, 3H; AlCH_3), -0.05 (s, 3H; AlCH_3), -0.08 (s, 6H; AlCH_3), -0.19 (s, 9H; AlCH_3), -0.38 (s, 3H; AlCH_3); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): $\delta = 48.9$; $^{27}\text{Al}\{^1\text{H}\}$ NMR (C_6D_6): $\delta = 62.0$ (br s); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): $\delta = 312.8$ (s; Ti(C)Al₃), 109.9 (s; Cp), 64.9 (s; OCH_2CH_3), 27.2 (d, $^1J_{\text{PC}} = 58.8\text{ Hz}$; $\text{PCH}(\text{CH}_3)_2$), 17.2 (d, $^2J_{\text{PC}} = 44.1\text{ Hz}$; $\text{PCH}(\text{CH}_3)_2$), 13.3 (s; OCH_2CH_3), 10.6 (s; Ti-CH₃), -1.1 , -2.4 , -3.7 , -4.9 , -7.8 (s; AlCH_3).

6: Addition of a solution of AlMe_3 (2.0 M, 0.810 mmol) in hexane (0.41 mL) to a stirred pale yellow solution of **5** (68 mg, 0.162 mmol) in benzene (4 mL)

led to the immediate formation of orange solution formed immediately. Stirring for 16 h gave a dark orange-rust solution which was evaporated to dryness to give an orange-red solid. Yield: 0.088 g (88 %). ^1H NMR (C_6D_6): δ = 7.43 (m, 6H; C_6H_5), 7.01 (m, 9H; C_6H_5), 5.83 (s, 5H; Cp), 0.50 (br s, 3H; CH_3), -0.03 (br s, 12H; AlCH_3), -0.21 (s, 6H; AlCH_3), -0.39 (m, 9H; AlCH_3); ^{31}P [^1H] (C_6D_6): δ = 25.2 (s); ^{13}C [^1H] NMR (C_6D_6): δ = 134.2, 132.1, 129.3, 127.3 (PC_6H_5), 113.2 (s; Cp), Al- and Ti-bound methyl resonances are unobserved. Elemental analysis calcd (%) for $\text{C}_{34}\text{H}_{50}\text{Al}_4\text{NPTi}$: C 61.91, H 7.64, N 2.12; found: C 61.65, H 7.37, N 2.01.

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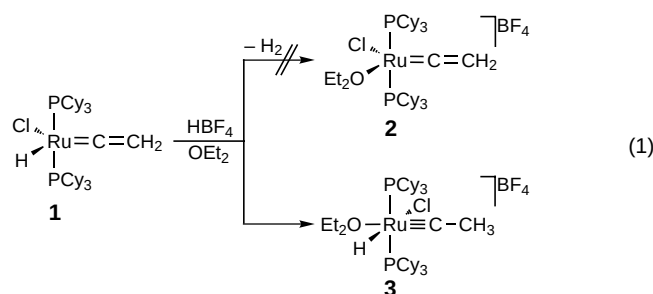
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- [12] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-141319, and -141320. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk). Diffraction experiments were performed on a Siemens SMART System CCD diffractometer and solved by employing the SHELX-TL software package. Data for complex **3**: Orthorhombic, space group *Fdd2*; a = 33.064(5) Å, b = 36.522(7) Å, c = 9.8244(19) Å, V = 11 863(4) Å³, Z = 16, R = 0.0449, R_w = 0.1103, GOF = 1.104. Data for complex **6**: Orthorhombic, space group *Pbca*; a = 18.825 (3) Å, b = 18.7742(3) Å, c = 23.9904(4) Å, V = 8479.0(2) Å³, Z = 8, R = 0.0517, R_w = 0.1291, GOF = 1.034.
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The First Example of an Equilibrium between a Carbene and an Isomeric Carbyne Transition Metal Complex**

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Dedicated to Professor Herbert W. Roesky on the occasion of his 65th birthday

By attempting to generate a cationic, possibly solvated, vinylideneruthenium(II) complex with a 14-electron configuration at the metal center, we recently found that the reaction of the starting material **1** with HBF_4 in dichloromethane/diethyl ether leads to the formation of the ruthenium carbyne **3** instead of the anticipated ruthenium vinylidene **2** [Eq. (1)].^[1] Although compound **3** is a highly efficient catalyst



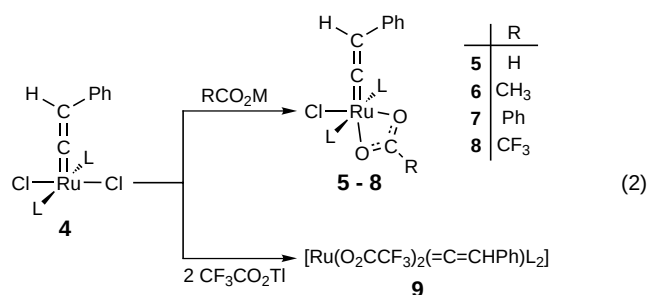
for olefin metathesis, including the cross-olefin metathesis of cyclopentene with methyl acrylate to afford multiply unsaturated esters $\text{CH}_2(\text{C}_5\text{H}_8)_n\text{CHCO}_2\text{Me}$ ($n = 1-3$), the lifetime of the cationic species containing a $\text{Ru}\equiv\text{C}$ bond is rather limited.^[1, 2] We therefore set out to prepare more stable ruthenium(II) carbynes and discovered in the course of these studies the first example of an equilibrium between a cationic metal carbene and the isomeric cationic metal carbyne.

The dichloro(vinylidene)ruthenium(II) complex **4**^[3] reacts with excess of HCO_2Na or $\text{CH}_3\text{CO}_2\text{Na}$ in THF or acetone to give exclusively the monosubstituted products **5** and **6** in 77 and 82 % yield, respectively [Eq. (2); $\text{L} = \text{P}(\text{Pr})_3$]. The corresponding benzoato(chloro) complex **7** can be prepared from **4** and PhCO_2H (ratio 1:1) in the presence of NEt_3 . The reaction of **4** with either $\text{CF}_3\text{CO}_2\text{Na}$ or $\text{CF}_3\text{CO}_2\text{K}$ in THF or acetone leads, however, even after prolonged stirring, to a mixture containing **4**, **8** (major components), and the disubstituted compound **9** (minor component). Treatment of **4** with one

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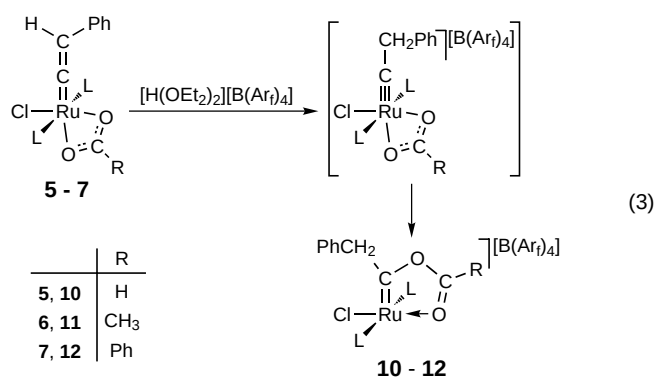
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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



equivalent of CF₃CO₂Ti in THF affords predominantly **8** (ca. 84 %) together with small amounts (ca. 8 % each) of **4** and **9**, which could not be separated either by fractional crystallization or column chromatography. An analytically pure sample of the bis(carboxylato) compound **9** was prepared in high yield from **4** and CF₃CO₂Ti (ratio 1:2.5) in acetone. The ¹³C NMR spectra of **5–9** display, due to ¹³C–³¹P coupling, a triplet for the α-carbon atom of the vinylidene ligand at δ ≈ 351–353 (for **5–8**) and δ = 362.1 (for **9**), the low-field chemical shift being typical for mononuclear vinylideneruthenium(II) complexes.^[3, 4]

Protonation of **5–7** with [H(OEt₂)₂][B(Ar_f)₄] (Ar_f = 3,5-C₆H₃(CF₃)₂)^[5] in dichloromethane gave the cationic complexes **10–12** in nearly quantitative yield [Eq. (3); L = P*i*Pr₃]. While the ¹H and ¹³C NMR spectra of **10–12** (typical features



are given in the Supporting Information) could not provide conclusive proof as to whether a carbene- or a carbyneruthenium derivative was generated, the single-crystal X-ray structure analysis of **10** confirmed the formation of a metal carbene (Figure 1).^[6] The coordination geometry around the ruthenium center is distorted square-pyramidal with the carbene carbon at the apical and the oxygen, the chlorine, and the two phosphorus atoms at the basal positions. In contrast to the Cl–Ru–O1 axis, which is almost linear, the P1–Ru–P2 axis is significantly bent, both phosphorus atoms pointing away from the Ru–C1 bond. An analogous square-pyramidal configuration has been found for the ruthenium carbenes [RuCl₂(=CHCH₂Ph)(P*i*Pr₃)₂]^[7] and [RuCl₂(=CHR)(PCy₃)₂] (R = 4-C₆H₄Cl, CH=CPh₂)^[8] as well as for the related vinylidenes [RuCl₂(=C=CHPh)L₂] (L = P*i*Pr₃, PCy₃).^[3] Compared with the Ru–C bonds in [RuCl₂(=CHCH₂Ph)(P*i*Pr₃)₂] and [RuCl₂(=CHR)(PCy₃)₂], the Ru–C1 bond length in **10** is

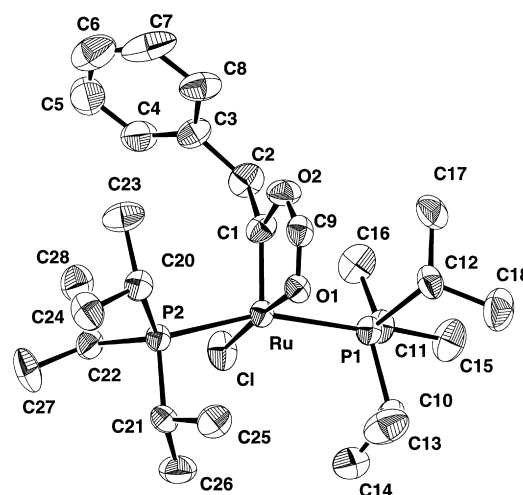
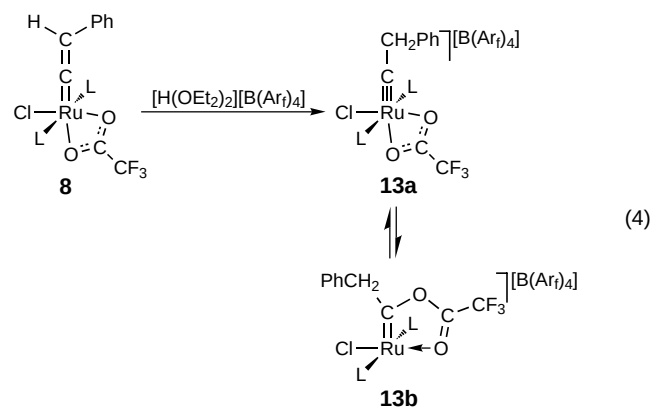


Figure 1. Molecular structure of the cation of **10**. Selected bond lengths [Å] and angles [°] with estimated standard deviations: Ru–C1 1.787(7), Ru–Cl 2.3306(17), Ru–O1 2.108(4), Ru–P1 2.4291(19), Ru–P2 2.4569(19), O1–C9 1.211(8), O2–C9 1.309(8), C1–O2 1.453(7), C1–C2 1.521(10); C1–Ru–O1 79.6(2), C1–Ru–Cl 105.5(2), C1–Ru–P1 97.2(2), C1–Ru–P2 103.4(2), Cl–Ru–O1 174.93(14), P1–Ru–P2 159.11(6), Cl–Ru–P1 88.36(6), Cl–Ru–P2 89.55(6), O1–Ru–P1 91.42(12), O1–Ru–P2 88.85(12), Ru–Cl–C2 135.6(5), Ru–Cl–O2 117.0(5), C2–C1–O2 107.2(6), C1–O2–C9 111.2(5), O1–C9–O2 122.3(6), Ru–O1–C9 109.9(4), C1–C2–C3 117.1(6).

rather short, whereas the C1–O2 distance is about 0.07–0.15 Å longer than in Fischer-type ruthenium carbenes containing a Ru=C(OR)R' unit.^[9] The plane of the chelate ring (Ru, C1, O2, C9, O1) (main deviation from planarity 0.017 Å) lies essentially perpendicular to the plane containing the ruthenium and the phosphorus atoms, which probably minimizes the steric repulsion between the ring atoms and the isopropyl groups. With regard to the mechanism of formation of **10–12**, we assume that in the initial step a cationic six-coordinate carbyne complex is generated as an intermediate [see Eq. (3)] which affords the more stable metal carbene by an intramolecular 1,2-shift of the weakly bound carboxylate oxygen atom to the carbyne carbon atom.

Following the hypothesis that nucleophilic attack at the carbyne carbon atom would be less favored with an electron-poor carboxylato ligand, we also treated compound **8** with [H(OEt₂)₂][B(Ar_f)₄] [Eq. (4); L = P*i*Pr₃]. Despite the presence of small amounts of **4** and **9** in the starting material, an analytically pure product **13** could be isolated by crystallization from dichloromethane at –78 °C. The composition of **13**



was analogous to that of **10**–**12** and corresponded to that of a 1:1 adduct of **8** and $\text{HB}(\text{Ar}_t)_4$. However, in contrast to **10**–**12**, the signals in the ^1H and ^{31}P NMR spectra of **13** at room temperature are somewhat broad, indicating that in solution a dynamic process takes place. A variable-temperature NMR study (using CD_2Cl_2 as the solvent) confirmed that indeed in solution two different species are present. Thus, at 190 K the ^{31}P NMR spectrum displays two sharp singlets at $\delta = 61.7$ and 50.8 which coalesce at 230 K and give rise to one singlet at $\delta = 56.5$ at 293 K. Similarly, in the ^1H NMR spectrum two signals are observed for the CH_2Ph protons at $\delta = 5.20$ and 4.59 (intensity ratio 1:2) at 190 K which coalesce at about 225 K and afford one singlet at $\delta = 4.88$ at 290 K. Since the chemical shift of the more intense resonance signal at $\delta = 4.59$ is almost identical to that of the ruthenium carbyne $[\text{RuCl}_2(\equiv\text{CCH}_2\text{Ph})(\text{P}i\text{Pr}_3)_2][\text{B}(\text{Ar}_t)_4]$,^[10] and the chemical shift of the less intense resonance signal at $\delta = 5.20$ is comparable to that of the ruthenium carbenes **10**–**12**, we conclude that in solution an equilibrium between the two isomers **13a** and **13b** [Eq. (4)] exists. The difference in free energy between **13a** and **13b** has been calculated as $0.25 \text{ kcal mol}^{-1}$ at 180 K.^[11]

An X-ray diffraction study revealed that the single crystals, which precipitated from a solution of **13** in dichloromethane at -78°C , consisted of the dominating isomer **13a**.^[6] The coordination geometry around the metal center (see Figure 2) can be described as distorted octahedral with a significant bending of the C1-Ru-O1 axis. The Ru-C1 bond length of $1.660(4) \text{ \AA}$ is one of the shortest M-C bond lengths found for carbyne transition metal complexes.^[12] Apart from the nearly linear Ru-C1-C2 axis, the most characteristic structural feature of **13a** is the difference between the distances Ru-O1 ($2.336(3) \text{ \AA}$) and Ru-O2 ($2.133(3) \text{ \AA}$). This significant difference not only indicates that the Ru-O1 interaction is rather weak, but also explains why there is only a low energy barrier

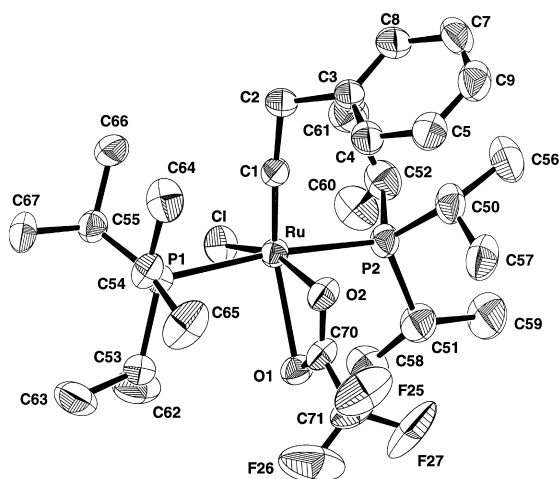


Figure 2. Molecular structure of the cation of **13a**. Selected bond lengths [$\text{\AA}$] and angles [$^\circ$] with estimated standard deviations: Ru-C1 $1.660(4)$, Ru-Cl $2.3143(11)$, Ru-O1 $2.336(3)$, Ru-O2 $2.133(3)$, Ru-P1 $2.4823(11)$, Ru-P2 $2.4922(11)$, C1-C2 $1.489(6)$, C2-C3 $1.524(7)$, O1-C70 $1.244(5)$, O2-C70 $1.253(5)$; C1-Ru-O1 $154.74(16)$, C1-Ru-O2 $96.18(16)$, C1-Ru-Cl $102.35(15)$, C1-Ru-P1 $95.06(14)$, C1-Ru-P2 $93.40(14)$, O1-Ru-O2 $58.56(11)$, O1-Ru-Cl $102.90(8)$, O1-Ru-P1 $86.58(8)$, O1-Ru-P2 $88.09(8)$, O2-Ru-Cl $161.45(9)$, O2-Ru-P1 $92.23(9)$, O2-Ru-P2 $92.26(9)$, Cl-Ru-P1 $85.23(4)$, Cl-Ru-P2 $87.66(4)$, P1-Ru-P2 $169.94(4)$, Ru-Cl-C2 $173.0(4)$, C1-C2-C3 $110.9(4)$, Ru-O1-C70 $84.8(2)$, Ru-O2-C70 $93.8(3)$, O1-C70-O2 $122.9(4)$.

for the interconversion between the two isomers **13a** and **13b**. A similar bonding situation of the trifluoroacetato ligand exists in the enynylruthenium(II) compound $[\text{Ru}\{\text{C}(\equiv\text{CHPh})(\text{C}\equiv\text{CPh})\}(\kappa^2\text{-O}_2\text{CCF}_3)(\text{CO})(\text{PPh}_3)_2]$, in which the bond lengths Ru-O1 and Ru-O2 are 2.353 and $2.234 \text{ \AA}$, respectively.^[13] It should be emphasized that if the single crystals studied crystallographically are dissolved in CD_2Cl_2 , the ^1H and ^{31}P NMR spectra of the solution equally display somewhat broadened signals indicating that the equilibrium between **13a** and **13b** is regenerated. Moreover, it is worth mentioning that to the best of our knowledge **13a** is the first carbyneruthenium complex which has been studied crystallographically.

In summary, we have shown that, by using $[\text{H}(\text{OEt}_2)_2][\text{B}(\text{Ar}_t)_4]$ as the proton source, vinylideneruthenium(II) complexes of the general composition $[\text{RuCl}(\kappa^2\text{-O}_2\text{CR})(\text{C}=\text{CHPh})(\text{P}i\text{Pr}_3)_2]$ ($\text{R} = \text{H}, \text{CH}_3, \text{Ph}, \text{CF}_3$) can be readily protonated at the β -carbon atom of the vinylidene unit to give the corresponding cationic carbyneruthenium derivatives. The main conclusion is that it critically depends on the substituent R of the carboxylate whether a subsequent intramolecular shift of the carboxylate oxygen atom to the carbyne carbon atom to give a carbene is energetically preferred. For $\text{R} = \text{CF}_3$, the difference in energy between the metal carbyne and the metal carbene is quite small and therefore it is possible to observe—as far as we are aware for the first time—^[12, 14] an equilibrium between two isomers containing a $\text{M}\equiv\text{C}$ and a $\text{M}=\text{C}$ bond.

Experimental Section

All reactions were carried out under argon. The NMR data of the phenyl groups and the $\text{B}(\text{Ar}_t)_4^-$ ion are omitted for clarity. Abbreviations: ν = virtual coupling, $N = {}^3J(\text{P,H}) + {}^5J(\text{P,H})$ or ${}^1J(\text{P,H}) + {}^3J(\text{P,H})$.

The syntheses and spectroscopic data of compounds **5**–**8** and **10**–**12** are given in the Supporting Information.

13a: A mixture of **8** (and small amounts of **4** and **9**; in total 181 mg, proportion of **8** 84%, 0.27 mmol of Ru) and $[\text{H}(\text{OEt}_2)_2][\text{B}(\text{Ar}_t)_4]$ (268 mg, 0.27 mmol) was treated with CH_2Cl_2 (2 mL) at -78°C . The resulting suspension was stirred and allowed to warm to room temperature for about 15 min. Addition of pentane (20 mL) to the brown-orange solution led to the precipitation of a yellow solid which was recrystallized from CH_2Cl_2 (2 mL) at -78°C ; yield 347 mg (99% based on initial content of **8** in the starting material); m.p. 97°C (decomp); ^1H NMR (400 MHz, CD_2Cl_2 , -20°C): $\delta = 4.86$ (br, 2H; CH_2Ph), 2.62 (m, 6H; PCHCH_3), 1.30 (dvt, $N = 14.8$, ${}^3J(\text{H,H}) = 7.2 \text{ Hz}$, 18H; PCHCH_3), 1.27 (dvt, $N = 15.3$, ${}^3J(\text{H,H}) = 7.2 \text{ Hz}$, 18H; PCHCH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (100.6 MHz, CD_2Cl_2 , -20°C): $\delta = 64.9$ (s; CH_2Ph), 24.8 (vt, $N = 10.2 \text{ Hz}$; PCHCH_3), 19.6, 19.0 (both s; PCHCH_3), $\text{Ru}=\text{C}$, CF_3CO_2 and CF_3CO_2 not observed; $^{31}\text{P}\{^1\text{H}\}$ NMR (162.0 MHz, CD_2Cl_2 , -20°C): $\delta = 56.5$ (br); ^{19}F NMR (376.4 MHz, CD_2Cl_2 , -20°C): $\delta = -73.96$ (br; CF_3CO_2).

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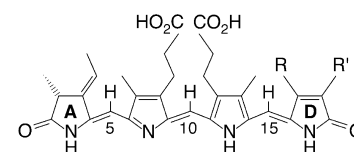
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Model Studies of Phytochrome Photochromism: Protein-Mediated Photoisomerization of a Linear Tetrapyrrole in the Absence of Covalent Bonding**

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Dedicated to Professor Günther Wilke on the occasion of his 75th birthday

Open-chain tetrapyrroles, such as phytochromobilin (**1**) and phycocyanobilin (**2**), serve as chromophores in a number of chromoproteins. Their photoreactivity is largely regulated by the protein environment. Whereas **2** functions as an



1	R = CH_3 ,	R' = $\text{CH}=\text{CH}_2$
2	R = CH_3 ,	R' = CH_2CH_3
3	R = $\text{CH}=\text{CH}_2$,	R' = CH_3

antenna chromophore in the light-harvesting pigments of cyanobacteria,^[1] **1** is the chromophore of the plant photo-receptor phytochrome,^[2] where it reversibly photoisomerizes around the $\text{C}_{15}-\text{C}_{16}$ double bond thereby converting the physiologically dormant P_r form into the active P_{fr} state.^[2, 3] This photochromic control, triggered by a double-bond isomerization, is not restricted to **1**. For example, **2** in the phytochrome of the alga *Mesotaenium caldarium* functions as a photochemical trigger analogous to **1**.^[4] Furthermore, **2** can also assume this role in recombinant phytochromes of higher plants.^[5] In all these photoreceptors, the chromophore is invariably bound by a covalent thioether bond to a cysteine residue of the protein.

We have now explored whether covalent chromophore bonding to the apoprotein is a prerequisite to the phototrigger function. We find, for the first time, that such is not the case. Rather, the chromophore binding pocket of the apoprotein can accommodate a chromophore sufficiently well to control the host–guest interactions driving the $\text{P}_r \rightleftharpoons \text{P}_{fr}$ photocycle, without the need for covalent bonding.

The boundary conditions for the influence of the protein matrix on the absorption and photochemical properties of the phytochrome chromophore have not yet been studied thoroughly. The regioselective double-bond photoisomerization at

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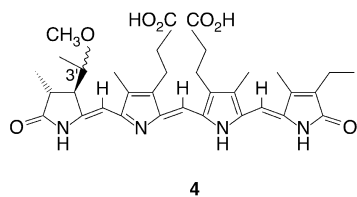
[**] This work is part of the PhD thesis of I. Lindner, Max-Planck-Institut für Strahlenchemie, Mülheim an der Ruhr, and Gerhard-Mercator-Universität, Duisburg, **2000**. We thank Tanja Berndsen, Gül Koç, and Helene Steffen for technical assistance.

C-15 in the protein-bound form of open-chain tetrapyrroles, such as **1** and **2**, is attributed to a steering effect of the surrounding protein matrix. In homogeneous organic solutions they are presumed to preferentially photoisomerize around the C-10 double bond.^[6] Moreover, the 10*E* isomer of **2** is thermally unstable and reverts to the *Z* form within nanoseconds at ambient temperature.^[6, 7]

It has also been found that the phytochrome A (phyA) photochromism is influenced by only a few amino acid residues,^[8] and that both ligation of **2** to the apoprotein and photochromism depend critically on the two propionate side chains of **2**, which appear to position the chromophore prior to binding and to maintain its conformation.^[8a] Furthermore, we have shown^[9] that variation of ring D substitution in the phytochromobilin isomer **3** is tolerated in the P_r/P_{fr} photocycle, while the absorption maximum of the P_{fr} form is selectively blue shifted (that is, shifted to shorter wavelengths).

Finally, the significance of this study using a phytochrome-like protein with a covalently nonbonding chromophore is accentuated by the fact that a *bacteriophytochrome* has recently been found^[10] which possesses a histidine rather than the usual cysteine at the site for covalent bonding and, thus, broadens the native structural variability of the phytochrome family.

When recombinant oat apophyA 65^[11] was incubated with **4**, a methanol adduct to **2**,^[12] the chromophore was embedded in the apoprotein. The resulting complex, apophyA 65/**4**,



proved capable of undergoing a photoreversible transformation reminiscent of the phytochrome photocycle ($\lambda_{\text{max}} = 643$ and 705 nm, somewhat blue shifted but analogous to the P_r and P_{fr} forms of recombinant phyA65-2, respectively; Figure 1A), even though the methanol addition blocks the

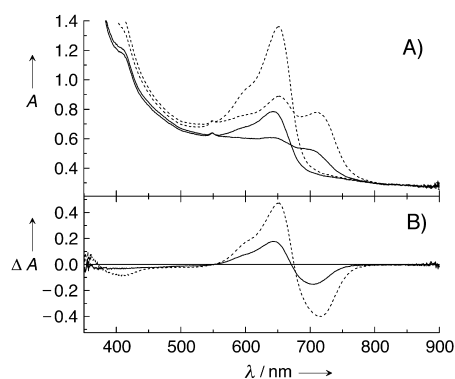
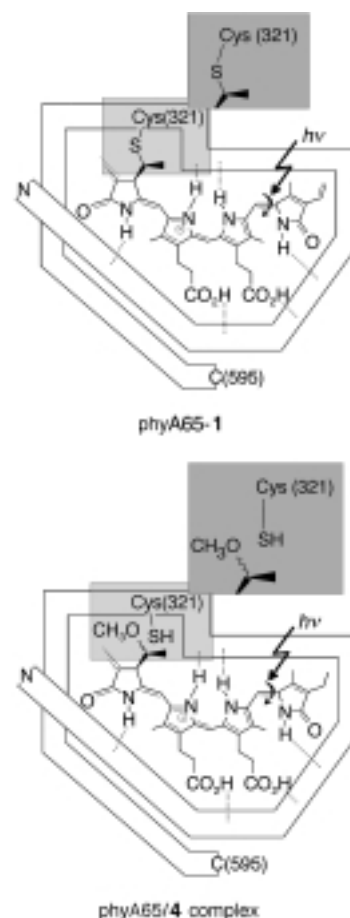


Figure 1. P_r and P_{fr} absorption (A) and P_r - P_{fr} difference absorption spectra (B) of recombinant oat phyA65-2 (dashed lines) and apophyA65/4 complex (complete lines), after saturating red and far-red irradiations. The spectra of phyA65-2, as shown here, were recorded in the competition experiment with apophyA65/4 and 2.

attachment site (C-3') on the chromophore and prevents covalent bonding of **4** to the apoprotein (Scheme 1^[14]).^[15] Repetitional cycling at appropriate irradiation wavelengths ($\lambda = 614$ and 714 nm for excitation of the P_r- and P_{tr}-like forms, respectively) did not cause any loss of the 643 and 705 nm absorbances.



Scheme 1. Schematic representations of the chromophore pockets of P_r phytochrome^[14] (top) and the P_r-like apophyA 65/4 complex (bottom). The arrows indicate the *Z* → *E* double-bond photoisomerization that leads to the P_{fr} forms.

The noncovalent incorporation of the methanol adduct in the apophyA65/4 complex was demonstrated conclusively by a competition experiment. Upon addition of **2** to the P_r-like form of the complex under conditions known^[11b,c] to bring about spontaneous covalent bonding to the apoprotein, the methanol adduct **4** was promptly replaced in the binding pocket. The familiar chromoprotein, the P_r form of phyA65-2,^[11] was formed instead (Figure 1) and was capable of undergoing the characteristic P_r/P_{fr} photocycle (recombinant phyA65-2: λ_{max} = 653 nm for P_r, and 718 nm for P_{fr}^[11c]).

The absorption intensity of the P_{fr}-like form of apophyA65/4 is somewhat lower, relative to P_r, than is customary for the P_{fr} forms with covalently bound chromophores (**1** or **2**), and the spectral shape suggests the presence of more than just two absorbing components (Figure 1 A). Nevertheless, in the difference absorption spectrum of apophyA 65/4 (Figure 1 B), the intensities of the P_r- and P_{fr}-like features are comparable

to those of wild-type phytochromes. The greater breadth of the absorption bands, relative to those of P_r and P_{fr} , possibly reflects a wider conformational population of chromophores in the binding pocket than is encountered in the phytochromes. It transpires that the covalent bonding to Cys321 (the binding position in the oat phytochrome) is essential for a conformationally uniform incorporation of the chromophore into the protein. Undoubtedly, this arrangement is further supported by hydrogen bridges and dipolar interactions. In the absence of the covalent bonding, these nonbonding forces are insufficient to selectively provide the optimum conformation. Nevertheless they are good enough to ensure an arrangement which is reasonably stable—albeit one which allows exchange of the methanol adduct **4** with the unsaturated phycocyanobilin (**2**)—and is capable of performing a phototrigger function that mimics the phytochrome photochromism.

Experimental Section

Compound **4**: **2** and **4** were obtained from *Spirulina platensis* upon methanolysis in the routine preparation of **2**.^[16] After treating the lyophilized cyanobacteria (500 g) for 16 hr with refluxing MeOH under argon in the dark, the bilin extract was purified by reverse-phase column chromatography. Preparative HPLC (Chromasil RP-18, 1 mL min⁻¹, MeCN:phosphate buffer(7:18, 7.5 mM, pH 7.6)) separated **2** and **4** (12:1). Acetonitrile was removed from the latter fraction in vacuo and the residue was extracted with CH₂Cl₂:*n*-hexane at -70 °C. UV/Vis (100 mM Tris, 200 mM NaCl, 10% glycerol, 0.2% DMSO, pH 8.0): λ_{max} = 348, 593 nm. ¹H NMR (500 MHz, C₅D₅N, ¹H/¹H-COSY): δ = 1.24 (t, ³J(H,H) = 7.52 Hz, 3H; H₃C-18³), 1.31 (d, ³J(H,H) = 6.30 Hz, 3H; H₃C-3²), 1.43 (d, ³J(H,H) = 7.35 Hz, 3H; H₃C-2¹), 1.97, 2.07, 2.12, 3.30 (4s, 3H each; H₃C-7¹, -17¹, -13¹, and H₃CO-3¹, respectively), 2.49 (m, 2H; H₂C-18¹), 2.78, 3.06 (2m, 1H each; HC-2 and -3, respectively), 2.85 (m, 4H; H₂C-8² and -12²), 3.10, 3.18 (2t, ³J(H,H) = 7.42, 7.23 Hz, 2H each; H₂C-8¹ and -12¹, respectively), 3.75 (m, 1H; HC-3¹), 5.68, 6.06, 7.30 (3s, 1H each; HC-5, -15, and -10, respectively); the signals were assigned by decoupling and NOESY experiments. HR-MS (ESI positive ion mode): C₃₄H₄₂N₄O₇Na [M^+ Na]: calcd 641.2951, found 641.2999.

The structure of **4**^[12] was confirmed by MS and comparison of the ¹H NMR spectra with in-house recorded data for **2**, and literature data for the dimethylesters of **2**,^[17] **4**,^[13] and adducts of **2** with short Cys-containing peptides.^[18] The sample of **4** that was used for the assembly with apophyA 65 was homogeneous by HPLC, MS, and ¹H NMR spectroscopy. In particular, it was not contaminated with other tetrapyrrole components, such as **2**.

Assembly of the apophyA 65/**4** complex and irradiation: Oat apophyA 65^[11] (crude lysate of *Hansenula polymorpha* yeast cells;^[11a] 4.5 nmol in 600 μ L phosphate buffer, pH 7.6) was incubated with **4** (5 nmol in 1 μ L DMSO) at ambient temperature. The apoprotein concentration was determined prior to incubation, by assembly of an aliquot of the apoprotein solution with excess **2**.

The solution of apophyA 65/**4** complex was irradiated with light from a slide projector bulb (250 W; distance between sample cell and bulb was approximately 20 cm; excitation wavelengths of λ = 614 $\pm$ 7 and 714 $\pm$ 7 nm were determined for $P_r \rightarrow P_{fr}$ and $P_{fr} \rightarrow P_r$, respectively, with interference filters). Complete conversions (maximum absorption values) required irradiation periods of about 10 min. Absorption spectra were recorded with a Shimadzu spectrophotometer UV2102PC.

Competition experiment with apophyA 65/**4** complex and **2**: Compound **2** (1.7 nmol in 1 μ L DMSO) was added to a sample of the P_r -like form of the

apophyA 65/**4** complex (600 μ L). The mixture was kept in the dark for several min prior to consecutive irradiations at λ = 614 $\pm$ 7 (interference filter; $P_r \rightarrow P_{fr}$) and approximately 720 nm (cut-off filter; $P_{fr} \rightarrow P_r$). Absorption spectroscopic analyses were performed prior to the first and after each irradiation.

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C₆₀F₁₈, a Flattened Fullerene: Alias a Hexa-Substituted Benzene**

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The aromaticity of fullerenes^[1] is restricted by the increase in (bond-shortening) strain within the pentagonal rings that arises from the degree of double-bond character they must acquire following electron delocalization.^[2] Nevertheless, some degree of delocalization in the carbon cage exists, as shown by the sequential pattern of polyadditions to fullerenes. Addition to one 6–6 “double” bond localizes the electrons in the remaining double bonds of the same hexagon and causes them to undergo preferential addition,^[3] as exemplified by the products of hydrogenation,^[4] and fluorination.^[5]

The strain caused by the presence of a delocalized double bond within a pentagon is diminished when any of the remaining three carbon atoms in the pentagon changes its hybridization from sp² to sp³; this change facilitates delocalization in the adjacent hexagons. This principle was invoked when the tetrahedral (*T*) molecular structure for C₆₀H₃₆ was suggested^[6] and accounts for the octahedral addition patterns to [60]fullerene,^[7] for example, in the formation of [C₆₀{Pt(PEt₃)₂}]₆.^[8] In each case more-delocalized benzenoid rings are created. A novel *D*₃ addition pattern resulting from the addition of tetramethylazomethine ylide to [60]fullerene was observed recently by Rubin and co-workers and is likewise a consequence of the creation of a combination of benzenoid and naphthalenoid rings.^[9]

Relatively few single-crystal X-ray structures have been determined for fullerenes, and only three are known for halogenofullerenes, namely, C₆₀Br₂₄,^[10] C₆₀Br₈, and C₆₀Br₆.^[11] Two X-ray structures have provided evidence for the addition-driven delocalization of electrons described above. The lengths of the 6–6 and 6–5 bonds are 1.39 and 1.42 Å, respectively, in the eight semi-benzenoid rings of C₆₆(COOEt)₁₂, produced by the octahedral addition of six

diethyl malonyl moieties to [60]fullerene. Similar values have been obtained for a related tethered hexakis adduct.^[12] The alternation is thus less than in [60]fullerene itself (about 1.40 and 1.45 Å).^[13]

A few fully characterized fluorofullerenes have been produced, for example, C₆₀F₄₈,^[14] C₆₀F₃₆ (both *T* and *C*₃ isomers),^[15] and C_{3v}-C₆₀F₁₈.^[16] The high stability of the latter three derivatives is attributed to the presence of four-, three-, and one π-delocalized benzenoid rings, respectively. We now report the first single-crystal X-ray structure of a fluorinated fullerene, C₆₀F₁₈ (as a toluene solvate), which reveals that one half of the [60]fullerene cage is flattened (Figure 1). The

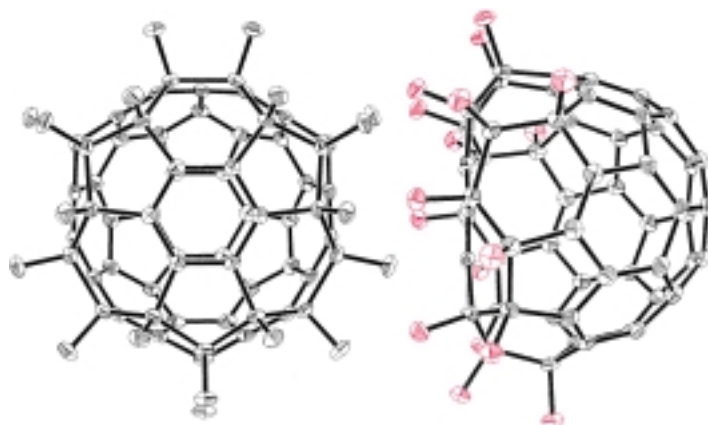


Figure 1. Two projections of C₆₀F₁₈ (ORTEP).

hexagonal face at the center of the fluorinated crown has equal bond lengths, which shows it is fully aromatic. It is the first truly benzenoid ring identified on a fullerene surface. The unique geometry of the molecule makes it also a hexa-substituted benzene (specifically here a “pseudocyclophane” with a nondistorted fullerene hemisphere opposite to the arene ring).

The single-crystal X-ray crystallographic study confirms the conclusions reached previously from the ¹⁹F NMR spectrum,^[16] namely, that all the fluorine atoms are bound to one hemisphere of the [60]fullerene cage. The “upper” six-membered ring of sp² carbon atoms is isolated from the residual molecular π system by a “belt” of sp³-hybridized carbon atoms. The Schlegel diagram (Figure 2) shows the positions of the fluorine atoms, together with the notation for the symmetry-independent C–F and C–C bonds and the conformations of the nonplanar polygons. A similar addition pattern was earlier predicted for C₆₀H₁₈ by semiempirical AM1 quantum chemistry calculations,^[17] and confirmed by ¹H NMR spectroscopy.^[18] Although such calculations (and indeed simple molecular models) indicate substantial cage flattening, the X-ray data (Figure 1) now reveal the actual extent to which this occurs.

The distances (Δ_{*i*}) of the C atoms from the center of the carbon cage,^[19] and the spherical excesses (φ_{*i*}) of the “surface” environments of the carbon atoms were used, respectively, to characterize the radial atomic displacements and surface puckering in C₆₀F₁₈. The latter φ_{*i*} parameter was defined as 360° minus the sum of three C–C–C bond angles at the *i*th

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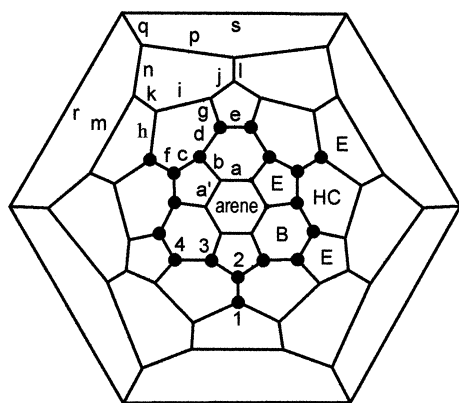


Figure 2. Schlegel diagram for $C_{60}F_{18}$ showing the symmetry-independent fluorine atoms (1–4), C–C bonds (a–s) and ring conformations (see text) within the idealized C_{3v} symmetry.

carbon atom. Standard values in [60]fullerene itself are $\Delta = 3.57 \text{ \AA}$ and $\varphi = 12^\circ$ for all atoms. Using these parameters four regions on the surface of the distorted cage may be distinguished in $C_{60}F_{18}$: 1) a planar six-membered “upper” cycle with $\Delta = 2.97 \text{ \AA}$ and $\varphi < 0.1^\circ$; 2) a puckered fluorinated belt of eighteen $C(sp^3)$ atoms ($3.8 < \Delta < 4.0 \text{ \AA}$; $26.4 < \varphi < 29.9^\circ$); 3) a flattened equatorial belt of twelve $C(sp^2)$ atoms bonded directly to $C(sp^3)$ atoms ($3.5 < \varphi < 5.2^\circ$); 4) a residual bottom hemisphere made up of 24 $C(sp^2)$ atoms which is close to the normal fullerene geometry ($7.8 < \varphi < 13.3^\circ$). The Δ_i parameter, which is strongly dependent on the choice of the molecule center, is not informative in the last two regions because of the small radial distortions ($2\text{--}3 \text{ pm}$).^[19]

Table 1 shows the bond lengths in $C_{60}F_{18}$ averaged within the ideal C_{3v} symmetry (the lengths of symmetry-related bonds mostly agree within a few pm). The “upper” hexagonal ring, planar within $0.003 \text{ \AA}$, shows no alternation in the 6–5

Table 1. Bond lengths [$\text{\AA}$] in $C_{60}F_{18}$ (for notations, see Figure 2).

C–C				C–F	
a,a'	1.372	j	1.428	1	1.396
b	1.476	k	1.435	2	1.361
c	1.623	l	1.386	3	1.385
d	1.557	m	1.437	4	1.377
e	1.672	n	1.387		
f	1.558	p	1.436		
g	1.500	q	1.453		
h	1.524	r	1.387		
i	1.363	s	1.447		

and 6–6 carbon–carbon bond lengths and is thus fully aromatic. Neighboring $C(sp^3)$ atoms deviate from the mean plane of this ring towards the rest of the molecule by $0.055\text{--}0.075 \text{ \AA}$. The average C–C bond lengths in the arene ring ($1.372 \text{ \AA}$) and those attached to it ($1.476 \text{ \AA}$) are noticeably shorter than in either the related fluorocarbon species, perfluorinated indacene $C_{15}F_{18}$ (1.389 and $1.499 \text{ \AA}$),^[20] or hexa(trifluoromethyl)benzene (1.405 and $1.541 \text{ \AA}$).^[21] The bond lengths of the ring are essentially the same as the average CSD value in hexafluorobenzene ($1.368 \text{ \AA}$), which is indicative of the strong electron-withdrawing effect of the eighteen fluorine atoms in the “upper” hemisphere.

The pentagons and hexagons adjacent to the planar face have envelope and boat conformations, respectively. Their neighboring pentagons and hexagons are flattened envelopes and half-chairs, respectively (Figure 2). Single $C(sp^3)\text{--}C(sp^3)$ bonds in the fluorinated belt are elongated to $1.623 \text{ \AA}$ (bonds c) and $1.672 \text{ \AA}$ (bonds e). Such elongation is typical for fluoro-substituted hydrocarbons and polycyclic fluorocarbons.^[22]

The lengths of four symmetrically independent C–F bonds (1–4) differ slightly, but significantly ($1.361\text{--}1.396 \text{ \AA}$, Table 1). The averaged C–C–F bond angles have normal tetrahedral values of $106.9\text{--}111.3^\circ$ with a slight asymmetry relative to the C_{60} framework; this observation reflects the stronger asymmetry of their opposite C–C–C angles in the puckered five-membered ($100.4\text{--}109.9^\circ$) and six-membered rings ($108.3\text{--}122.6^\circ$), composed of sp^2 and sp^3 carbon atoms.

The alterations in the cage C–C bonds in $C_{60}F_{18}$ relative to those in C_{60} (mapped^[23] in Figure 3) are: a) a small shortening in the aromatic ring from the standard value of $1.399 \text{ \AA}$ in arenes;^[24] b) a strong elongation of the $C(sp^3)\text{--}C(sp^3)$ bonds in the fluorinated belt; c) a smaller but significant ($0.01\text{--}0.03 \text{ \AA}$) shortening of the $C(sp^2)\text{--}C(sp^3)$ bonds in the adjacent “flattened” belt; and d) no significant bond redistribution in the “lower” hemisphere. The distribution of the charge mostly in the vicinity of the fluorinated belt agrees with the generally observed poor relay of electronic effects through the [60]fullerene framework, which is necessitated by the need to avoid the energetically unfavorable reduction in bond lengths in the pentagons.^[25] Thus, for example, the unpaired electrons resulting from radical additions are highly localized,^[26] and electron addition produces only minor changes in the Raman spectrum since the general cage structure is retained.^[27]

The $C_{60}F_{18}$ molecules are linked by inversion centers in anti-parallel zigzag chains along the [101] direction of the crystal lattice (Figure 4). The planar “upper” arene face of each molecule in these chains has normal van der Waals contacts with the “lower” fullerene hemisphere of the neighboring chain, with the shortest center-to-center distance in the chain being $9.4 \text{ \AA}$. The shortest intermolecular $CF \cdots C(\text{cage})$ contacts between neighboring chains are 2.75 and $2.92 \text{ \AA}$ (from the type 4 C–F bond to the *i*-type $C(sp^2)\text{--}C(sp^2)$ bond). From the observation of a lack of elongation of these bonds and the slightly lower spherical excess φ_i values of the surrounding $C(sp^2)$ (3.5 and 4.4°) and $C(sp^3)$ (26.4°) atoms we suggest that these shortened $F \cdots C$ contacts are forced and therefore

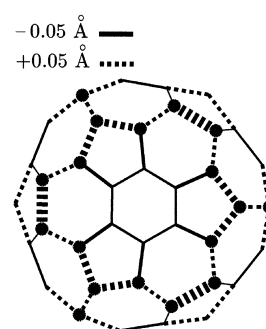


Figure 3. Shortening (filled lines) and elongation (dotted lines) of the C–C bonds in $C_{60}F_{18}$, averaged within a C_{3v} symmetry, from their standard lengths of $1.397 \text{ \AA}$ ($sp^2\text{--}sp^2$), $1.507 \text{ \AA}$ ($sp^2\text{--}sp^3$), and $1.530 \text{ \AA}$ ($sp^3\text{--}sp^3$; see ref. [24]). The thickness of the bonds is proportional to the extent of their shortening/elongation; fluorinated sp^3 carbon atoms are marked by black circles. Bond lengths in the opposite hemisphere (not shown) coincide within $0.02 \text{ \AA}$ with those in C_{60} .

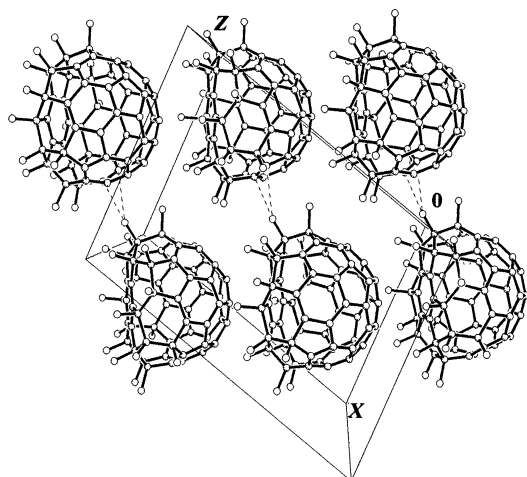


Figure 4. Molecules of $C_{60}F_{18}$ in the crystal lattice.

repulsive ones. Attractive electrostatic interactions between the strongly polarized $C_{60}F_{18}$ molecules probably account for the closeness of the molecules and the unusually high degree of molecular ordering in $C_{60}F_{18} \cdot C_6H_5CH_3$. Such electrostatic forces, expressed in multipole interactions, are believed to be a reason for a weak specific bonding to donors in molecular complexes of hexafluorobenzene.^[28]

The distorted geometry of the $C_{60}F_{18}$ molecule is quantitatively reproduced by quantum chemistry calculations, even on a semiempirical PM-3 level. Ab initio HF calculations (GAUSSIAN-94, 3-21G* and 6-31G basis sets, geometry optimized) convergently predict a substantial polarization of the C–F bonds with charges of -0.34 to -0.39 on the fluorine atoms and charges of $+0.25$ to $+0.40$ on the sp^3 carbon atoms. The calculated charges are mainly located in the “fluorinated” belt of the C_{60} moiety, which is in agreement with the experimental redistribution of the C–C bond lengths (Figure 3). The unique arene ring carries a small positive charge per carbon atom ($+0.04$ in 3-21G* and $+0.03$ in 6-31G). Both HF approximations used ascribe a large dipole moment to the molecule (12.4 D in 3-21G* and 15.7 D in 6-31G); however, smaller values of 6–7 D have been predicted independently.^[29] DFT calculations (B3LYP/6-31G) give similar values to those obtained by HF, but with the C–F bond distance overestimated by $0.04 \text{ \AA}$, a slightly more uniform charge distribution, and a predicted dipole moment of 13.3 D.

The very strongly electron-deficient planar region suggests that this molecule may find important applications in intramolecular electron-transfer complexes, whilst η^6 coordination with transition metal atoms may also be possible. These aspects are to be investigated.

Experimental Section

$C_{60}F_{18}$ was prepared and purified as described previously.^[16] Light-yellow diamond-shaped plates of $C_{60}F_{18} \cdot C_6H_5Me$ with a good X-ray quality were grown from toluene. The structure was determined by X-ray diffraction.^[30] Errors (esd) for the C–F and C–C bond lengths were 0.002 – 0.003 and 0.003 – $0.004 \text{ \AA}$, respectively.

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[30] Crystal data for $\text{Ce}_{60}\text{F}_{18} \cdot (\text{C}_7\text{H}_8)_x$, $M_r = 1154$, monoclinic, space group $P2_1/n$, $a = 11.532(2)$, $b = 21.501(3)$, $c = 16.261(2)$ Å, $\beta = 101.798(5)^\circ$, $V = 3947$ Å³, $Z = 4$, $\mu(\text{MoK}\alpha) = 0.17$ mm⁻¹, $T = 100$ K. 49263 Reflections measured (Siemens SMART diffractometer, $2\theta_{\text{max}} = 60^\circ$) 11565 unique, refined on F^2 using SHELXTL, $R1 = 0.049$ (for 6426 reflections with $I > 2\sigma(I)$), $wR2 = 0.118$ (for all 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-142291. 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).

The First Microporous Framework Cerium Silicate**

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Recently, much research has been carried out aimed at preparing inorganic microporous zeotype solids composed of interconnected octahedral- and tetrahedral-oxide polyhedra. One such family of materials of considerable interest embraces microporous titanosilicates and their derivatives which contain Ti^{IV} usually in octahedral coordination.^[1, 2] As part of a systematic search for novel microporous framework inorganic materials we have prepared (among others) AV-1, a synthetic analogue of the rare sodium yttrium silicate mineral montregianite ($\text{Na}_4\text{K}_2\text{Y}_2\text{Si}_{16}\text{O}_{38} \cdot 10\text{H}_2\text{O}$).^[3, 4] In nature, montregianite usually occurs with framework substitution of some yttrium by cerium and this raised the possibility of obtaining a purely cerous form of AV-1. Here we report the synthesis and characterization of AV-5, a microporous framework sodium cerium silicate with the structure of montregianite.

It is well known that Ce^{III} -containing materials present potential for applications in optoelectronics. For example, Ce^{III} in a LiYF_6 matrix has been shown to lase.^[5] This system may find important applications since Ce^{III} lasers emit in the high-energy region of the spectrum (blue or UV) and they exhibit tunability due to the inherently broad emission lines. Glass-supported cerium is used in Faraday rotators, which protect lasers from back-reflected light.^[6] As for other lanthanides, cerium has been used in transducers designed to detect or modify IR, UV, X-ray, and gamma radiation.^[6] The luminescence of Ce^{III} /silica systems prepared by sol-gel methods has also been studied.^[7] Our main aim with this work

was to explore the possibility of preparing porous cerium silicates with potential for use in optoelectronics.

The crystal structure of montregianite (and AV-5) consists of two different types of layers alternating along the [010] direction (Figure 1): a) a double silicate sheet, in which the

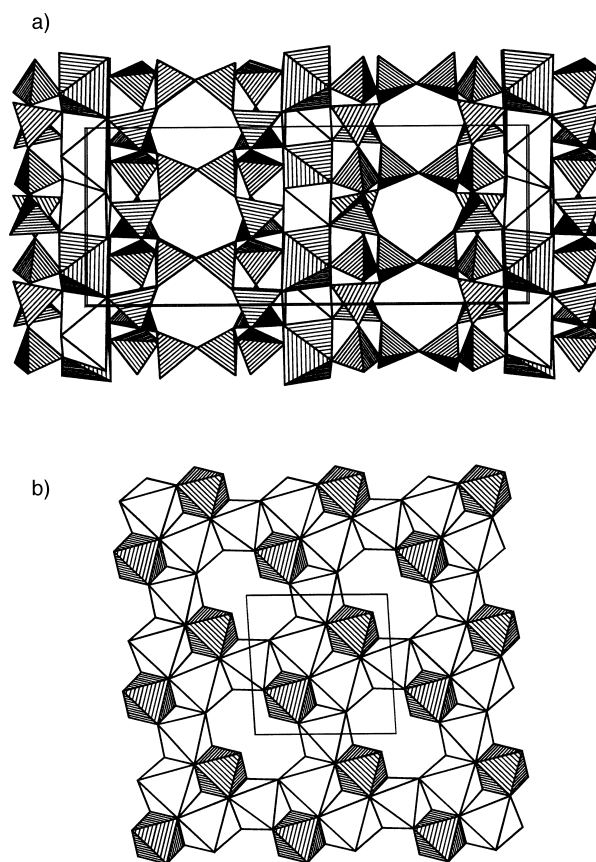


Figure 1. Polyhedral representation of the montregianite structure: a) viewed along [100] showing the alternating octahedral sheet and double silicate sheet along [010]; b) open octahedral sheet, viewed along [010], consisting of Y and three different Na^+ octahedra (unfilled octahedra).

single silicate sheet is of the apophyllite type with four- and eight-membered rings, and b) an open octahedral sheet composed of $[\text{YO}_6]$ (or $[\text{CeO}_6]$ in AV-5) and three distinct $[\text{NaO}_4(\text{H}_2\text{O})_2]$ octahedra.^[8] The layers are parallel to the (010) plane. The potassium ions are ten-coordinate and the six water molecules (not shown) are located within large channels formed by the planar eight-membered silicate rings.

Figure 2 shows the experimental and simulated powder X-ray diffraction (XRD) patterns of AV-5. The differences observed are due to the presence of a small amount of an, as-yet, unknown impurity. The unit cell parameters have been refined by assuming a monoclinic unit cell, space group $P2_1/n$, and cell dimensions $a = 9.6906(9)$, $b = 24.055(2)$, $c = 9.5759(8)$ Å, and $\beta = 93.683(7)^\circ$ and are similar to those reported for montregianite, dimensions $a = 9.512$, $b = 23.956$, $c = 9.617$ Å, and $\beta = 93.85^\circ$.^[9]

Presumably owing to the presence of paramagnetic Ce^{III} centers we were unable to record a ^{29}Si magic-angle spinning (MAS) NMR spectrum of AV-5. It is, however, possible to oxidize Ce^{III} to Ce^{IV} by calcining AV-5 in air at 300°C for 3 h.

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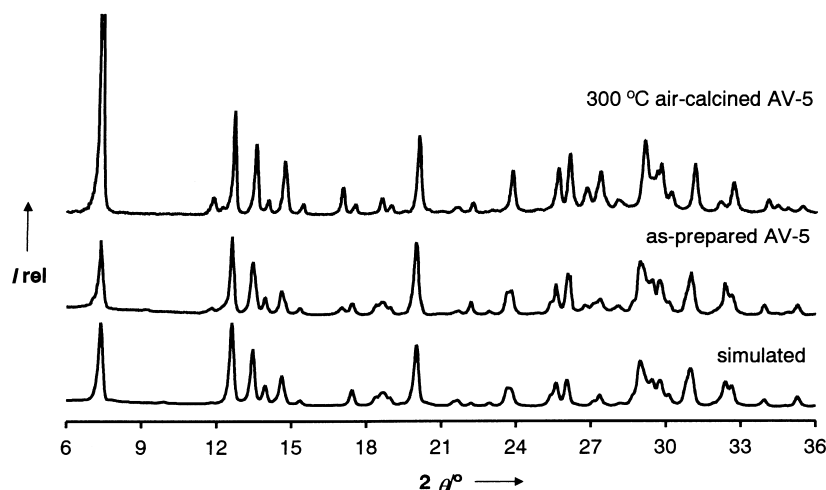


Figure 2. Experimental and simulated powder X-ray diffraction patterns of as-prepared AV-5 and 300 °C air-calcined AV-5.

In the process, the faint pink powder becomes light yellow. As shown by powder XRD (Figure 2), this treatment does not compromise the materials structural integrity. The oxidation of Ce^{III} to Ce^{IV} is accompanied by the reduction of O_2 to O^{2-} . The potassium ions which are not necessary to balance the new (less negative) AV-5 framework charge likely form an (unidentified, probably amorphous) oxide phase. The ^{29}Si MAS NMR spectrum of calcined AV-5 (Figure 3a) suggests

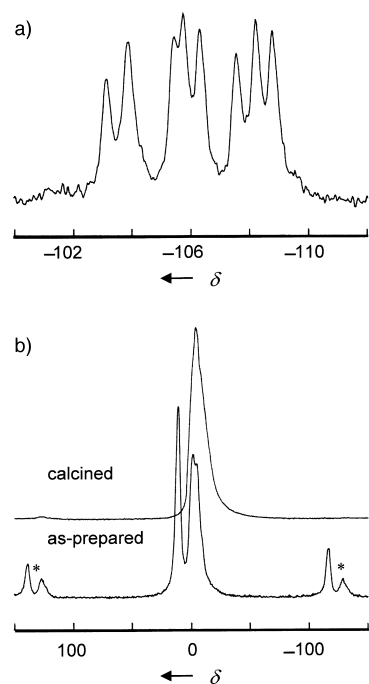


Figure 3. a) The ^{29}Si MAS NMR spectrum of as-prepared AV-5 displays eight resonance signals indicating that this solid possesses the structure of montregianite which contains an equal number of nonequivalent Si sites. The spectrum was recorded at 79.5 MHz on a Bruker MSL 400P with 45° pulses, a 5 kHz spinning rate, and 50 s recycle delays. Chemical shifts are quoted in ppm (referenced to TMS). b) Single-quantum ^{23}Na MAS NMR spectra of as-prepared and 300 °C air-calcined AV-5 recorded at 105.5 MHz with 12° pulses, 13.5 kHz spinning rates, and 2 s recycle delays. Asterisks denote spinning side bands attributed to the presence of paramagnetic Ce^{III} centers.

that AV-5 and montregianite have the same structure. Indeed, this spectrum displays eight resonance signals (deconvoluted intensities in parentheses) centered at $\delta = -103.1$ (0.9), -103.9 (1.1), -105.4 (0.9), -105.7 (1.0), -106.3 (1.1), -107.5 (1.0), -108.2 (1.1), -108.8 (1.0) in accord with the crystal structure of montregianite which reveals eight unique Si sites with equal populations. Based on the crystal structure of montregianite we attempted to assign the AV-5 ^{29}Si MAS NMR resonance signals. Two silicate tetrahedra (Si1 and Si4), one each within the four-membered rings belonging to a single sheet, have upward-pointing apices (O1) which are shared with the apices of the two downward-pointing corresponding tetrahedra (Si4 and Si1, respectively) in the second silicate sheet.^[8] Si1 and Si4 are Si(4Si) environments (Si linked to four

other Si through oxygen bridges) and the ^{29}Si MAS NMR resonance signals at $\delta = -108.8$ and -108.2 are tentatively assigned to them. The assignment of the peaks at high frequency is more difficult. Si6 is of the type Si(3Si, 1Ce). Si6–O12 is the shortest nonbridging bond length (1.562 Å in montregianite) involving the most charge-deficient oxygen atom which is bonded to a Ce atom. Considering the well known correlation between the ^{29}Si chemical shifts and the Si–O bond lengths in silicates,^[10] we tentatively assign the peak at $\delta = -107.5$ to Si6. The assignment of the other resonance signals is difficult because the NMR data available for cerium silicates is very limited.

It was possible to record a single-quantum (“conventional”) ^{23}Na MAS NMR spectrum of as-prepared AV-5. This observation immediately suggests that the solid contains some Ce^{IV} . The spectrum depicted in Figure 3b (MAS at 13.5 kHz) displays strong spinning side bands (much more so than those observed in the spectrum of synthetic montregianite) indicating the presence of Ce^{III} centers. When this spectrum is recorded with a MAS rate of 30 kHz (not shown) the prominent spinning side bands are not observed. The two central NMR resonance signals observed exhibit characteristic second-order quadrupole powder patterns centered at $\delta = 12$ and 0. The triple-quantum (3Q) MAS NMR spectrum^[11] of as-prepared AV-5 (Figure 4a) confirms that only two resonance signals are present. The AV-5 sample calcined in air at 300 °C gives a much less resolved single-quantum ^{23}Na MAS NMR spectrum (Figure 3b) and, because most Ce^{III} has been oxidized, the spinning side bands seen with MAS at 13.5 kHz (essentially due to second-order quadrupole effects) are very much fainter. The 3Q MAS NMR spectrum (Figure 4b), however, shows that two main ^{23}Na resonance signals are still present, with somewhat different quadrupole parameters. Although montregianite contains three nonequivalent sodium sites the $[\text{Na}(2\text{A})\text{O}_4(\text{H}_2\text{O})_2]$ and $[\text{Na}(2\text{B})\text{O}_4(\text{H}_2\text{O})_2]$ octahedra (populations 0.47 and 0.45, respectively) are very similar in configuration and it is unlikely that they can be distinguished by solid-state ^{23}Na NMR spectroscopy.^[3, 4, 8] The $[\text{Na}(1)\text{O}_4(\text{H}_2\text{O})_2]$ site (population 0.95) forms a weak seventh bond with O5B and is, thus, distorted. The 3Q peak S1 given

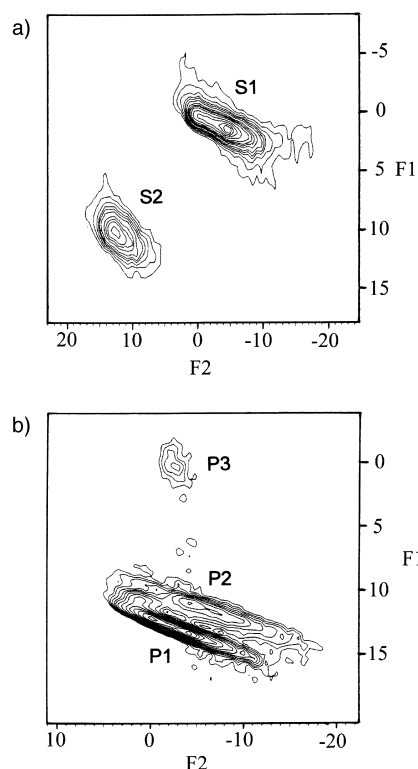


Figure 4. The unsheared 3Q ^{23}Na MAS NMR spectra of a) as-prepared and b) 300 °C air-calcined AV-5 show the presence of two main types of Na sites which are to be expected if the solids possess the structure of montregianite. A simple two-pulse sequence was used for recording the spectra.^[12] The spectrum of as-prepared AV-5 was recorded on a 2.5 mm double-bearing Bruker probe with a B_1 field amplitude of 250 kHz and a spinning rate of 30 kHz. The spectrum of the 300 °C air-calcined sample was recorded on a 4 mm double-bearing Bruker probe with a B_1 field amplitude of 125 kHz and a spinning rate of 13 kHz.

by as-prepared AV-5 displays a relatively broad powder pattern and, hence, is tentatively ascribed to the distorted NaI site. S2 is assigned to sites Na2A and Na2B. The single-quantum ^{23}Na MAS NMR spectrum of as-prepared AV-5 (Figure 3b) was simulated by assuming the presence of two resonance signals, ascribed to sites NaI and (Na2A and Na2B), with intensities of about 1:0.9 (measured from the 30 kHz MAS spectrum), respectively. The former resonance signal has an isotropic chemical shift $\delta_{\text{iso}} = 4.5$, a quadrupole coupling constant, C_Q , of 1.8 MHz, and an asymmetry parameter, η , of 0.40. The quadrupole parameters ($\delta_{\text{iso}} = 4.5$, $C_Q = 0.6\text{--}0.8$ MHz, η not determined) for the other resonance signal were estimated from the 3Q MAS NMR spectrum and used in the simulation of the single-quantum spectrum. These results are similar to those obtained previously for synthetic montregianite. The 3Q MAS NMR spectrum of AV-5 calcined at 300 °C in air (Figure 4b) exhibits, apart from the main resonance signals P1 and P2, a third faint peak P3.

The $\text{Ce}^{\text{III}}/\text{Ce}^{\text{IV}}$ conversion in AV-5 opens up several future lines of research. On one hand, controlling the amount of Ce^{III} present may be achieved by framework oxidation/reduction. For applications in the field of optoelectronics this would be important because when two Ce^{III} centers are too close the luminescence is quenched. We have carried out a preliminary luminescence study of as-prepared AV-5. Figure 5 shows the

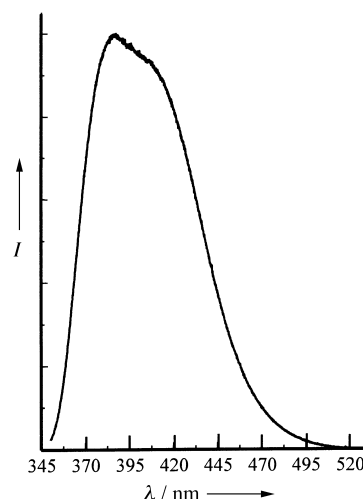


Figure 5. Emission spectrum of AV-5 recorded at 14 K. The spectrum was recorded on a 1 m spectrometer (1704 Spex) coupled to a R928 Hamamatsu photomultiplier. The 325 nm line of a He–Cd laser was used as the excitation source. The spectrum was corrected for the response of the detector.

14 K emission spectrum of this material recorded for the 325 nm He–Cd excitation line, corresponding to the main peak detected in the excitation spectrum (not shown). The blue emission observed clearly shows a broad band with two overlapping peaks centred at about 377 and about 410 nm. This is typical of Ce^{III} -activated phosphors and corresponds to transitions between the lowest Stark component of the $5d^1$ excited states and the two levels of the $4f^1$ ground state ($^2F_{5/2}$ and $^2F_{7/2}$).^[13, 14] In general, these two levels are separated by 2000 cm^{-1} , and this is indeed the result obtained by fitting the spectrum with two Gaussian lines using commercial nonlinear least-squares-fitting software. Owing to different crystal-field splitting of the 5d level the luminescence energy range strongly depends on the structure of the host crystal, varying from the near ultraviolet to the green region.^[13, 14] In the present case, the blue Ce^{III} emission is strongly dependent on the temperature (not shown). A detailed characterization of this emission is in progress.

The tuning of the adsorption properties of AV-5 is another interesting challenge. Indeed, as the Ce^{IV} content increases less channel cations are required to balance the charge of the anionic framework, thus changing the adsorption characteristics of the material. Preliminary work shows that, for example, as-prepared AV-5 calcined at 300 °C in an inert atmosphere reversibly loses and gains about 9.3 wt % water. In contrast, when heated up at 300 °C in air the material first loses 9.3 wt % but subsequent rehydration followed by calcination leads to a loss of about 15 wt %, presumably due to the oxidation of Ce^{III} to Ce^{IV} . Catalysis and ion exchange are also areas where AV-5 may find useful applications.

Experimental Section

AV-5 was prepared in Teflon-lined autoclaves under hydrothermal conditions. An alkaline solution was made by mixing sodium silicate solution (6.05 g; 27 % m/m SiO_2 , 8 % m/m Na_2O , Merck), H_2O (5.04 g), and KOH (0.91 g; Merck). A second solution was prepared by mixing $\text{Ce}_2(\text{SO}_4)_3 \cdot 8\text{H}_2\text{O}$ (1.04 g, Aldrich), H_2O (10.02 g), and HCl solution (2 M;

2.00 g). The two solutions were mixed and stirred thoroughly. The gel, with a composition $0.29\text{Na}_2\text{O}:0.30\text{K}_2\text{O}:1.0\text{SiO}_2:0.054\text{Ce}_2\text{O}_3:34.8\text{H}_2\text{O}$, was placed in an autoclave for eight days at 230°C . The crystalline product was filtered, washed with distilled water, and dried at ambient temperature. Bulk chemical analysis (ICP) yielded an $\text{Si/Ce}/(\text{Na} + \text{K})$ ratio of $8.6:1.0:2.3$. The Si/Ce molar ratio of $8.6:1$ is slightly larger than expected assuming a montregianite-type structure for which $\text{Si/Ce} = 8:1$. This is partially due to the presence of some amorphous siliceous phase. Indeed, the baseline of the ^{29}Si MAS NMR spectrum is slightly raised, although (due to the horizontal expansion) this is not clearly seen in Figure 3a. In addition, powder X-ray diffraction exhibits faint reflections from unknown impurities, while scanning electron microscopy (SEM) shows the presence of some poorly defined particles of very small size. The $(\text{Na} + \text{K})/\text{Ce}$ ratio of $2.3:1$ is between 2 and 3, as expected for samples containing only Ce^{IV} or Ce^{III} , respectively. The K/Na ratio is $0.4:1$.

Although small ($40\text{ }\mu\text{m}$ at best) some of the AV-5 crystals seemed to be of good quality. It was, however, not possible to solve the structure by single-crystal methods. The sample was ground prior to data collection for Rietveld refinement. Powder XRD data were collected on a X'Pert MPD Philipps diffractometer ($\text{CuK}\alpha$ radiation) with a curved graphite monochromator, constant slit, and a flat plate sample holder, in a Bragg-Brentano para-focusing optics configuration. Different grinding times were used and several data sets obtained. The unit cell parameters were first obtained with program TREOR90.^[15] The Rietveld refinement was carried out with program Fullprof,^[16] in the space group $P2_1/n$, range $5-100\text{ }2\theta$, using the atomic coordinates of montregianite and assuming full Ce for Y substitution. The background, peak shapes, and cell parameters were refined. The refinement reliability factors were relatively poor ($R_p = 0.138$, $R_{wp} = 0.177$), probably due to the presence of unknown impurities and to orientation effects.

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Is Hydrogen Tunneling Involved in AcylCoA Desaturase Reactions? The Case of a Δ^9 Desaturase That Transforms (*E*)-11-Tetradecenoic Acid into (*Z,E*)-9,11-Tetradecadienoic Acid**

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Introduction of double bonds into the unactivated aliphatic chains of fatty acids is one of the most remarkable transformations in living organisms. This reaction is catalyzed by specific desaturases, which are oxygen-dependent enzymes containing a non-heme diiron cluster at their active site.^[1] The mechanism of enzymatic desaturation of fatty acids is a subject of currently active research. It has been proposed^[2-5] that desaturation reactions begin with an initial hydrogen atom abstraction step by a hypervalent iron-oxo species to generate a very short lived intermediate, that is then transformed into the olefin via a one-electron oxidation/deprotonation or simple disproportion process. Large activation energies are required to cleave the stable C-H bonds in the substrates as the first step of desaturation. How the enzyme manages to perform such a difficult reaction is an interesting question and the contribution of hydrogen tunneling (H-tunneling) is an attractive possibility. It has been proposed^[2, 3] that enzymatic desaturation and hydroxylation of unactivated aliphatic chains do share common mechanistic features and H-tunneling has been invoked in the oxidation of linoleic acid catalyzed by soybean lipoxygenase.^[6] H-tunneling is defined as a phenomenon by which an atom transfers through a reaction barrier as a result of its wave-like properties.^[7] Reports that show H-tunneling in enzymes are relatively recent.^[7-9] This is an important event in enzyme catalysis, since it contributes to circumvent the thermodynamic difficulties associated with classical descriptions of C-H bond cleavage.

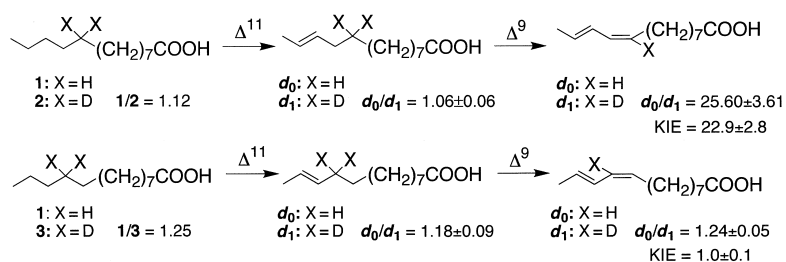
To investigate the putative effect of hydrogen tunneling in fatty acid desaturase reactions, we report herein on a mechanistic study on one of these catalysts, namely the Δ^9 desaturase of (*E*)-11-tetradecenoic acid,^[10] using the moth *Spodoptera littoralis* as biological model.

To determine the cryptoregiochemistry or initial oxidation site of the reaction, the same methodology previously validated for other desaturases^[3-5, 11] was followed. This procedure is based on the calculation of intermolecular, primary kinetic isotope effects (KIE) in competitive experi-

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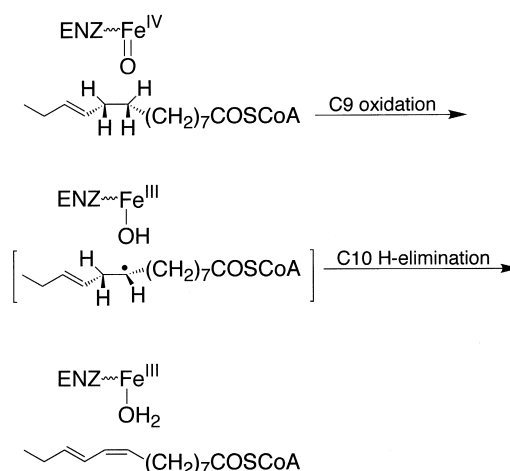
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Scheme 1. Transformation of nonlabeled (**1**) and labeled (**2**, **3**) tridecanoic acids into nonlabeled (**d₀**) and labeled (**d₁**) (*E*)-11-tridecenoic acids and (*Z,E*)-9,11-tridecadienoic acids in the competitive experiments for KIE determination. Ratios between nonlabeled and labeled substrates in the administered mixture and ratios between nonlabeled and labeled metabolites are shown under the chemical structures. For the substrates, the ratio corresponds to a single determination with a $\text{BF}_3 \cdot \text{MeOH}$ -derivatized sample of the used mixture. For the metabolites, ratios correspond to mean $\pm$ SD of seven or eight replicates. Δ^{11} means Δ^{11} desaturase and Δ^9 means Δ^9 desaturase. Product KIEs were calculated as described in the Experimental Section.

ments between a substrate and the same substrate dideuterated at either C9 or C10. In this case, tridecanoic acid (**1**), [9,9- D_2] tridecanoic acid (**2**), and [10,10- D_2] tridecanoic acid (**3**) were used as probes (Scheme 1). Although tridecanoic acid was not the actual enzyme substrate, the approach is valid because tridecanoic acid is transformed, after activation to the corresponding coenzyme A (CoA) ester, into (*E*)-11-tridecenoic acid^[5] in the whole tissue assay. Furthermore, (*E*)-11 desaturation of tridecanoic acid **1** and of both the dideuterated probes, **2** and **3**, takes place at equal rates (Scheme 1). The base methanolized lipidic extracts, prepared after incubation of the moth pheromone sex glands with the probes, were analyzed by gas chromatography coupled to mass spectrometry under the selected ion monitoring mode (SIM-GC-MS). Integration of peaks corresponding to the nonlabeled and monodeuterated methyl (*Z,E*)-9,11-tridecadienoates formed from each mixture afforded the data required to determine the KIEs and, subsequently, the site of initial oxidation in the desaturation reaction.

A very large isotope effect was observed for the carbon–hydrogen bond cleavage at C9 (KIE (25 °C) = 22.9 ± 2.8 [mean $\pm$ standard deviation], $n = 8$), but no isotope discrimination occurred in the H abstraction at C10 (KIE (25 °C) = 1.0 ± 0.1 , $n = 7$). These results indicated that abstraction of C9–H is rate limiting in the desaturation reaction, in accordance to those previously reported for the (*Z*)-9 stearoyl CoA desaturase,^[3] (*Z*)-12 oleoyl CoA desaturase^[4] and Δ^{11} myristoyl CoA desaturase.^[5] In all of these cases, large KIEs were found for the abstraction of the hydrogen atom located at the carbon atom nearer to the carboxyl group, whereas negligible isotope effects were obtained for the removal of the other hydrogen atom to form the double bond. In agreement with the model proposed by Buist et al.,^[2, 3] the Δ^9 desaturation studied here occurs by an initial rate-limiting abstraction of C9–H^[12] to give a carbon-centered radical intermediate, which affords the final olefin by fast elimination of C10–H^[12] (Scheme 2). This result contrasts with what would have been expected from the chemical point of view: That cleavage of the allylic C10–H bond occurred more easily than that of the homoallylic C9–H. Proximity of C9 to the oxidizing iron-oxo species at the enzyme active site^[2, 3] can account for initial



Scheme 2. Proposed mechanism for the (*Z*)-9 desaturation of (*E*)-11-tetradecenoic acid.

oxidation of atom C9. On the other hand, formation of the homoallylic radical intermediate would avoid putative rearrangements that could occur from the allylic species,^[13] that result from initial C10–H bond cleavage and it does, therefore, contribute to the regioselectivity of the enzymatic reaction.

The inflated KIE found for the Δ^9 desaturase studied here is not unprecedented in the literature. Large KIEs have been reported for several enzymes, especially those cleaving C–H bonds.^[14–16] Although inflated isotope effects are suggestive of H-tunneling, the temperature dependence of the reaction rates and KIEs have to be determined and the results must be compared with the behavior expected for tunneling regimes in an Arrhenius plot.^[17] Therefore, to confirm the contribution of H-tunneling to the Δ^9 desaturase of (*E*)-11-tetradecenoic acid, competitive experiments with tridecanoic acid and **2** were also performed in vitro at different temperatures. As shown in Figure 1, the resulting Arrhenius plots afforded almost parallel lines for the nonlabeled and the deuterated sub-

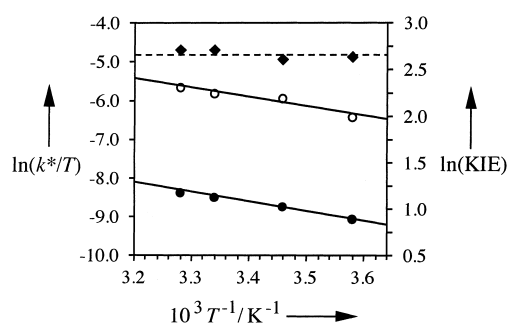


Figure 1. Rate and KIE dependence on temperature as Arrhenius plots for the Δ^9 desaturase of (*E*)-11-tetradecenoic acid. The measurements were performed in competitive experiments with tridecanoic acid and [9,9- D_2]tridecanoic acid as substrates. Data were collected under conditions of substrate saturation using a gland culture assay (see Experimental Section). Reaction rates (k^*) were defined as the relative amounts of nonlabeled (●) or labeled (○) diene with respect to the internal standard, methyl pentadecanoate. Each point was obtained from 6–8 replicates. Linear regression yields: $y = 1.58 - 2.22x$ (correlation coefficient $R^2 = 0.96$; non-labeled diene) and $y = -0.60 - 2.37x$ ($R^2 = 0.98$; deuterated diene). Product KIEs (◆) were calculated as described in the Experimental Section.

strates, which indicates that the difference in activation enthalpies are near zero and that the KIEs are almost independent of temperature. Extrapolation of the data in Figure 1 to a zero reciprocal temperature leads to an isotope effect on the pre-exponential factor of the Arrhenius function, $A_H/A_D = 19.8 \pm 2.9$, which is markedly different from that expected for a classical transition-state barrier model ($A_H/A_D \approx 1$). These results are consistent with contribution of H-tunneling to the C9–H abstraction step.

Whether H-tunneling is a general event in desaturase reactions is now raised as an interesting question. In a recent review, Scrutton et al.^[17] warned against the assumption that a measured KIE < 7 is indicative of classical hydrogen-atom transfer and emphasized that extensive tunneling could also occur in cases with low KIE values. Therefore, although KIEs in the range of 5.5–7.0 have been reported for other fatty acid desaturases thus far studied, it is possible that H-tunneling is a common mechanism of hydrogen-atom transfer in these oxidation reactions. Therefore, temperature dependence of the reaction rate and KIE should be determined in other desaturase enzymes to confirm that H-tunneling occurs. Further work along this line is in progress in our laboratory.

Experimental Section

The probes (9,9-²H₂) tridecanoic acid (**2**) and (10,10-²H₂) tridecanoic acid (**3**) were prepared following reported procedures.^[5] The deuterium contents of the labeled substrates were determined by GC-MS analysis of their respective methyl esters and were found to be as follows: **2**: 89.0% ²D, 8.7% ¹D, and 1.1% ⁰D; **3**: 88.2% ²D, 8.7% ¹D, and 3.1% ⁰D. Cryptoregiochemistry was determined following the previously reported procedure.^[5]

In vitro temperature dependence of reaction rates and KIE values were determined using a gland culture procedure. These experiments were carried out using round bottom, 96-well plates. To each well, a 5 μ L drop of incubation medium was added and the plates were placed in an incubator at the desired temperature (6, 15, 23 and 30 °C). The incubation medium consisted of Grace's saline (135 μ L) and a 1:1 mixture of tridecanoic acid and **2** (10 mg mL⁻¹ each) in a dimethyl sulfoxide solution (15 μ L). One-day-old virgin *S. littoralis* females, reared as reported elsewhere,^[10] were briefly anesthetized on ice and the pheromone glands were excised, carefully cleaned, and immersed into a drop of the incubation medium at the given temperatures. Plates were sealed with adherent plastic covers and the incubation proceeded for 3 h. After this time, pheromone glands were collected and soaked in chloroform/methanol (2/1) at 25 °C for 1 h. The lipidic extracts thus obtained were base methanolized as described elsewhere^[10] to obtain the fatty acid methyl esters. Methyl pentadecanoate was added as internal standard for quantification.

The extracts were analyzed by GC-MS at 70 eV on a Fisons gas chromatograph (8000 series) coupled to a Fisons MD-800 mass selective detector. The system was equipped with a nonpolar Hewlett Packard HP-1 capillary column (30 m $\times$ 0.20 mm i.d.) using the following temperature program: After an initial delay of 2 min at 120 °C, the temperature increased from 120 °C to 180 °C at 5 K min⁻¹ and then to 260 °C at 2 K min⁻¹. Analyses were carried out under selected ion monitoring (SIM) mode. Selected ions were 223, 224, 225, 226, 227, 228, 229, 230, and 231. The dwell was set at 0.02 and mass span at 0.5.

KIEs were calculated from the ratios of product formed from unlabeled substrate and that from the deuterated analogs, and were based on the abundance of the respective molecular ions of the various isotopomers of methyl (*Z,E*) tridecadienoate (**d**₀, 224, **d**₁, 225). Isotope effects were corrected for the exact proportion of unlabeled and labeled substrates administered, which was determined by GC-MS analysis of a BF₃·MeOH derivatized sample of the applied mixture. Corrections were also made for incomplete deuterium incorporation in the substrates and for the natural

abundance of carbon and oxygen isotopes in the ions monitored. These latter values were obtained from the GC-MS chromatograms of gland extracts incubated with the individual substrates.

Reaction rates (k^*) were defined as the relative amounts of nonlabeled or labeled diene formed with respect to the internal standard.

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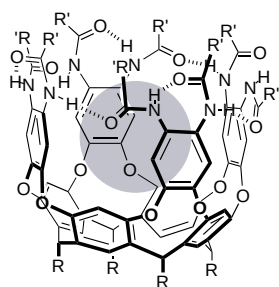
Molecular Recognition with Introverted Functionality**

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Both chemical and physical forces play roles in molecular recognition. Functional group complementarity influences selectivity and affinity in chemical recognition, and these interactions can be arranged using synthetic receptors with open, cleftlike structures. For receptors that completely

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1 • guest

Figure 1. Structure of the octa-amide cavitand **1** with a generic guest species encapsulated, R = C₁₁H₂₃, R' = C₇H₁₅.

resist changes in the conformation of the cavitand; the peripheral alkyl groups of the amide units enhance the solubility of the cavitand in organic media.^[1] The interior surface of the cavitand is bleak and featureless. Most of the atoms that line the receptor are sp² hybridized with their p orbitals directed inward, as though the cavity was coated with a thin layer of negative charge. This offers weak C–H/π interactions and polarizability to size- and shape-complementary guests. Neutral molecules such as adamantane are recognized and bound reversibly as guests in the open-ended cavity host. The binding affinities are low ($\Delta G \sim 2$ – 3 kcal mol^{−1}) but the energetic barriers to guest exchange are high ($\Delta G^\ddagger \sim 17$ kcal mol^{−1}). The aromatic rings provide physical barriers to the entrance and exit of guests, and the seam of hydrogen bonds must be disrupted for guest exchange to occur.

We incorporated chemical recognition features by installing a carboxyl group on the rim of the vaselike cavitand. The condensation^[2] of a Kemp's triacid^[3] module **2** (Scheme 1) with the known diamine **3**^[4] in boiling toluene gave the new receptor **4**. The benzimidazole nucleus formed raises the rim of the cavitand and maintains the space available in the cavity, and the carboxyl function may have an "inwardly" directed (introverted) OH bond as in *endo*-**4** or an outwardly directed one as in *exo*-**4**. Two geometrical isomers were obtained in roughly a 5:1 ratio from the condensation and both products displayed *m/z* signals in the mass spectrum that were consistent with the expected structures.^[5] Given the affinity of *endo*-**4** for triethylamine (see below), it is likely that

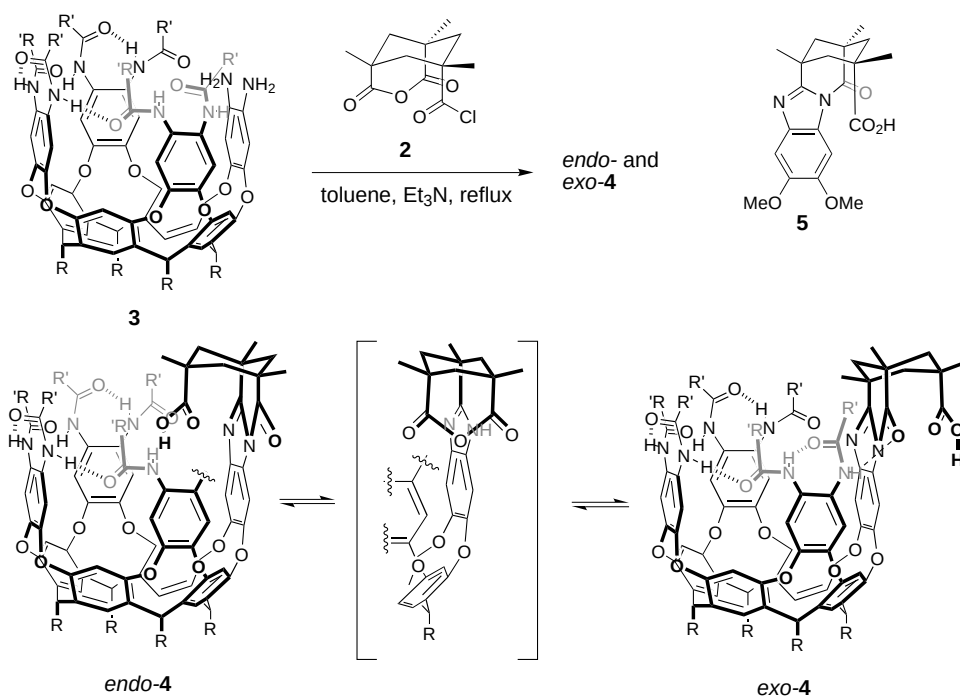
surround their targets, simple size and shape complementarity can distinguish one molecule from another. Herein, we present a synthetic receptor that surrounds a smaller target within physical barriers and offers a carboxyl function for contact with amines such as nicotine.

The cavitand **1** (Figure 1) has a vaselike shape and eight secondary amide functions on its upper rim. These groups provide a chain of intramolecular hydrogen bonds that

the presence of this base in the condensation reaction exerted a template effect and drove the presumed equilibrium (Scheme 1 bottom) toward the production of *endo*-**4**. The structures are inherently chiral, with the seam of head-to-tail secondary amides most likely to be that shown.^[6]

The molecular recognition capabilities of the major product were revealed during its isolation, when signals of the adventitious guest, triethylamine, were identified in the NMR spectrum (Figure 2a). Signals in the region upfield of 0 ppm are characteristic of bound species and reflect the anisotropy provided by the eight aromatic rings that line the interior of the gloomy capsule.^[1] Exchange rates are slow on the NMR timescale (600 MHz, room temperature), and separate signals are seen for the free and bound guests. The binding is tight ($> 5 \times 10^4$ kcal mol^{−1}) and beyond the limits for accurate determination by NMR titrations. The asymmetric environment of the cavity results in the splitting of the methylene signals of the triethylamine guest. In contrast, no upfield signals were observed in solutions of triethylamine with the minor product *exo*-**4**, the model compound **5**, or the parent structure **1**.

The interplay of mechanical and chemical recognition in *endo*-**4** was revealed through its binding behavior with amine guests (Figure 2). Primary amines such as isobutylamine (b) and 1-aminoadamantane (c), and tertiary amines such as triethylamine (a), (*S*)-(–)-nicotine (d), and 3-picoline (e) form stoichiometric complexes. In contrast, cavitand **1** does not bind triethylamine, isobutylamine, nicotine, picoline, or even the corresponding acetate salts under these conditions. Only 1-aminoadamantane is bound by both *endo*-**4** and **1**, but even in this case there are differences. The guest is bound with its amine function directed at the floor of the cavity in **1**^[7] but



Scheme 1. Synthesis of the cavitands *endo*-**4** and *exo*-**4** from the diamine **3** and the Kemp's triacid module **2**. The model compound **5** was prepared similarly. The introverted carboxyl function is shown in bold. R = C₁₁H₂₃, R' = C₇H₁₅.

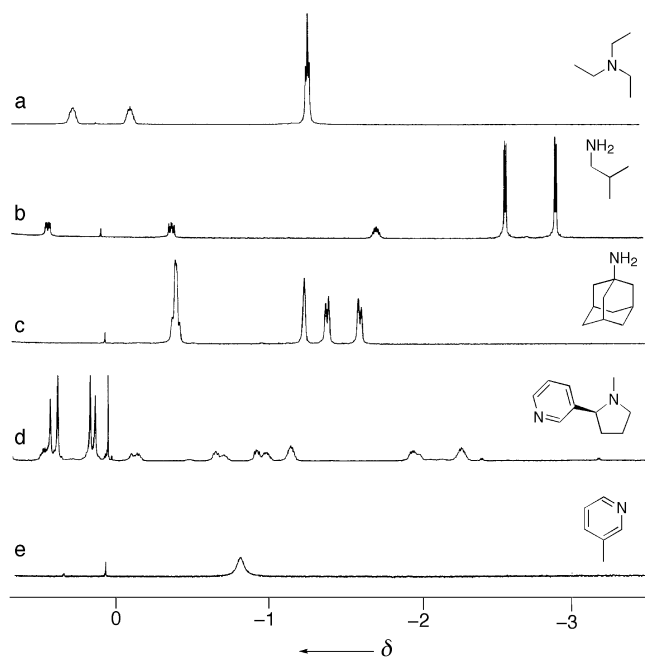


Figure 2. Signals of encapsulated guests from the upfield region of the ^1H NMR spectra (600 MHz, CDCl_3 , 295 K) of the receptor *endo-4*. The guest species are a) triethylamine, b) isobutylamine, c) 1-aminoadamantane, d) (*S*)-(-)-nicotine, and e) 3-picoline.

with the opposite orientation, namely with the amino group in contact with the acid, in *endo-4*. Adamantane itself is not bound by *endo-4* but is bound by **1**. Accordingly, the chemical recognition of *endo-4* is involved with amine guests. The high affinity is likely to involve the formation of a contact ion pair between the introverted carboxyl group and the guest amine.^[8] The binding of 1-aminoadamantane represents the size limit for guests of *endo-4*. The slightly larger 1-adamantanylmethylamine was only weakly bound by *endo-4* and gave rise to very broad signals in the ^1H NMR spectrum. The Kemp's triacid group partially obstructs the opening and provides size limitations in recognition: the guest is mechanically bound in all three dimensions within the host.

Diastereoselective binding in receptor *endo-4* was probed with a chiral guest. When an excess of (*R*)-(-)-1-cyclohexylethylamine was added to (racemic) *endo-4*, two equal-intensity sets of host signals were observed in the NMR spectrum; these signals correspond to the two diastereomeric complexes formed (Figure 3a). When a racemic mixture of 1-cyclohexylethylamine was added to *endo-4*, the same two sets of host signals were observed, but in a 3:1 ratio (Figure 3b). The binding diastereoselectivity for this guest is approximately 50% *de* at room temperature. In the case of (*S*)-(-)-nicotine, the pyrrolidine nitrogen atom is an additional stereogenic center and it is this atom that is in contact with the acid. A ROESY NMR experiment established that the pyrrolidine ring is bound deep in the cavity of *endo-4* and gave an exchange rate constant of $k \approx 0.5 \text{ s}^{-1}$. The presence of a number of isomers led to unresolved signals and precluded the determination of diastereoselectivity for this case.

In summary, the receptor *endo-4* features both mechanical and chemical bonding. The concave surface provides a cavity

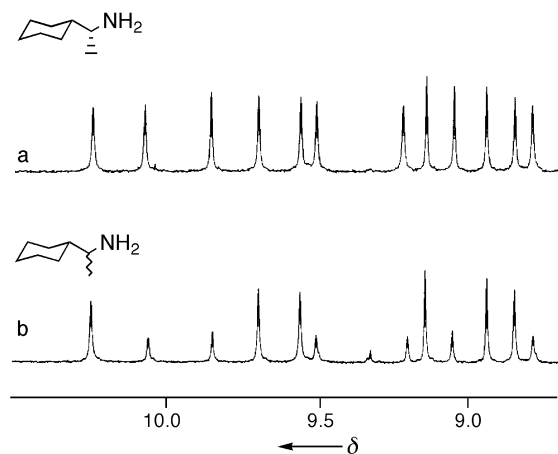


Figure 3. Downfield amide resonances for the receptor *endo-4* with either a) (*R*)-(-)-1-cyclohexylethylamine or b) racemic ($\pm$)-1-cyclohexylethylamine as the guest.

isolated from bulk solution and the introverted carboxyl group is directed towards the guest inside. The cavity is asymmetric and capable of diastereoselection in the binding of chiral substrates. Convergent functional groups,^[9] concave reagents,^[10, 11] molecular tweezers,^[12] macrocyclic compounds,^[13, 14] and endohedral functions^[15] also feature reactive atoms in sterically constrained environments, but typically there is not enough room to both surround a guest completely and present useful functionality to it. Additionally, there is the often insurmountable problem of doing chemistry on concave molecular surfaces. The combination of the recognition attributes of **4**, many of which are shared by enzymes, augurs well for the development of synthetic molecules that approach the functional features of naturally occurring macromolecules.

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Towards Synthetic Molecular Muscles: Contraction and Stretching of a Linear Rotaxane Dimer**

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In memoriam Olivier Kahn

Molecules whose shape and dynamic properties can be controlled at will have recently triggered great interest. Most of the systems specially designed and constructed for their movement properties contain interlocking rings (catenanes) or rings threaded by molecular stringlike components (pseudo-rotaxanes and rotaxanes). These compounds are often described as machines^[1] or motors.^[1, 2] The latter term applies more specifically to systems for which continuous movement can be induced while energy is consumed. Biological molecular motors are especially important.^[3] They can be classified as rotary motors, ATP synthase being the most important and well understood example,^[4] or linear motors.^[5] Among the few natural linear motors identified to date, skeletal muscles^[6] occupy a special position due to their universal character and to the importance of the mechanical properties they confer to vertebrates.

To date, several interesting artificial systems have been described which aim at reproducing some essential properties of skeletal muscles. Polypyrrole-based devices are particularly promising, since they allow one to bend a solid polymer film in one direction or another depending on the sign of an electric current applied to the film.^[7] A more recent approach relies on single-walled nanotube sheets.^[8]

To the best of our knowledge, unimolecular linear arrays capable of undergoing contraction or stretching have not been made as yet. Herein we describe such a multicomponent system, capable of contracting or stretching under the action of a chemical signal. It is based on a doubly threaded structure recently described.^[9]

The principle of the contraction/stretching process is indicated in a very schematic fashion in Figure 1.

By analogy with real muscles, we designed a molecular assembly for which two filaments can glide along one another. This is the very process taking place in the sarcomere, in which the thick filament (containing myosin) moves along the thin

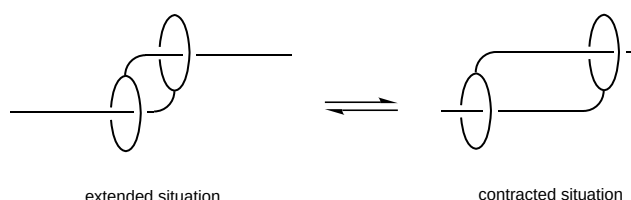


Figure 1. The topology and the shape of a linearly arranged pseudo-rotaxane dimer is adapted to a contraction/stretching motion.

filament (actin polymer) in one direction or the other so as to induce contraction or stretching. In the double-threaded object of Figure 1, the motion is easy to visualize. Both strings (mimicking the muscle filaments) move along one another but stay together thanks to the rotaxane nature of the system.

So far, the machinelike compounds elaborated and studied in our group are mostly copper complexes, the motion being triggered by an electrochemical reaction ($\text{Cu}^{\text{I}}/\text{Cu}^{\text{II}}$), although photochemically driven processes based on ruthenium(II) compounds seem also to be promising.^[10] The present system contains copper(I) as the assembling and templating metal, but the movement is induced by a *chemical reaction*, corresponding to metal exchange. As shown in Figure 2, the doubly

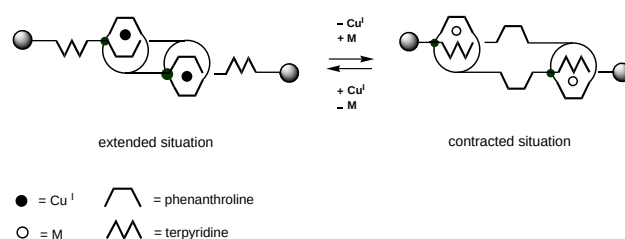


Figure 2. Functioning principle of the unimolecular synthetic "muscle" reported herein. The two-component rotaxane dimer contains identical ring-and-string conjugates. Each component consists of a bidentate chelate (U-shaped symbol) embedded in a ring, the ring being covalently attached to a filamentlike part. This string contains another bidentate ligand, a terdentate coordinating unit (schematically represented by a W-shaped symbol), and a bulky stopper (sphere) whose function is to prevent dethreading of the filaments from the rings through which they are threaded. The four-coordinate situation (left-hand side) is such that the metal (black disk) is coordinated to two bidentate chelates. If the bidentate chelate belonging to the string is replaced by a terdentate fragment, a five-coordinate situation is reached, which corresponds to an overall contracted situation (right-hand side). A representative geometrical parameter measuring the level of contraction or stretching of the system can be the center-to-center distance between the two stoppers. The contracted situation is obtained by replacing the four-coordinate metal of the compound represented on the left (copper(I) in the present work) by a five-coordinate center (white disk; zinc(II) in the present report).

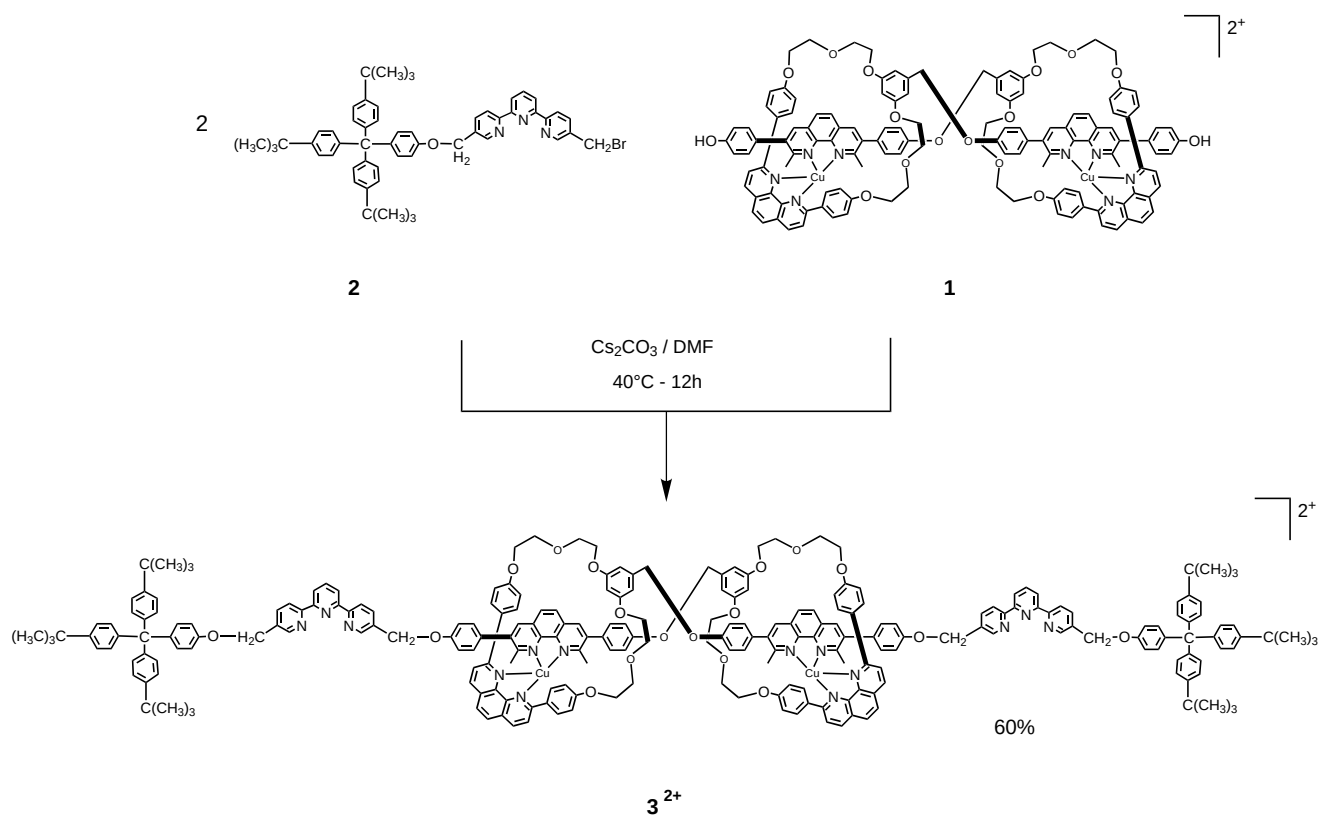
threaded compound reported herein can bind simultaneously two metal centers, either in a four-coordinate or a five-coordinate geometry. The four-coordinate situation corresponds to an extended geometry, whereas the five-coordinate situation leads to a contracted geometry.

The molecular "muscle" of the present report was synthesized in its extended conformation (i.e., with a geometry corresponding to the left-hand side of Figure 2), from the dicopper(I) precursor **1** (Scheme 1). Compound **1** displays an interesting topology^[11] which prompted us to call it a

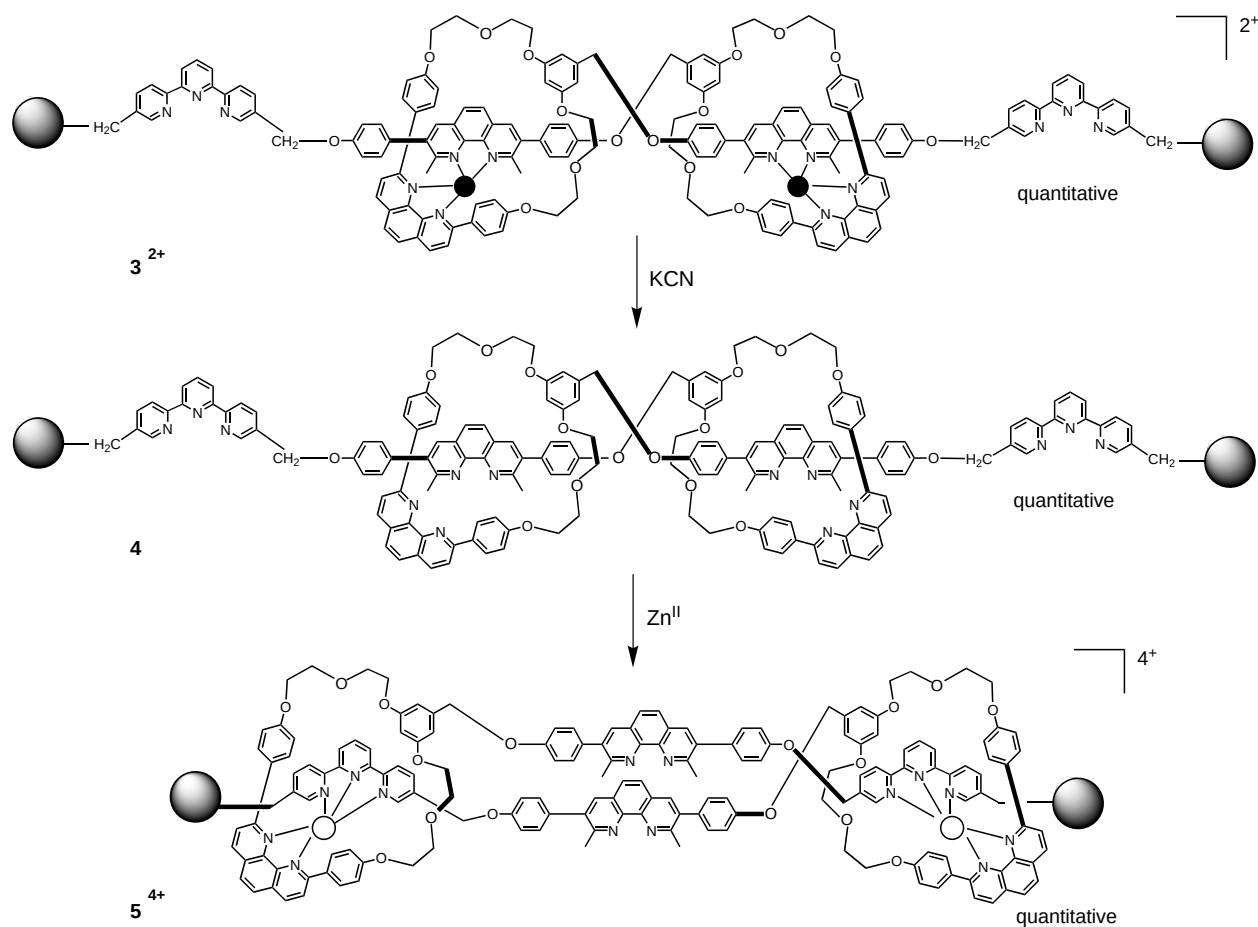
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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



Scheme 1. Synthesis of the “muscle” 3^{2+} in its extended situation.



Scheme 2. Reversible chemically induced motions between extended 3^{2+} and contracted 5^{4+} .

“hermaphrodite” molecule.^[9] Reaction of the phenolic functions of **1** in DMF and Cs₂CO₃ at 40 °C with two equivalents of bromide **2** (a stopper-bearing disymmetrical 2,2',6',2''-terpyridine whose synthesis will be described elsewhere), led to the desired complex **3**²⁺ in 60% yield (Scheme 1).

2D-ROESY NMR experiments as well as high-resolution mass spectrometry (FAB) showed clearly that the stoppering procedure leading to **3**²⁺ occurred without significant dethreading as evidenced by large interfragment interactions in the NMR spectrum and by the expected molecular ion peak at *m/z* 3834.2 (calcd *m/z* 3834.3). The free ligand **4** obtained in quantitative yield by reacting **3**²⁺ with a large excess of KCN (in CH₂Cl₂/H₂O at room temperature) was subsequently remetalated with Zn(NO₃)₂ (in CH₂Cl₂/MeOH) affording quantitatively **5**⁴⁺ as a colorless solid, in the contracted situation (Scheme 2, page 3885). The reverse motion, leading back to the extended situation, that is back to **3**²⁺, could be easily induced upon addition of excess Cu(CH₃CN)₄·PF₆ in CH₂Cl₂/CH₃CN at room temperature. Although the dimeric nature of **3**²⁺, **4**, and **5**⁴⁺ could be easily demonstrated by mass spectrometry (FAB or ES),^[13] demonstration of the contraction/stretching phenomenon accompanying the metal exchange (Cu^I/Zn^{II}) required extensive NMR studies. Some of the most characteristic chemical shifts are collected in Table 1.

Table 1. ¹H NMR spectroscopy: chemical shifts (δ, in ppm) of a few selected protons.^[a]

Compounds	H _o	H _m	CH ₃	6 _{terpy}	6'' _{terpy}	4' _{terpy}
2	—	—	—	8.68	8.72	7.96
3 ²⁺	7.48	6.04	2.09; 2.21	8.79	8.75	7.97
4	8.44	7.17	2.66; 2.72	8.80	8.71	7.97
5 ⁴⁺	6.85	6.29	2.71; 2.75	7.39	8.13	8.58

[a] 400 MHz in CD₂Cl₂.

From previous studies on the doubly threaded dicopper(I) precursor, it is clear that **3**²⁺ contains two pseudo-tetrahedral complexes, each metal center being coordinated to a dpp motif embedded in the ring (dpp = 2,9-diphenyl-1,10-phenanthroline) and a dmp fragment belonging to the filament (dmp = 2,9-dimethyl-1,10-phenanthroline). The data in Table 1 shows that various NMR probes can be considered (see Figure 3).

1) H_o and H_m are very indicative of the dpp situation. If this unit is free, the chemical shifts of the two protons are “normal” for an aromatic group attached to a 1,10-phenanthroline (phen) nucleus (δ = 7–8.5). On the other hand, once coordinated to a metal center in a 2-aromatic polyimine complex, H_o and H_m

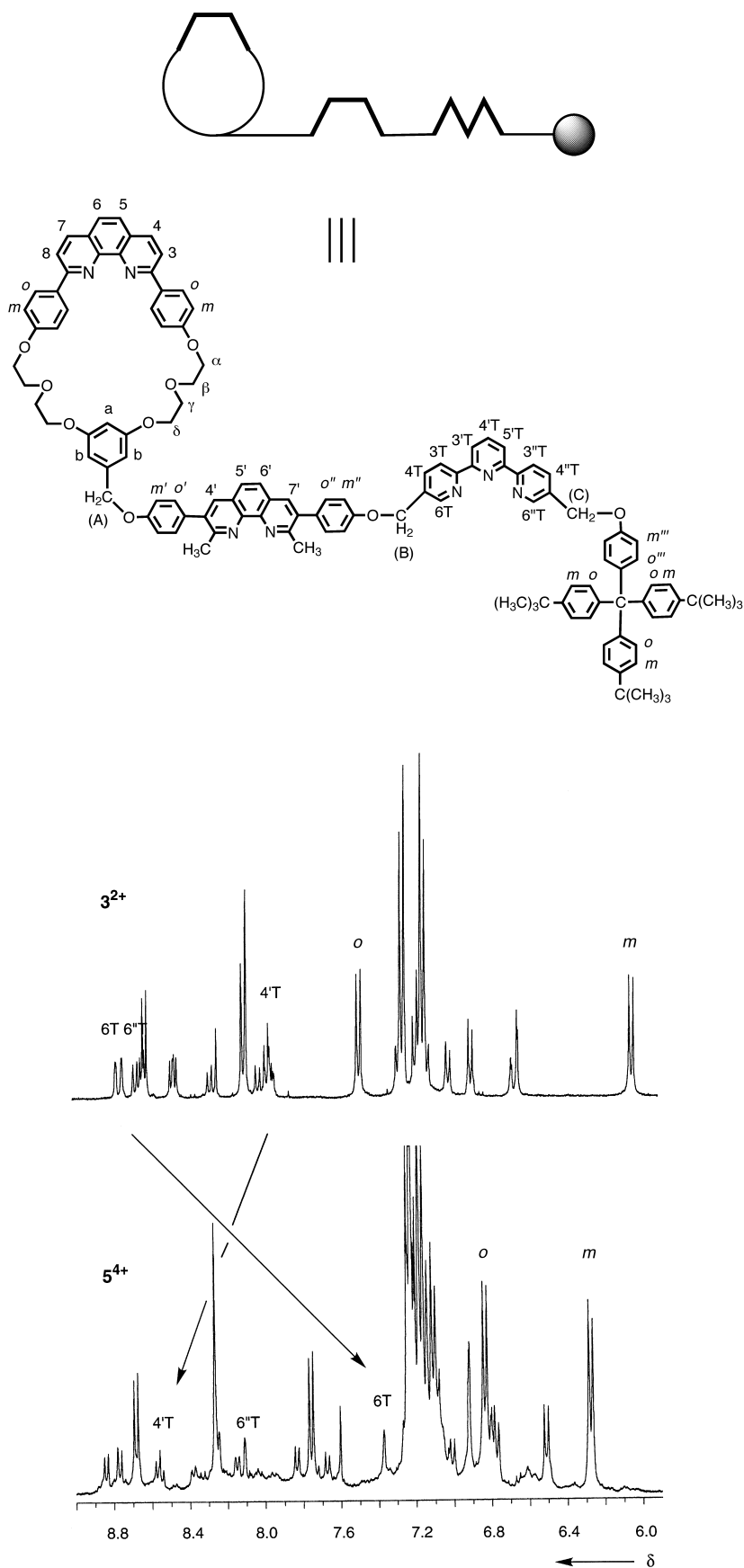


Figure 3. ¹H NMR spectra (aromatic part) of **3**²⁺ and **5**⁴⁺ (400 MHz in CD₂Cl₂).

undergo a strong upfield shift characteristic of “entwined” compounds, as evidenced long ago.^[12] From the data in Table 1, it is evident that dpp is coordinated in both complexes **3**²⁺ and **5**⁴⁺. For instance, H_m undergoes an upfield shift with respect to the free ligand of $\Delta\delta = -1.13$ and -0.88 ppm for **3**²⁺ and **5**⁴⁺, respectively. For H_o, the shift is even more spectacular in **5**⁴⁺ ($\Delta\delta = -1.59$ ppm) probably because of the strong ring current exerted by the terpyridine (terpy) unit onto this proton.

- 2) The CH₃ groups borne by the phen chelate included in the filament are also strongly upfield shifted by complexation, owing to a similar “entwining” effect as that observed for H_o and H_m. The chemical shifts are strikingly similar for **4** and **5**⁴⁺, but they are significantly different from those of **3**²⁺: $\Delta\delta \sim -0.6$ ppm for **3**²⁺ with respect to **4** or **5**⁴⁺; thus, the dmp unit of **5**⁴⁺ is free.
- 3) H₆, H_{6'}, and H₄ of the terpy part also undergo substantial chemical shift changes upon complexation. Whereas the corresponding chemical shift values are similar in **2**, **3**²⁺, and **4** ($\delta = 8.76 \pm 0.08$ for H₆; $\delta = 8.73 \pm 0.02$ for H_{6'}, and $\delta = 7.97 \pm 0.01$ for H₄), they are noticeably different in **5**⁴⁺ (strong upfield shift for H₆ and H_{6'}; relatively strong downfield shift for H₄). These observations are consistent with the “entwining” effect, placing H₆ and H_{6'} in the shielding region of the dpp unit belonging to the ring for a (dpp)(terpy) complex. They also unambiguously demonstrate that both Zn²⁺ centers in **5**⁴⁺ are coordinated to the terpy fragments.

From the ¹H NMR data, it can be concluded that each Zn²⁺ ion of **5**⁴⁺ is coordinated to a dpp chelate and a terpy unit, that is the molecule is in the contracted situation, as represented in Scheme 2.

In conclusion, the present rotaxane dimer is the first unimolecular linear array capable of stretching and contracting at will under the action of a chemical stimulus. From CPK model estimations, the length of the compound changes from 83 Å to 65 Å between both situations, that is roughly by the same relative amount as natural muscles ($\sim 27\%$).

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Conformation Control of Oligosilanes Based on Configurationally Constrained Bicyclic Disilane Units**

Kohei Tamao,* Hayato Tsuji, Masayoshi Terada, Masahiro Asahara, Shigehiro Yamaguchi, and Akio Toshimitsu

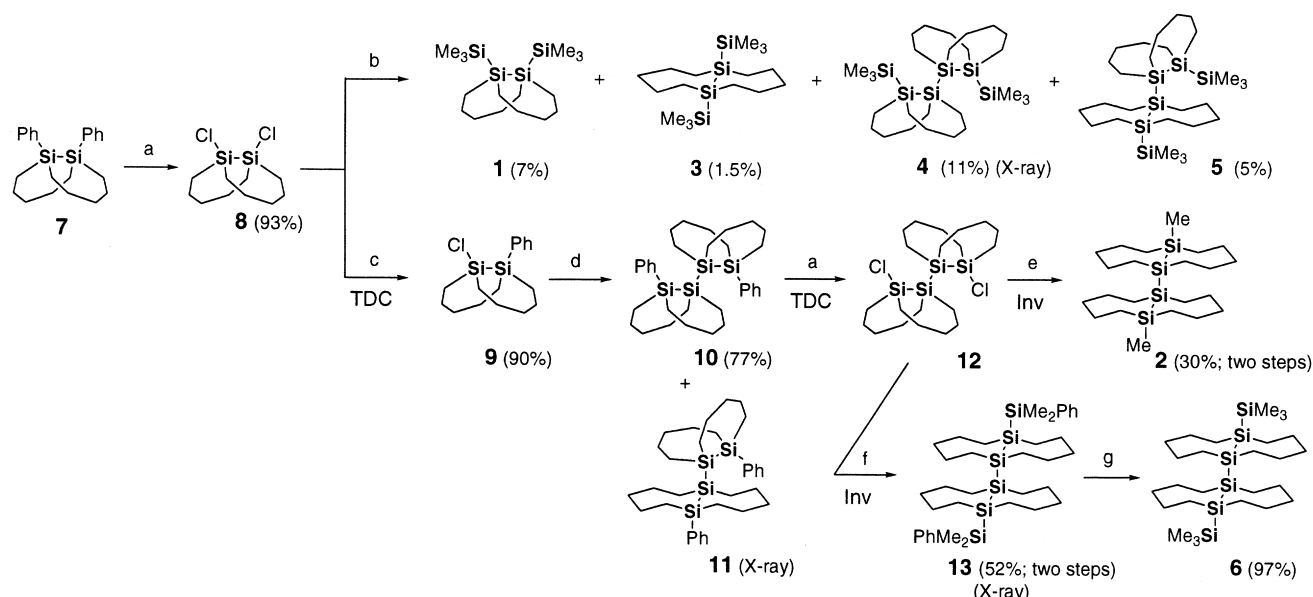
One of the most important properties of polysilane compounds is their electronic absorption in the UV region that provides a representative example of the σ -conjugation systems.^[1] It is generally accepted that *both* the absorption wavelengths, λ_{max} , and the intensities depend on the chain length of the silicon backbone in the *anti* conformation, while the *gauche* conformations behave as defects in the σ conjugation. This idea has been successfully used to explain the thermochromic behavior of long-chain polysilanes.

However, little is understood about the origin of the conformation-dependency of the electronic absorptions. Recently, a new explanation has been proposed by Michl and co-workers^[2] based on their pioneering theoretical and experimental studies on tetrasilane model systems. These systems

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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.



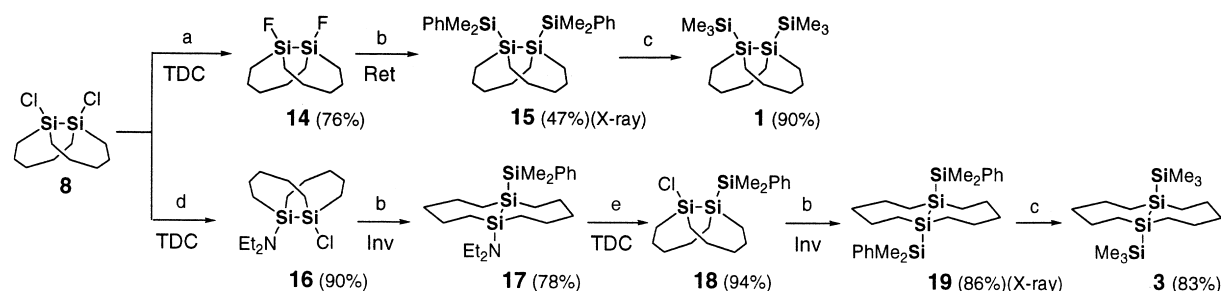
Scheme 1. Synthesis of **1–6**. a) HCl(gas)/ AlCl₃, C₆H₆; b) Li/Me₃SiCl, THF; c) PhMgCl, THF; d) Li, THF; e) MeLi, Et₂O; f) PhMe₂SiLi, THF; g) 1) TFOH, C₆H₆; 2) MeMgI, Et₂O. TDC = thermodynamically controlled conditions; Inv = inversion of configuration. Experimental details and workup procedures are available in the Supporting Information.

are the shortest polysilane units to have conformational isomers defined by the dihedral angle (ω) around the central Si–Si bond. According to this new explanation there are two absorptions assigned to the $\sigma-\sigma^*$ and the $\sigma-\pi^*$ transitions in which the σ and σ^* orbitals are of the silicon backbone and the π^* orbitals primarily consist of the σ^* orbitals of the two substituents on each silicon atom; the energies of these two absorptions are not conformation dependent but the intensities are. Thus, in the *anti* conformation ($\omega = 180^\circ$), the low-energy absorption ($\sigma-\sigma^*$) is strong and the high-energy absorption ($\sigma-\pi^*$) is very weak, while in the *syn* conformation ($\omega = 0^\circ$), the high-energy absorption ($\sigma-\sigma^*$) is strong and the low-energy absorption ($\sigma-\pi^*$) is very weak; the intensities of the two absorptions would be medium and comparable to each other in conformers in which $\omega = 90-135^\circ$. Experimental support of this important theory is essential to fully understand the σ conjugation and should best be obtained with conformationally defined oligosilanes. However, it is one of the most difficult tasks in organic chemistry to obtain desired conformational isomers at will. The energy-minimum stable conformers, that is, the *anti*, and the *gauche* ($\omega = 60^\circ$), or similar conformers in the oligosilane case, can be

fixed under thermodynamically controlled conditions such as in low-temperature matrices^[2] or in crystals.^[3, 4] Whereas, to fix the conformers which do not correspond to the local energy minima requires special techniques. For instance, by introducing the tetrasilane skeleton into five- to eight-membered cyclic carbosilane compounds Michl and co-workers have prepared several conformers in which $\omega = 0-80^\circ$.^[5]

Herein we report the syntheses and UV absorption spectra of tetrasilane and hexasilane compounds in which the dihedral angles ω are controlled to *syn* (*S*), *anti* (*A*), and “*second-eclipsed*”^[6] (*E*) ($\approx 120^\circ$) by the use of disilane units that are configurationally constrained to *syn* or *anti* by two pentamethylene tethers.^[7]

The syntheses of the tetrasilane compounds **1–3** and the hexasilane compounds **4–6** from the single key compound **7** (which is readily converted into **8**) are summarized in Schemes 1 and 2.^[8] The tetrasilane compounds **1** and **3** which contain one disilane unit (*syn* or *anti*) and the hexasilanes **4** and **5** which contain two disilane units (*syn, syn* and *anti, syn*) were formed by the reductive coupling reaction of the dichlorodisilane **8** with lithium (Scheme 1) and were separated by gel permeation chromatography (GPC). The tetra-



Scheme 2. Selective synthesis of **1** and **3**. a) ZnF₂, Et₂O; b) PhMe₂SiLi, THF; c) 1) TFOH, C₆H₆; 2) MeMgI, Et₂O; d) Et₂NH/Et₃N, THF; e) HCl(gas), Et₂O. TDC = thermodynamically controlled conditions; Inv = inversion of configuration; Ret = retention of configuration. Experimental details and workup procedures are available in the Supporting Information.

silane compound **2** and the hexasilane compound **6** which contain two *anti*-disilane units were prepared by stereo-defined transformations including the selective formation of the *syn*-chlorodisilane units under thermodynamically controlled conditions (TDC) and stereospecific substitution reactions with inversion of configuration (Inv)^[9] (Scheme 1). By use of a similar methodology we also developed alternative selective synthetic routes to **1** and **3** (Scheme 2).^[10]

The UV absorption spectral data of the tetrasilane compounds **1–3** and of the hexasilane compounds **4–6** are summarized in Tables 1 and 2, respectively, together with the particular dihedral angles determined by X-ray crystallogra-

Table 1. UV absorptions and dihedral angles of Si-Si-Si-Si framework of tetrasilane compounds **1–3**.

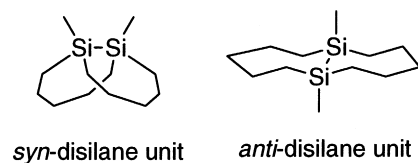
Com- pound	UV absorption $\lambda_{\max}$ [nm] (ϵ)	Dihedral angles determined by X-ray crystallography		
		Com- pound	ω [°]	Conforma- tion ^[a]
1	≈ 210 (sh ^[b] , 1.3×10^4)	15	7.1	<i>S</i>
2	204 (2.2×10^4) 230 (1.7×10^4)	13	117.5	<i>E</i>
3	238 (3.0×10^4)	19	180	<i>A</i>

Table 2. UV absorptions and dihedral angles of Si-Si-Si-Si framework of the hexasilane compounds **4–6**.

Com- pound	UV absorption $\lambda_{\max}$ [nm] (ϵ)	Com- pound	Dihedral angles determined by X-ray crystallography			Conforma- tion ^[a]
			ω_1 [°]	ω_2 [°]	ω_3 [°]	
4	239 (2.2×10^4)	4	13.1(30.0) ^[b]	180(180) ^[b]	13.1(30.0) ^[b]	<i>SAS</i>
5	255 (3.1×10^4)	11	179.2 ^[c]	177.5	8.0 ^[c]	<i>AAS</i>
6	≈ 225 (sh ^[d] , 1.2×10^4) 259 (5.3×10^4)	13	171.1	117.5	170.2	<i>AEA</i>

[a] *S* = *syn*, *A* = *anti*, *E* = second-eclipsed. [b] There are two independent molecules in crystals. [c] The Si-Si-Si-C dihedral angle in **11**. [d] sh = shoulder.

phy for the five compounds **4**, **11**, **13**, **15**, and **19**.^[11] The dihedral angles of the *syn*-disilane units (ω in **15**, ω_1 and ω_3 in **4**, and ω_3 in **11**) were found to be constrained between 7–30° (*S*) and those of the *anti*-disilane units (ω in **19**, ω_1 and ω_3 in **13**, and ω_1 in **11**) between 170–180° (*A*). In **4** and **11**, the unconstrained dihedral angles (ω_2) around the central Si-Si bond between the two disilane units, *syn*, *syn* and *anti*, *syn*,



were found to be 180° (*A*) and 177.5° (*A*), respectively. In **13**, the dihedral angle ω_2 between the two *anti*-disilane units was found to be 117.5° (*E*). PM3 calculations indicate that the most stable conformer of **6** has a dihedral angle ω_2 between

the two *anti*-disilane units of 115°, while in the most stable conformer of **4** the dihedral angle ω_2 between the two *syn*-disilane units is 180°.

The UV absorption spectra of tetrasilanes **1–3** measured at room temperature in 2,2,4-trimethylpentane are shown in Figure 1; the spectrum of decamethyltetrasilane $\text{Si}_4\text{Me}_{10}$ is included for comparison. The *A*-tetrasilane compound **3** has an absorption maximum at 238 nm with an absorbance of

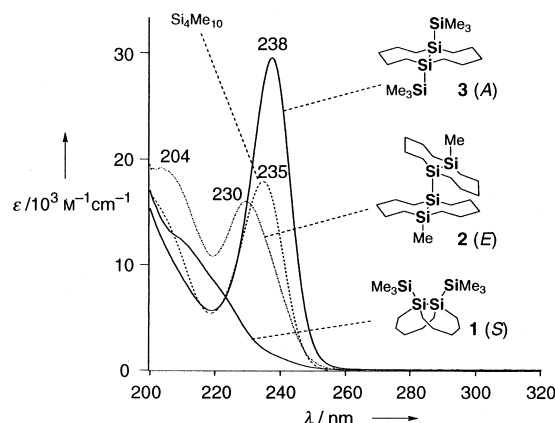


Figure 1. UV absorption spectra of the tetrasilane compounds **1–3** and $\text{Si}_4\text{Me}_{10}$.

greater intensity than that of $\text{Si}_4\text{Me}_{10}$. The *E*-tetrasilane compound **2** with a dihedral angle of $\approx 120^\circ$ has “twin” absorption maxima of medium intensity at 204 and 230 nm. The absorption of the *S*-tetrasilane compound **1** appears as a shoulder at around 210 nm. These results seem to be consistent with the theoretical prediction.^[2] The strong absorption in the *A*-tetrasilane compound **3** is assigned to the low-energy σ – σ^* transition, the shoulder-like absorption around 210 nm of the *S*-tetrasilane compound **1** is assigned to the high-energy σ – σ^* transition, and the σ – π^* transition of compounds **1** and **3** is

expected at around 230–240 nm for **1** and at around 210 nm for **3**; however, this transition is hardly recognizable presumably because of its very low intensity. The “twin” absorption maxima in **2** are assigned to the high- and low-energy transitions of the tetrasilane unit which has a dihedral angle of approximately 120°.

Figure 2 shows the UV absorption spectra of hexasilanes **4**, **5**, and **6**, in the sequence **4**, **5**, **6** the $\lambda_{\max}$ shifts from 239 for **4** to 259 nm for **6** and the intensity almost triples. The absorption spectrum of the *SAS*-hexasilane **4** has a $\lambda_{\max}$ at 239 nm which corresponds to the combined spectra of the *S*-tetrasilane compound **1** and the *A*-tetrasilane compound **3**. This result is best understood by assuming that this absorption maximum arises from the tetrasilane skeleton of the *anti* conformation and that the additional *syn* segments do not contribute to the elongation of the σ conjugation. A comparison of the spectra of the *SAS*-hexasilane compound **4** and *AAS*-hexasilane compound **5** clearly shows the significant contribution of the additional *anti* segment to the elongation of the σ conjugation. The strong but slightly red-shifted absorption observed in the

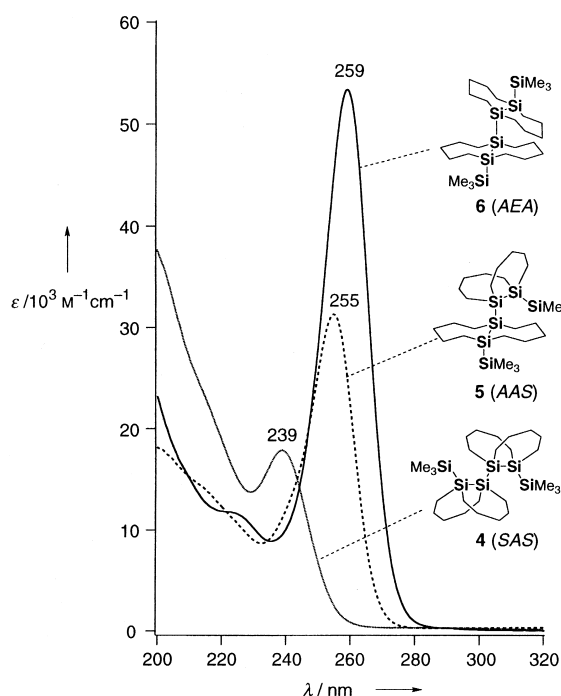


Figure 2. UV absorption spectra of the hexasilane compounds 4–6.

AEA-hexasilane compound **6** indicates that the connection of two constrained *A* conformations with one *E* conformation is the main contribution to the enhancement in the intensity.^[12]

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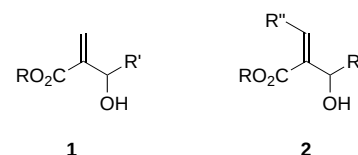
Crystallographic Data Centre as supplementary publication nos. CCDC-141477 (**4**), -141479 (**11**), -141478 (**13**), -141475 (**15**), and -141476 (**19**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

- [12] The λ_{max} of **6** in the solid state was confirmed to be the same as that in solution, and suggests that the dihedral angle ω_2 around the “non-constrained” Si-Si bond is comparable in both the solution and the solid state.

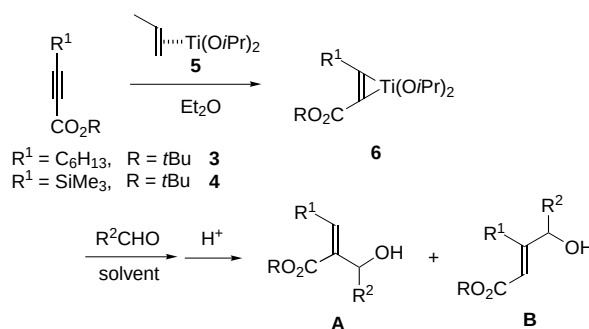
Asymmetric Synthesis of Baylis–Hillman-Type Allyl Alcohols via a Chiral Acetylenic Ester Titanium Alkoxide Complex**

Daisuke Suzuki, Hirokazu Urabe, and Fumie Sato*

Functionalized allyl alcohols such as **1** and **2** are useful intermediates in organic synthesis.^[1, 2] Several recent reports have revealed that the asymmetric Baylis–Hillman reaction



afforded alcohols **1** in a highly optically active form.^[1, 3] However, asymmetric synthesis of the homologues **2**, which contain a substituent β to the acrylate group, has not been successful by this method.^[4] We recently reported the first generation of the acetylenic ester Group 4 transition metal complexes such as **6**^[5] from esters **3** or **4** and a low-valent titanium complex, $[(\eta^2\text{-propene})\text{Ti}(\text{O}i\text{Pr})_2]$ (**5**)^[6] (Scheme 1).

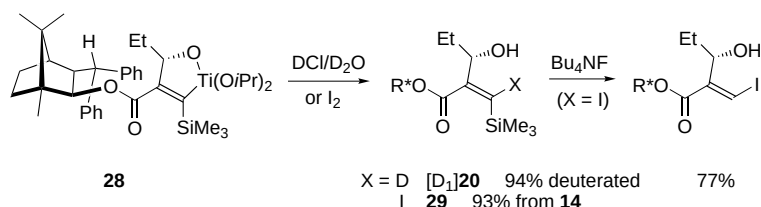


Scheme 1. Reaction of acetylene complex **6** with aldehydes.

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In the above transformations, the reaction intermediates were simply hydrolyzed. However, the remaining carbon–metal bond could be used for further synthesis. In fact, deuterolysis or iodolysis of the intermediate oxatitanacycle **28** afforded the corresponding deuterated product [D_1]**20** or vinylic iodide **29** (Scheme 3), the latter of which may be useful for further transformation as such or after fluoride-mediated desilylation.^[13]



Scheme 3. Preparation of functionalized Baylis–Hillman-type products.

In conclusion, a new chiral dimetallic species, the acetylenic ester titanium alkoxide complex with a camphor-derived auxiliary, enabled the preparation of highly optically active, stereodefined hydroxyacrylates. The scope and limitations of this reaction will be reported in due course.

Experimental Section

Typical procedure for the asymmetric addition to aldehydes (entry 7, Table 2): *i*PrMgCl (1.45 M in Et₂O, 0.323 mL, 0.468 mmol) at -78°C under argon was added to a stirred solution of acetylenic ester **14** (52 mg, 0.117 mmol) and [Ti(O*i*Pr)₄] (0.069 mL, 0.234 mmol) in Et₂O (3 mL) to give a yellow homogeneous solution. The solution was warmed to -50°C over 30 min, during which period it turned red. After the mixture had been stirred at -50°C for an additional 5 h, THF (3 mL) was introduced to the reaction mixture at -50°C , followed 10 minutes later by benzaldehyde (1.0 M in Et₂O, 0.94 mL, 0.94 mmol). After the solution had been stirred at -50°C for 3 h, the reaction was terminated by the addition of 1 N HCl, and the organic products were extracted with diethyl ether. The combined organic layers were washed with an aqueous solution of NaHCO₃, dried over Na₂SO₄, and concentrated in vacuo to give a crude oil, which was chromatographed on silica gel (hexane/diethyl ether) to give (1*R*,2*R*,3*S*,3'*S*)-(Z)-3-(diphenylmethyl)-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl 3'-hydroxy-3'-phenyl-2'-[(trimethylsilyl)methylene]propanoate (**22**) (50.5 mg, 97%) as a colorless oil, which was fully characterized by IR, ¹H, and ¹³C NMR spectroscopy, and elemental analysis. The following NOE study of **22** by ¹H NMR spectroscopy confirmed the assigned regio- and stereochemistry. Irradiation of the proton at $\delta = 0.02$ (Me₃Si) and that at $\delta = 5.47$ (CHOH) showed 11% and 6% NOE enhancements to the peak at $\delta = 6.09$ (Me₃Si–CH=C), respectively. Optical rotations of products (for the isomeric purities or *ee* values, see Table 2 and Scheme 2): **20**: [α]_D²⁵ = -132.70 ($c = 1.00$ in CHCl₃); **21**: [α]_D²⁵ = -108.60 ($c = 1.00$ in CHCl₃); **22**: [α]_D²⁵ = -103.6 ($c = 1.00$ in CHCl₃); **23**: [α]_D²⁵ = -18.00 ($c = 1.20$ in CHCl₃); **24**: [α]_D²⁵ = $+6.67$ ($c = 0.60$ in CHCl₃).

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Analysis of Solid-Phase Reactions: Product Identification and Quantification by Use of UV-Chromophore-Containing Dual-Linker Analytical Constructs**

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The development of chemistry directly on solid phase is often hampered by difficulties associated with monitoring the progress of reactions and establishing product purity.^[1] One remedial approach has involved introduction of a dual-linker analytical construct comprising a linear sequence of two chemically orthogonal linkers separated by an analytical component.^[2,3] Cleavage at one linker releases only the substrate of interest in a conventional manner, whereas cleavage at the other affords the substrate attached to the analytical unit, which facilitates identification of that product. This principle was further developed in a recently reported construct^[3] that was designed to release an analytical fragment composed of an amine group (generated at the point of cleavage), which enhanced detection by electrospray mass spectrometry (MS; a MS sensitizer), and an isotope label which gave rise to a characteristic split-peak pattern in the mass spectrum (a MS splitter).

Herein, we report further enhancement of the construct properties by the incorporation of a UV chromophore (Figure 1). The intention is to provide a means by which components present in a product mixture may not only be identified by MS, but also be quantified quickly, and accurately by measurement at key UV wavelengths. Follow-

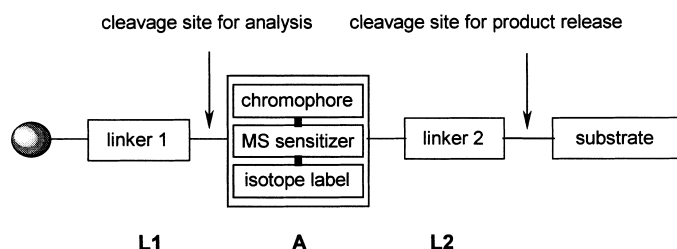


Figure 1. Schematic representation of the analytical construct. Cleavage at linker 2 (**L2**) releases the substrate; alternatively, cleavage at linker 1 (**L1**) releases the substrate bound to an analytical unit (**A**) that enables characterization by HPLC-MS.

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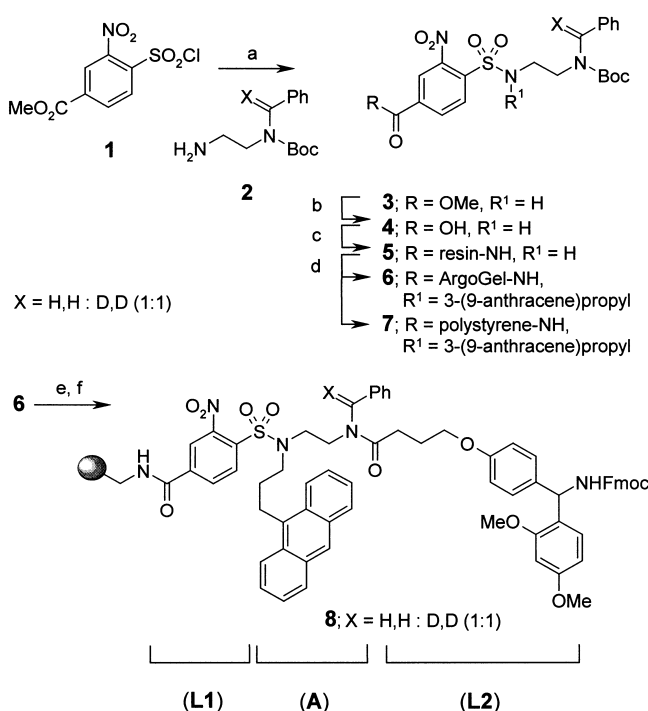
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ing validation of this idea, the construct was applied to the synthesis of an otherwise analytically problematic tetrapeptide.

In selecting the chromophore a number of factors were considered. Clearly the chromophore must be inert under a wide range of reaction conditions. Furthermore, for quantification purposes the absorption maxima of the UV spectrum must occur in a region generally free of absorbances from other species and also be intense enough to enable detection of material released from a single resin bead. The anthracene moiety, which possesses distinctive and intense absorbances at 254 nm (ϵ 180 000, CH₃CN) and at 386 nm (ϵ 9000, CH₃CN) appeared to satisfy these criteria.^[4]

The synthetic route to the new dual-linker analytical construct **7**^[5] is shown in Scheme 1 in which an *o*-nitrophenylsulfonamide group,^[6] chosen as linker 1 (**L1**) as this generates the MS sensitizing amine on cleavage, is tethered to the chromophore and the isotope label (**A**). Thus, the sulfonyl

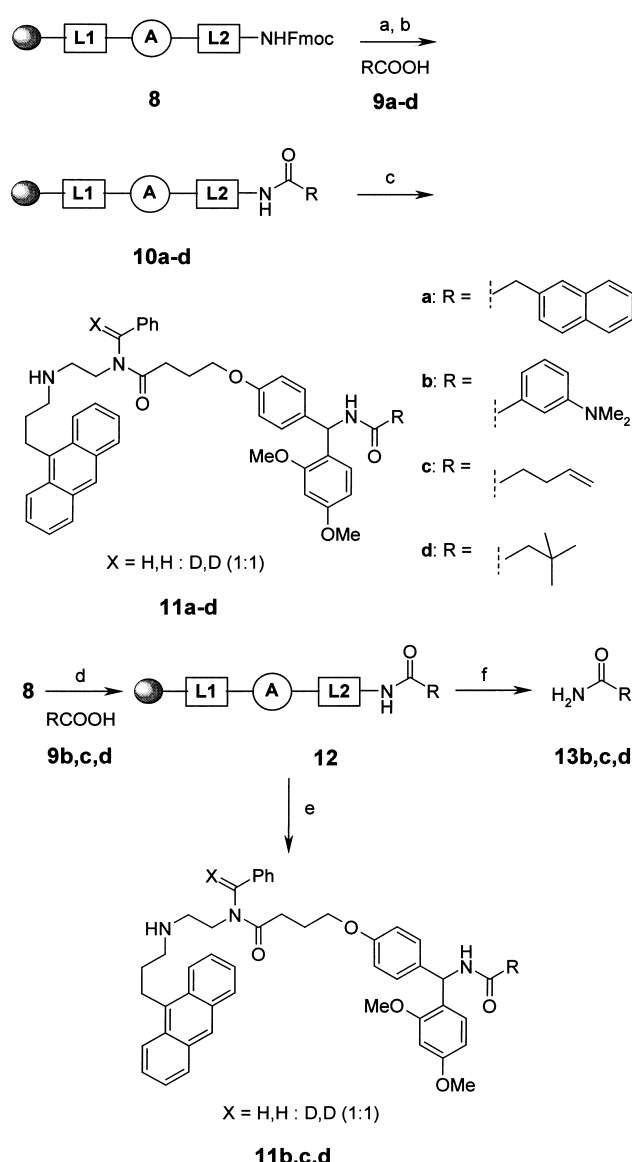


Scheme 1. a) NEt₃, CH₂Cl₂; b) NaOH, MeOH/dioxane; c) aminomethyl ArgoGel resin (0.4 mmol g⁻¹), PyBOP, HOBT, *i*Pr₂NEt, CH₂Cl₂/DMF; d) 3-(9-anthracene)propanol (5 equiv), PPh₃ (5 equiv), di-*tert*-butyl azodicarboxylate (5 equiv), CH₂Cl₂; e) TFA/CH₂Cl₂, phenol; f) 4-[4-[(2,4-dimethoxyphenyl)[[(9H-fluoren-9-ylmethoxy)carbonyl]amino]methyl]phenoxy]butanoic acid, PyBOP, HOBT, *i*Pr₂NEt, CH₂Cl₂/DMF. PyBOP = pyridine-benzotriazolylxytris(dimethylamino)phosphonium hexafluorophosphate; HOBT = 1-hydroxy-1H-benzotriazole.

chloride **1** and labeled *N*-benzyl-*N*-Boc ethylenediamine (**2**)^[7] were combined to give the sulfonamide **3**, the ester group of which was hydrolyzed to the acid **4** and then linked either to aminomethyl ArgoGel (0.4 mmol g⁻¹) or to aminomethyl polystyrene (1.0 mmol g⁻¹) to give the resins **5**. The chromophore was then introduced in quantitative yield by reaction of resins **5** with 3-(9-anthracene)propanol^[8] under Fukuyama Mitsunobu conditions^[9] to give the ArgoGel resin **6** and the polystyrene resin **7** in >98% purity. A second linker (**L2**,

Scheme 1, a modified Fmoc–Rink linker) was introduced on to the ArgoGel resin **6** to give the complete construct **8** with a purity of >96%.

The effectiveness of the chromophore as a tool for quantification was demonstrated by studying a model mixture of products. The Fmoc protective group of **8** was removed (Scheme 2) and four samples of this resin were derivatized by using the acids **9a–d** to produce the four resins **10a–d**. These acids, which possess a range of UV properties, were carefully chosen. First, on cleavage at **L1** and analysis by gradient HPLC, each of the product amines **11a–d** had a distinctive retention time which enables individual peak areas in the chromatogram of a mixture of **11a–d** to be easily measured. Second, each acid gave rise to at least one resonance signal in the ¹H NMR spectrum that was distinct from the others and



Scheme 2. a) 20% piperidine/CH₂Cl₂; b) **9a**, **b**, **c**, or **d** (4 equiv), PyBOP (8 equiv), HOBt (8 equiv), *i*Pr₂NEt (16 equiv), CH₂Cl₂/DMF; c) 4-mercaptobenzoic acid, *N*-methylmorpholine, CH₂Cl₂; d) **9b** (2 equiv), **9c** (1 equiv), **9d** (3 equiv), PyBOP (8 equiv), HOBt (8 equiv), *i*Pr₂NEt (16 equiv), CH₂Cl₂/DMF; e) 2-mercaptoethanol/DBU, acetonitrile (cleavage conditions A); f) 80% TFA/CH₂Cl₂, phenol. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

from the background resonance signals of the construct itself.^[10] Thus a correlation could now be made directly between the peak areas of a HPLC chromatogram, observed at the key analytical wavelengths, and the magnitudes of the integration of these resonance signals in the ¹H NMR spectrum.

Table 1 shows the results from the cleavage of a mixture of approximately equal quantities of the resins **10a–d** to release a mixture of **11a**, **11b**, **11c**, and **11d**. The ratio of the components (relative to **11c**) derived from both HPLC peak areas at 254 and 386 nm and from the integration of the ¹H NMR spectrum indicate an excellent correlation by using these separate techniques.

Table 1. A comparison of the ratios of components in a four-product mixture as measured by HPLC, relative peak areas at 254 nm and 386 nm, and by ¹H NMR spectroscopy.

	254	386	¹ H NMR ^[10]
11a	1.30	1.30	1.34
11b	0.87	0.84	0.85
11c	1.00	1.00	1.00
11d	1.00	0.99	1.05

Further validation of the construct was achieved by studying products synthesized as a mixture. A single sample of resin **8** was deprotected and treated (Scheme 2) with a mixture of three acids **9b**, **9c**, and **9d** to afford resin **12**. The ratio of the three components was ascertained by cleavage of a resin sample at **L1** to release a mixture of **11b**, **11c**, and **11d** and by measuring the peak area of each component by HPLC at 254 nm and 386 nm. Determination of the ratio from a single bead of resin in the same way established that the chromophore was suitable for detection at this low level at both wavelengths and that the bulk bead ratio was preserved. Cleavage at **L2** by using trifluoroacetic acid (TFA) released a mixture of the three relatively nonvolatile primary amides **13b**, **13c**, and **13d**. Comparison of the integration magnitudes at the key resonance signals in the ¹H NMR spectrum^[10] afforded a ratio that correlated well with that determined by HPLC (Table 2).

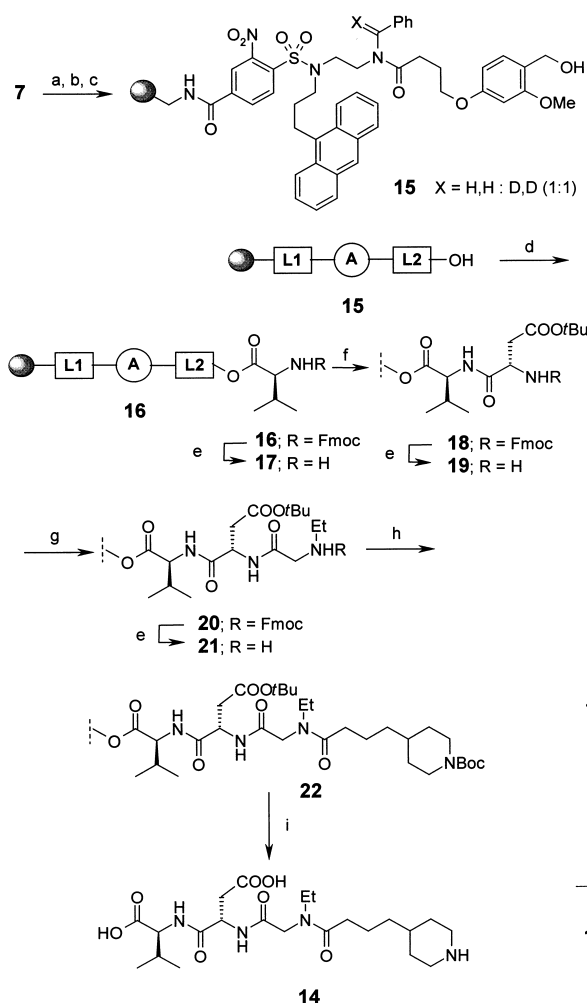
Table 2. A comparison of the ratios of components in a randomly derived three-product mixture as measured by HPLC, by analytical cleavage at **L1**, and by ¹H NMR spectroscopy of amide derivatives cleaved conventionally at **L2**.

	254(b) ^[a]	386(b) ^[a]	254(s) ^[a]	386(s) ^[a]	¹ H NMR ^[10]
11b	0.74	0.71	0.71	0.69	13b 0.74
11c	1.00	1.00	1.00	1.00	13c 1.00
11d	0.44	0.46	0.42	0.41	13d 0.42

[a] b = bulk beads, s = single bead; ArgoGel 150 μm beads, theoretical loading 0.29 mmol g^{−1}.

These data show that the anthracene-containing construct can be used to determine accurately the relative quantities of materials in a product mixture.^[11]

The utility of the construct was demonstrated in the synthesis of an otherwise analytically problematic target molecule. Owing to the absence of a diagnostic chromophore the fibrinogen receptor antagonist **14**^[12] (Scheme 3) presents a



Scheme 3. a) 50% TFA/CH₂Cl₂, phenol; b) 4-(4-formyl-3-methoxyphenoxy)butyric acid (2 equiv), PyBOP (4 equiv), HOBT (4 equiv), *i*Pr₂NEt (8 equiv), CH₂Cl₂/DMF; c) NaBH₄, THF; d) FmocVal-OH, PyBOP, HOBT, *i*Pr₂NEt, CH₂Cl₂/DMF; e) 20% piperidine/CH₂Cl₂; f) FmocAsp(OtBu)-OH, PyBOP, HOBT, *i*Pr₂NEt, CH₂Cl₂/DMF; g) Fmoc-N-EtGly-OH, PyBOP, HOBT, *i*Pr₂NEt, CH₂Cl₂; h) 4-(4-*N*-Boc-piperidinyl)carboxylic acid, PyBOP, HOBT, *i*Pr₂NEt, CH₂Cl₂/DMF; i) 80% TFA/CH₂Cl₂ (68% overall yield). Fmoc = 9-fluorenylmethoxycarbonyl.

number of synthetic challenges on solid phase, these challenges are: coupling of the hindered Val residue to resin, potentially poor incorporation of Asp, likely generation of a diketopiperazine (DKP), and coupling to a hindered secondary amine.

Beginning with resin **7**, an acid-labile modified Sasrin linker was introduced as **L2**, affording the analytical construct **15** (Scheme 3). The remainder of the synthesis was carried out (Scheme 3), with each step being monitored by the cleavages of a sample of each resin **15–22** at **L1** and the analysis of the analytical fragments from HPLC-MS. The chromatograms at each step are reproduced (Figure 2) together with the parent ion in the mass spectrum derived from each major peak. The spectra show the characteristic isotope doublet

and confirm the identity of the product in each case. By using this technique each step of the synthesis could be followed through to completion, allowing optimization of reaction conditions as necessary. Critically, the construct enabled the development of conditions for complete loading of the first amino acid on to resin and also provided a means of quantifying the extent of DKP formation during the synthesis of **19**. Reliable quantitative analysis of these processes by conventional methods with small quantities of resin would have been very difficult. Analysis of the resin **22** indicated that the purity of the peptide should exceed 90% on conventional cleavage. This was indeed found to be the case; the purity of the cleaved crude peptide was estimated as approximately 95% by ¹H NMR spectroscopy (in 68% overall yield) and was consistent with the literature data for the peptide synthesized in solution.^[12, 13]

In conclusion, we have developed an analytical construct that allows monitoring of reactions carried out on solid support and the relative quantification of products, even from a single resin bead, by use of a simple cleavage protocol monitored by standard HPLC-MS analysis.

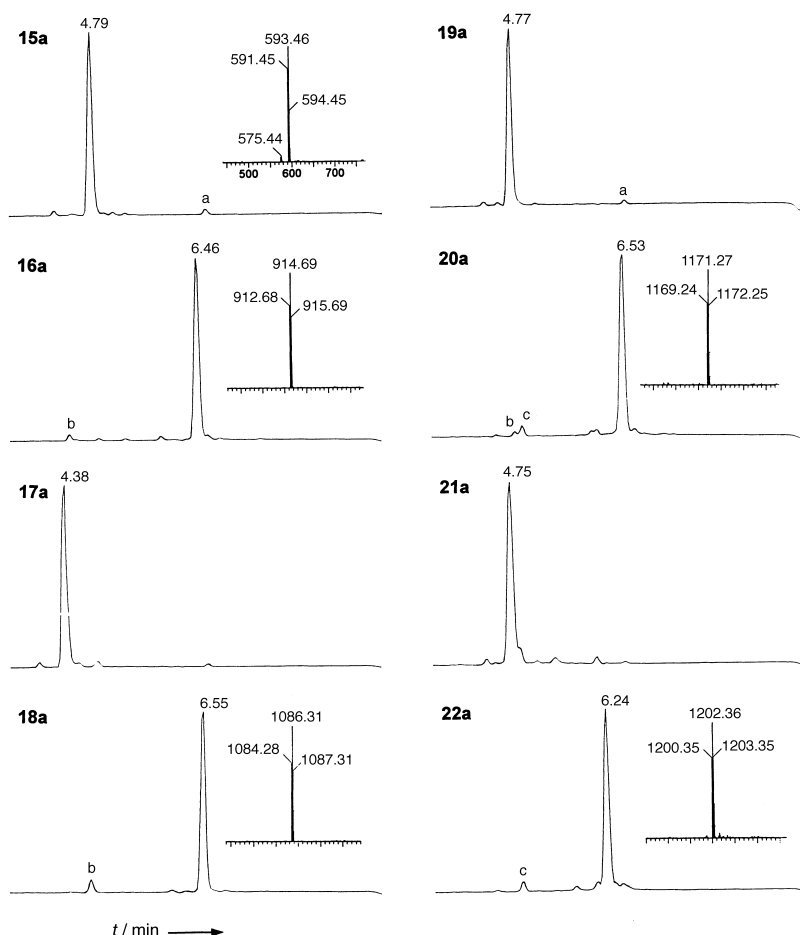


Figure 2. Representative HPLC chromatograms recorded at 386 nm of each amine **15a–22a**, obtained by cleavage of the resins **15–22** at **L1** (Scheme 3), indicating the progress of each step in the synthesis of **14**. The parent ion of the product in the mass spectrum corresponding to the main peak in selected chromatograms is also given. a: an impurity (2%) occurring after coupling of the second linker. b: indicates a loss (2–3%) of the Fmoc protective group during treatment of **16**, **18**, and **20** with the cleavage medium. c: indicates the reappearance of **15**, indicative of DKP formation in the previous step. Being relatively unreactive, this material is observed in subsequent chromatograms. Approximately 4% of the peptide is lost.

Experimental Section

The purity of materials on the solid support was assessed by cleavage at **L1** and analysis by using high-pressure liquid chromatography (HPLC) on a Hewlett Packard Series 1050 instrument. Column: Supercosil ABZ⁺PLUS 3.3 cm × 4.6 mm, 3 μm, reverse phase. Eluent A: water, 0.1 % trifluoroacetic acid; eluent B: acetonitrile 95 %, water 5 %, trifluoroacetic acid 0.05 %; flow rate: 1 mL min⁻¹; detection: UV (diode array: 215, 230, 254, 386 nm); typical method: gradient 10–95 % B in A, over 7 min. Liquid chromatography mass spectra (LCMS) were recorded on a Micromass Platform by using electrospray positive and negative ionization. Column: Supercosil ABZ⁺PLUS 3.3 cm × 4.6 mm, 3 μm, reverse phase. Eluent A: 10 mM solution of ammonium acetate in water, 0.1 % formic acid; eluent B: acetonitrile 95 %, water 5 %, formic acid 0.05 %; flow rate: 1 mL min⁻¹; detection: UV (diode array: 215, 230, 254 nm); typical method: gradient 0–100 % B in A, over 3.5 min, 100 % B 3.5 min.

Cleavage at **L1** was carried as follows: A) Modified Rink constructs **8–12**. A small resin sample was incubated for 5–10 min at room temperature with a solution (10 μL) composed of 2-mercaptoethanol (21 μL), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (70 μL) in acetonitrile (250 μL). After filtration the filtrate was diluted to a final volume of 20 μL with acetonitrile prior to analysis. B) Modified Sasrin constructs **15–22**. A small resin sample was incubated for 5–10 min at room temperature with a solution (10 μL) composed of thiophenol (25 μL), and diisopropylethylamine (50 μL) in acetonitrile (250 μL). After filtration the filtrate was evaporated under a stream of nitrogen and the residue dissolved in acetonitrile (20 μL) prior to analysis. Conditions B were chosen to avoid cleavage of the ester bond in compounds **16–22**.

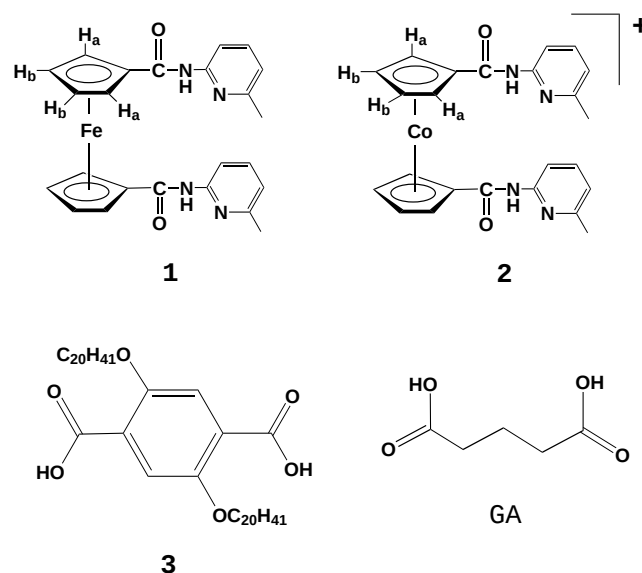
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Redox-Switched Control of Binding Strength in Hydrogen-Bonded Metallocene Complexes

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James H. R. Tucker,* and Joanna Westwood

Redox-switched binding of organic molecules by either organic^[1] or inorganic^[2] electroactive species is attracting continued interest in the area of supramolecular electrochemistry. Those examples in which the binding interaction is through hydrogen bonds are relevant to recent studies which indicate that hydrogen bonds play an important role in tuning the redox potential of certain proteins.^[3] Herein we compare the dicarboxylic acid binding properties of **1** with those of its charged cobaltocenium analogue **2**^[4] and show that the strength of the hydrogen bonding interaction can be controlled by changing the oxidation state of the metal in each receptor.



As found previously with **1**^[5] and with other organic^[6] and inorganic^[7] bis-amidopyridine receptors, **2** binds dicarboxylic acids in organic solvents. ¹H NMR studies in CDCl₃/[D₆]DMSO (0.5 %) revealed downfield shifts in the amide and Cp proton resonances of **2** upon addition of excess glutaric acid (GA). A titration of the H_a proton resonance against molar equivalents of GA revealed a complex stoi-

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chiometry of 1:1 (downfield shift in H_a protons upon complexation (ppm): **1**, +0.36; **2**, +0.19). The resulting binding constants (298 K), evaluated by EQNMR^[8] for a monomeric complex, (Scheme 1) show that the interaction of GA with **2** in CDCl₃/[D₆]DMSO(0.5%) is approximately twenty times stronger than that with **1** ($K_2^{\text{ox}} = 98\,000\text{ M}^{-1} \pm 10\%$ where $K_2^{\text{ox}} = [\mathbf{2}:\text{GA}]/[\mathbf{2}][\text{GA}]$; $K_1^{\text{red}} = 4600\text{ M}^{-1} \pm 5\%$ where $K_1^{\text{red}} = [\mathbf{1}:\text{GA}]/[\mathbf{1}][\text{GA}]$). A ¹H NMR competition experiment was carried out to corroborate these results. Figure 1 depicts the Cp regions of **1** and **2** in CDCl₃/[D₆]DMSO (0.5%) (each 0.80 mm) and the effect of adding one (Figure 1 b) and five molar equivalents (Figure 1 c) of GA to the solution. Clearly

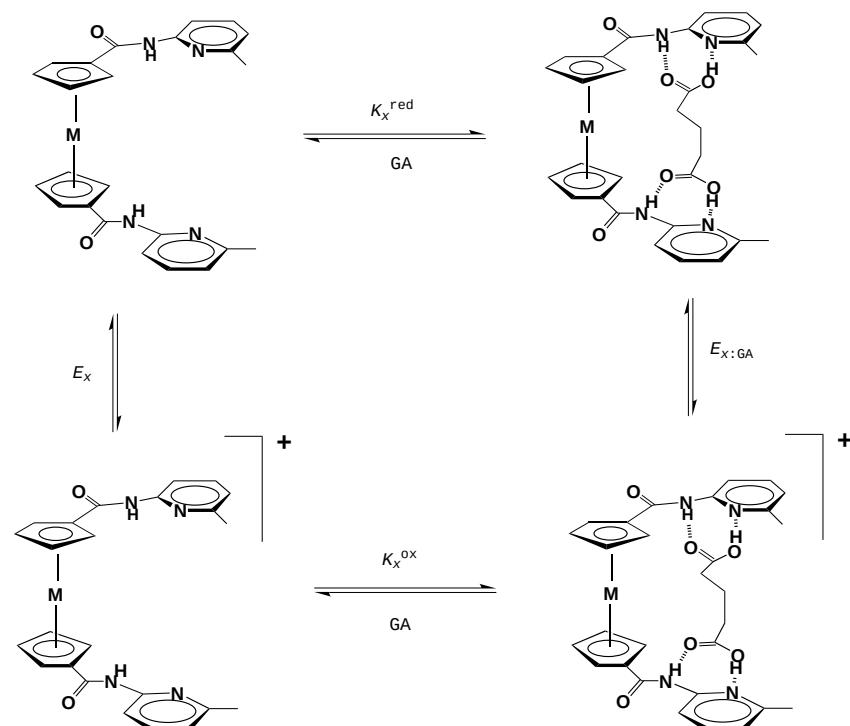
the first addition (Figure 1 b) shifts the Cp signals for **2** downfield (ca. 70% complexation), whereas those for **1** move only slightly (ca. 11% complexation). The binding constant K [Eq. (1)] evaluated from this data^[9] is virtually the same as that obtained by dividing K_2^{ox} by K_1^{red} . Only upon the addition of an excess of guest (Figure 1 c) does **1** bind GA to any great extent.



The X-ray structures of complexes [**1**:GA]₂ (Figure 2) and [**2**:GA]₂ (Figure 3) reveal dimeric metallocenes, formed by

intermolecular bonding through two GA units. The metallocene receptors form symmetric hydrogen bonds with the carboxylic acid groups on both GA molecules, where as expected, the amide protons associate with the carbonyl moiety and the pyridyl nitrogen atoms with the alcohol group. The functional groups on each metallocene are eclipsed in [**2**:GA]₂ and staggered in the 1,2-positions in [**1**:GA]₂. Interestingly, whereas the hydrogen bonds involving the pyridyl nitrogen atoms are of a similar length in both complexes, the amide hydrogen bonds in the cobalt complex are shorter (N–H...O_{av}/O–H...N_{av} 2.970(6)/2.662(3) and 2.897(4)/2.656(4) Å for [**1**:GA]₂ and [**2**:GA]₂, respectively). In addition, the amide N–H bond lengths within these hydrogen bonds in the cobalt complex are longer than those in the iron complex (0.945 and 0.880 Å, respectively). It is likely that both complexes are present as 2:2 dimers in the solid state due to favorable packing interactions, since there was no evidence for their formation in solution at millimolar concentrations. Solubility problems precluded vapor pressure osmometry (VPO) measurements but a 1:1 complex with **2**, expected on entropic (i.e. chelate formation) grounds and found for related complexes,^[6,7] was shown to be exclusively present under ESMS conditions (see Supporting Information). Furthermore, NMR studies revealed that the introduction of a rigid spacer group to the diacid guest (compound **3**) to preclude monomer formation due to steric hindrance and favor dimer formation resulted in only a very weak binding interaction.^[10] There was no evidence for the formation of mixed metallocene complexes from the competition studies.

Cyclic voltammetry studies^[11] in CH₂Cl₂ (0.5% DMSO) revealed identical negative shifts in the reversible Fe^{III}/Fe^{II} and Co^{III}/Co^{II} couples of the receptors (Scheme 1) upon the addition of excess GA (E



Scheme 1. Square scheme showing the relationship between the binding constants and the electrode potentials for receptors **1** (M = Fe, $x = 1$) and **2** (M = Co, $x = 2$).

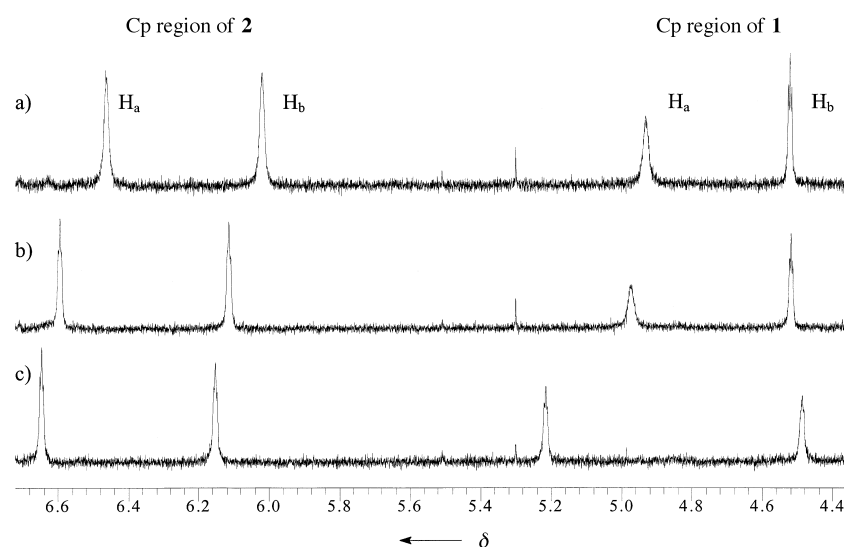


Figure 1. ¹H NMR spectra of an equimolar mixture of **1** and **2** (Cp region) in CDCl₃/[D₆]DMSO (0.5%) with a) no GA; b) 1 molar equivalent of GA; c) 5 molar equivalents of GA.

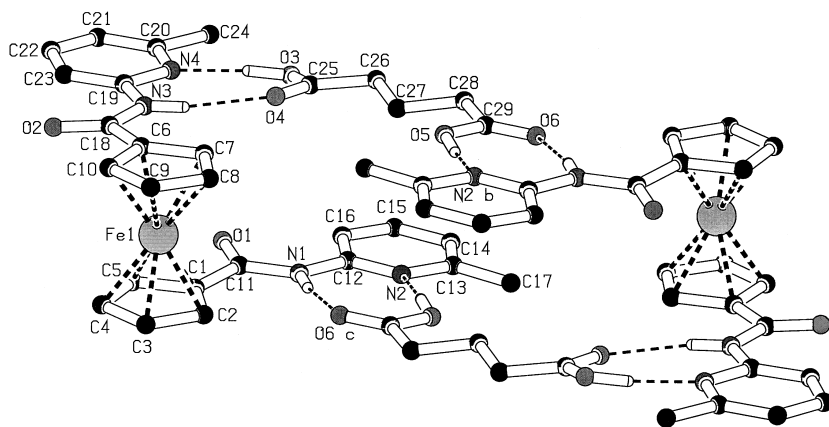


Figure 2. X-ray structure of the dimer $[1:GA]_2$. Selected bond lengths [Å] and angles [°]: C1–C11 1.478(10), C11–N1 1.373(8), N1–C12 1.402(8), C6–C18 1.505(9), C18–N3 1.350(8), N3–C19 1.402(8); C1–C11–N1 117.4(7), C11–N1–C12 126.4(6), C6–C18–N3 116.9(7), C18–N3–C19 126.1(6).

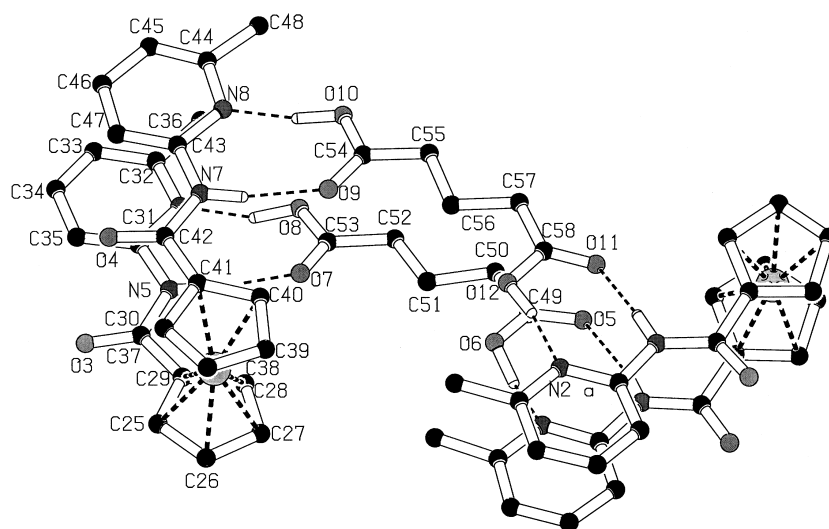


Figure 3. X-ray structure of $[2:GA]_2$ (PF_6^- counteranions omitted and only one independent molecule shown for clarity). Selected bond lengths [Å] and angles [°]: C29–C30 1.495(10), C30–N5 1.382(9), N5–C31 1.428(9), C41–C42 1.495(11), C42–N7 1.385(9), N7–C43 1.416(9); C29–C30–N5 114.5(8), C30–N5–C31 124.3(8), C41–C42–N7 115.9(8), C42–N7–C43 124.2(7).

(± 10 mV) vs. Fc^+/Fc internal couple: $E_1 + 0.45$ V, $E_{1:GA} + 0.36$ V; $E_2 - 0.83$ V; $E_{2:GA} - 0.92$ V; ΔE : **1**, -90 mV; **2**, -90 mV). Since the binding constant between GA and each receptor in one redox state is known, the binding constant in the other redox state, as shown in the square scheme (Scheme 1), can be evaluated^[1b] by using Equation (2) ($x = 1$ or **2**). Clearly the binding constants with GA are similar for

$$\frac{K_x^{ox}}{K_x^{red}} = \exp \left[\frac{-nF(\Delta E)}{RT} \right] \quad (2)$$

either of these metallocene receptors in their reduced (ferrocene, $\log K_1^{red} = 3.66 \pm 0.02$; cobaltocene, $\log K_2^{red} = 3.45 \pm 0.2$) or oxidized forms (ferrocenium $\log K_1^{ox} = 5.2 \pm 0.2$; cobaltocenium $\log K_2^{ox} = 4.99 \text{ M}^{-1} \pm 0.04$). Therefore, the factor responsible for this redox-switched effect must be the introduction of positive charge at the metallocene center, which, in pulling electron density away from the amide units, increases the acidity of the amide protons and makes hydro-

gen bonding stronger. The N–H stretching frequency for each receptor in CH_3CN (**1**: 3354 cm^{-1} ; **2**: 3178 cm^{-1}) and the bond lengths from the X-ray data are consistent with this explanation. In conclusion, we have shown how the ratio of binding strengths between two hydrogen-bonded complexes can be *reversibly inverted* by switching the charge of these receptors on or off.

Experimental Section

X-ray studies: $[1:GA]_2$: crystals from slow diffusion of diethyl ether into CH_2Cl_2 , $C_{24}H_{22}N_4O_2Fe \cdot C_5H_8O_4 \cdot 0.5(CH_3CH_2OH)$, $M_r = 609.45$; crystal dimensions $0.2 \times 0.16 \times 0.12 \text{ mm}^3$; monoclinic; space group $P2_1/n$; $a = 15.617(3)$, $b = 9.222(2)$, $c = 21.185(4)$ Å, $\beta = 103.43(3)^\circ$, $Z = 4$, $V = 2967.6(10) \text{ Å}^3$, $\rho_{calcd} = 1.364 \text{ mg m}^{-3}$, $T = 150(2)$ K; θ range = $1.98 - 24.96^\circ$; 11 075 measured reflections merged to 4357 unique reflections ($R_{int} = 0.1123$); $R_1 = 0.055$ and 0.1455 and $wR_2 = 0.1113$ and 0.1287 for observed data ($I > 2\sigma(I)$) and for all data, respectively; $\rho_{max}/\rho_{min} = 0.820$ and -0.319 e Å^{-3} . $[2:GA]_2$: crystals from slow diffusion of diethyl ether into CH_2Cl_2 , $C_{24}H_{22}N_4O_2Co \cdot C_5H_8O_4 \cdot PF_6$, $M_r = 734.47$; crystal dimensions $0.3 \times 0.18 \times 0.09 \text{ mm}^3$; triclinic; space group $P\bar{1}$; $a = 11.575(2)$, $b = 12.682(2)$, $c = 22.171(3)$ Å, $\alpha = 72.402(12)$, $\beta = 80.474(12)$, $\gamma = 87.362(11)^\circ$, $Z = 2$, $V = 3059.3(11) \text{ Å}^3$, $\rho_{calcd} = 1.595 \text{ mg m}^{-3}$, $T = 293(2)$ K; θ range = $1.78 - 25.05^\circ$; 11 985 measured reflections merged to 7893 unique ($R_{int} = 0.0796$); $R_1 = 0.0514$ and 0.1175 and $wR_2 = 0.0919$ and 0.1047 for observed data ($I > 2\sigma(I)$) and for all data, respectively; $\rho_{max}/\rho_{min} = 0.492$ and -0.338 e Å^{-3} . Both datasets were measured on a Nonius FAST TV area detector at the window of a Nonius FR591 rotating anode, MoK_{α} radiation ($\lambda = 0.71073$ Å); absorption correction applied using the RefDelf method of DIFABS; structures solved by Patterson methods using SHELXS-86 and refined with SHELXL-93. Non-hydrogen atoms anisotropic, hydrogen atoms isotropic riding model. 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-142347 ($[1:GA]_2$) and CCDC-142348 ($[2:GA]_2$). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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- [10] A ^1H NMR study revealed that the amide and Cp protons of **1** (1 mM in $CDCl_3/[D_6]DMSO$ (0.5%)) underwent very small downfield shifts upon the addition of excess **3** (e.g. H_a , +0.08 ppm) with 50% complexation only achieved after the addition of six molar equivalents of diacid.
- [11] For previous cyclic voltammetry studies with **1** and for experimental conditions, see reference [5].

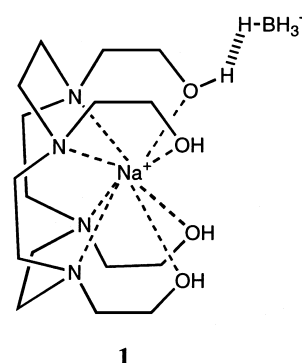
Toward Crystalline Covalent Solids: Crystal-to-Crystal Dihydrogen to Covalent Bonding Transformation in $NaBH_4 \cdot THEC^{**}$

Radu Custelcean,* Mircea Vlăssă, and James E. Jackson*

The hydridic-to-protonic interaction, or dihydrogen bonding, between negatively charged hydrogen atoms of $M-H$ ($M = Al, B, Ga, Ir, Mo, Mn, Os, Re, Ru, W$) bonds and conventional proton donors has been extensively studied since 1994.^[1] With characteristics similar to those found in classical hydrogen bonding, this unconventional interaction can influence reactivity and stereoselectivity in solution.^[2] In the solid state, dihydrogen bonds have the ability to direct crystal packing, thus finding utility in supramolecular synthesis.^[3] Along this line, we have employed $O-H \cdots H-B$ hydrogen bonds in the construction of novel one-, two-, and three-dimensional extended structures with various topologies.^[4] More interestingly, some of these systems can react topochemically in the solid state, releasing H_2 and exchanging the weak $O-H \cdots H-B$ interactions for strong $B-O$ covalent bonds, to form ordered, extended covalent materials.^[4] How-

ever, due to the cumulative shrinkage of the $B-O$ distances during decomposition, these topochemically assembled solids have shown poor crystallinity. In 1998 we suggested two strategies to circumvent this problem: a) design of cations to form closed loops in coordination with hydride-bearing anions, in which case the lattice distortion accompanying decomposition would not be cumulative, and b) selection of globular cations large enough that their close packing determines the lattice parameters, with the hydridic anions fitting into the interstitial holes, in which bond formation via flexible arms would induce minimal change in the unit cell.^[4a] We now describe a dihydrogen-bonded system that satisfies both of these geometrical criteria, leading as a result to a crystalline covalent product, in a crystal-to-crystal thermal decomposition.

N,N',N'',N''' -tetrakis-(2-hydroxyethyl)cyclen (THEC) and $NaBH_4$ form a 1:1 complex (**1**), which precipitates as a white crystalline solid from 2-propanol.^[5]



Single crystals suitable for X-ray crystallography were obtained by slow diffusion of diethyl ether into a solution of **1** in acetonitrile. The crystal structure is depicted in Figure 1.^[6] The THEC adopts a conformation with all hydroxyethyl arms oriented toward the same face of the azacrown ring, as observed in previously reported metal complexes containing this ligand.^[7] The nitrogen and oxygen atoms are thus organized in a pseudo-cubic geometry, encapsulating the octacoordinated Na^+ cation. The Na^+THEC units have thus the appropriate geometry for the formation of the desired closed-looped dihydrogen-bonded assembly. Indeed, these cations self-assemble into D_2 -symmetrical dimers, held together by four conventional $O(2)-H \cdots O(1)$ hydrogen bonds, complemented by four orthogonal $O(1)-H \cdots H-B$ proton-hydride interactions involving the borohydride anions (Figure 1a). There is no hydrogen bonding interconnecting the dimers, which are packed into two-dimensional layers (Figure 1b). The layers are stacked in a parallel fashion, thus creating one-dimensional channels along the a axis, in which the BH_4^- ions reside.

The association in closed loops, as well as the packing mode of the large globular $[Na_2(THEC)_2]^{2+}$ ions, appear to fulfill the two geometrical requirements that we predicted should favor the transfer of crystallinity from dihydrogen- to covalent-bonded networks.^[4a] Solid-state decomposition of **1**, induced by heating under an inert atmosphere at $160^\circ C$ for 20 h, results in complete elimination of H_2 , as indicated by the FT-

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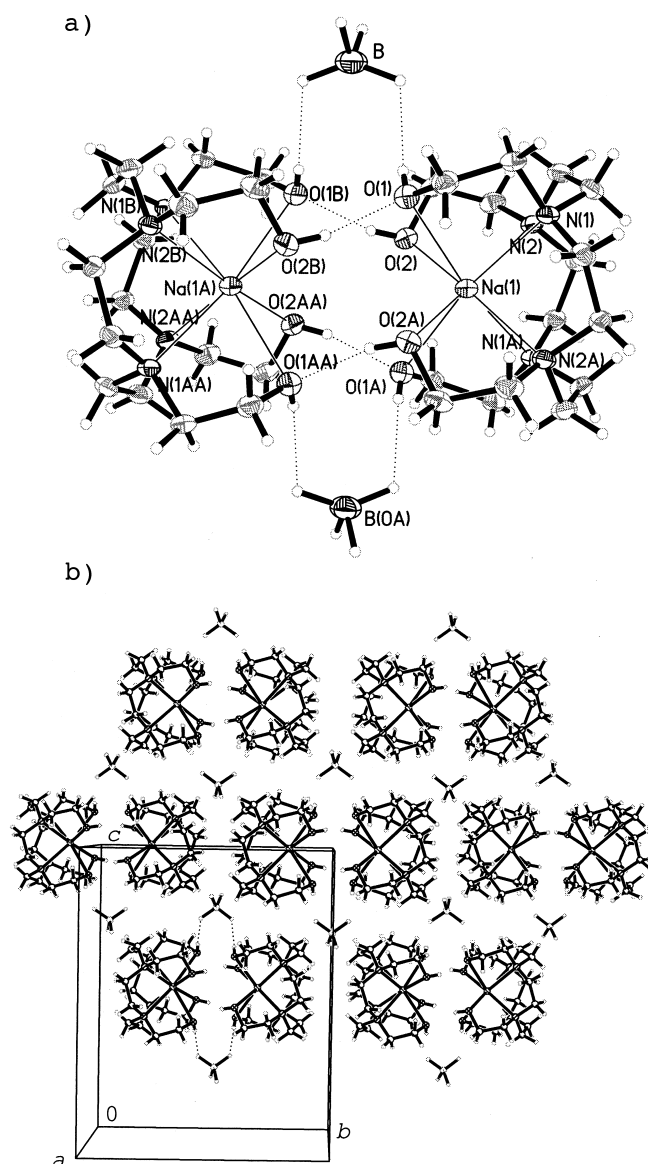


Figure 1. X-ray crystal structure of the dihydrogen-bonded complex **1**: a) self-assembled dimers; selected bond lengths [Å] and angles [°]: O(1)–H 0.72(3), B–H 1.17(2), O(1)H⋯HB 2.00(5), O(1)⋯B 3.352(3), O(2)–H 0.88(4), O(2)H⋯O(1) 1.94(5), O(2)⋯O(1) 2.801(6), N(1)–Na 2.706(2), N(2)–Na 2.634(2), O(1)–Na 2.690(2), O(2)–Na 2.474(2); O(1)–H–H 151(2), B–H–H 115(3), O(2)–H–O(1) 164(3); b) packing of dimers in layers.

IR and ^{11}B solid-state MAS NMR spectra of the resulting material.^[8] Thermogravimetric analysis shows a remarkably sharp 1.88% weight loss between 168.0 and 168.8°C, which corresponds to 3.63 mol H_2 . The solid-state decomposition product (**2**) exhibits high crystallinity, as indicated by microscopic examination, which showed good transparency to polarized light for the decomposed crystals (Figure 2). The reaction appears to be crystallographically homogeneous, in direct contrast to our previously reported dihydrogen-bonded systems, where the covalent product nucleated as a separate phase.^[4c] That the solid-state decomposition of **1** is a single-phase transformation is also supported by powder X-ray diffraction analysis, which shows a gradual shift of the diffraction pattern as the reaction progresses.^[9] The final covalent product exhibits high crystallinity, in agreement with

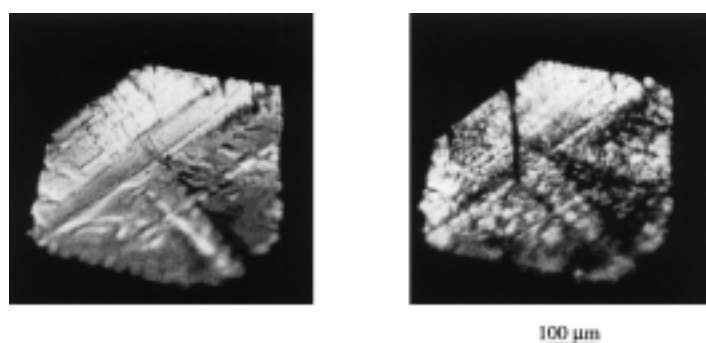


Figure 2. Polarizing microscopic view of $\text{NaBH}_4 \cdot \text{THEC}$ crystal-to-crystal decomposition: left) initial crystal; right) after 20 h at 160°C. Scale bar = 100 μm .

the microscopic observations (Figure 3). For comparison, an amorphous solid results when **1** is decomposed in the melt at 200°C for 10 min (Figure 3c).^[10] The unit cell parameters for **2**, calculated using powder X-ray diffraction data, are: $a = 8.600(34)$, $b = 13.864(74)$, $c = 16.895(27)$ Å, and $V = 2014(11)$ Å³. They correspond to 3.3, 2.5, 3.5, and 9.0% shrinkage, respectively, relative to **1**.^[11] We also monitored the

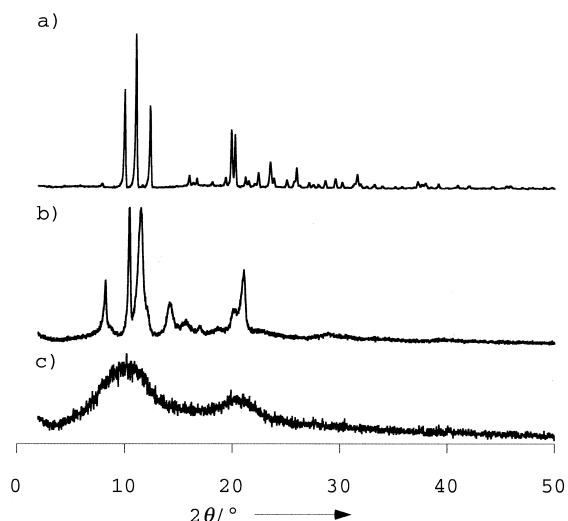


Figure 3. Powder X-ray diffraction patterns for: a) dihydrogen-bonded complex **1**; b) solid-state decomposition product **2**; c) product of decomposition in the melt.

evolution of unit cell parameters during the solid-state decomposition of **1** at 120°C. The initial crystal lattice smoothly transforms into the corresponding lattice of **2**, indicating a crystal-to-crystal process (Figure 4).^[9]

Despite our sustained efforts, the detailed structure of **2** has remained elusive to date. The relatively big change in the unit cell accompanying the solid-state decomposition ultimately results in deterioration of the single crystals' quality, precluding their X-ray structural investigation. Also, the unexpected insolubility of **2** in common organic solvents does not allow its recrystallization. Considering the crystal packing of **1**, with no interdimer hydrogen bonding present, formation of discrete molecular cages is expected from the solid-state decomposition. Crosslinking between dimers would require substantial intermolecular rearrangements, which is highly unlikely in a

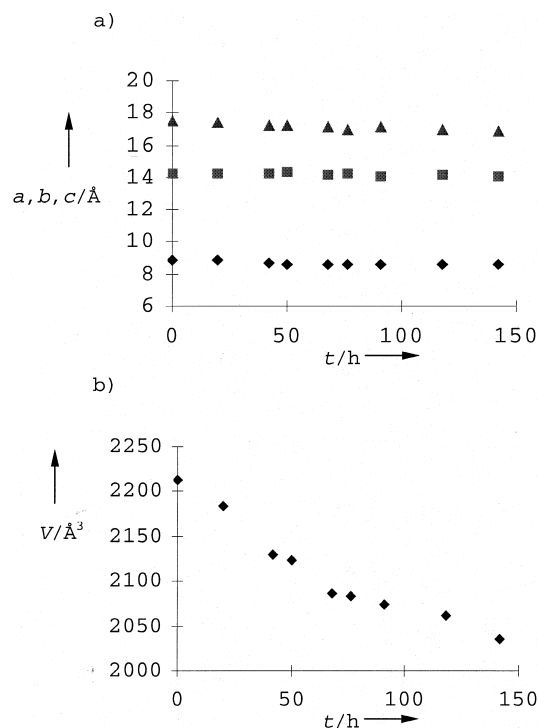


Figure 4. Evolution of unit cell axes (a; $a = \blacklozenge$, $b = \blacksquare$, $c = \blacktriangle$) and volume (b) during the solid-state decomposition of **1** at 120 °C, monitored by powder X-ray diffraction.

crystallographically homogeneous, lattice-controlled transformation. Figure 5 presents a possible structure for the molecular cages in **2**, optimized^[12] with the density functional theory (DFT) method at the B3LYP/6-31G* level.^[13] No unusual values for the bond lengths and angles were observed in the resulting C_2 -symmetrical structure, suggesting minimal strain in these covalent dimers.

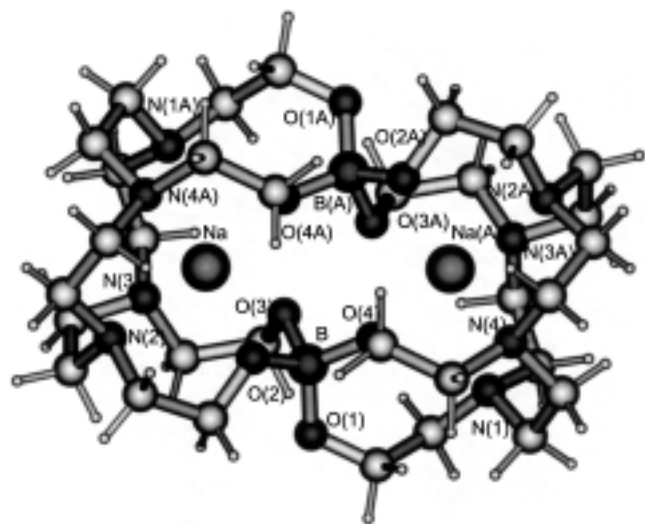


Figure 5. Molecular model of the solid-state decomposition product **2** calculated by using the DFT method at the B3LYP/6-31G* level; selected bond lengths [Å] and angles [°]: B-O(1) 1.458, B-O(2) 1.482, B-O(3) 1.497, B-O(4) 1.474, N(1)-Na(A) 3.003, N(2)-Na 2.714, N(3)-Na 2.435, N(4)-Na(A) 2.565, O(2)-Na 2.670, O(3)-Na 2.276, O(4)-Na(A) 2.228; O(1)-B-O(2) 112.5, O(1)-B-O(3) 109.1, O(1)-B-O(4) 114.6, B-O(1)-C 123.7, B-O(2)-C 122.3, B-O(3)-C 125.9, B-O(4)-C 125.6.

When **1** was decomposed in DMSO by heating at 120 °C for 48 h, a crystalline product^[14] (**3**) precipitated in high yield. X-ray structural analysis of the resulting crystals revealed a centrosymmetric molecular cage very similar to that expected from the solid-state reaction (Figure 6).^[6] However, one of the

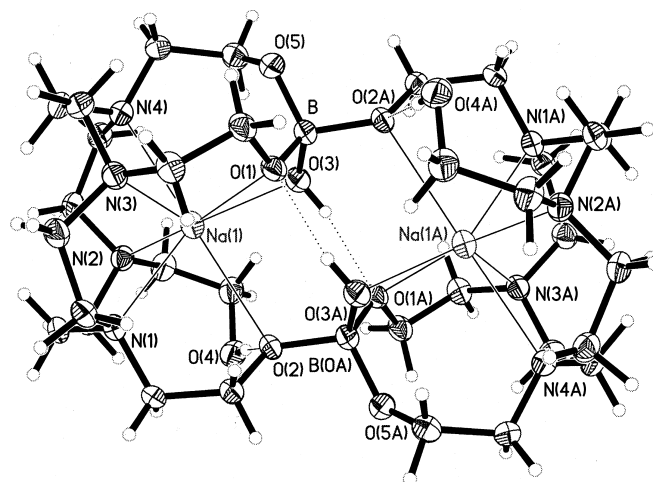


Figure 6. X-ray crystal structure of the major conformation found in the covalent dimer **3**, obtained by decomposing **1** in DMSO; selected bond lengths [Å] and angles [°]: B-O(1) 1.502(2), B-O(2) 1.511(2), B-O(3) 1.450(2), B-O(5) 1.457(2), N(1)-Na 2.519(1), N(2)-Na 2.540(1), N(3)-Na 2.565(1), N(4)-Na 2.682(1), O(1)-Na 2.371(1), O(2)-Na 2.796(1), O(3)-Na 2.391(1), O(1)-B-O(5) 111.6(1), O(2)-B-O(5) 109.8(1), O(3)-B-O(5) 111.6(1), B-O(1)-C 121.7(1), B-O(2)-C 113.2(1), B-O(5)-C 122.8(1).

hydroxyethyl arms in each of the two THEC ligands in the dimer does not bind the boron atom, nor does it complex the Na cation, being replaced by an OH group, presumably from the water present in the solvent. Except for the Na, the four N atoms, and C(4) and C(6), all the atoms in the structure are disordered over two sites, with 90.1 and 9.9% occupancies, respectively. Like **2**, **3** is surprisingly insoluble in common organic solvents, despite its relatively small size, overall neutral charge, and lack of intermolecular hydrogen bonding.

In summary, we report here the first deliberate crystal-to-crystal dihydrogen to covalent bonding transformation.^[15] Judicious engineering of dihydrogen-bonded crystals permits transfer of crystallinity to the covalent products resulting from their solid-state decompositions. Further elaboration of the dihydrogen-bonded building blocks described herein may ultimately lead to low-temperature, rational construction of extended crystalline covalent solids, a class of compounds to which few purposeful synthetic paths exist.

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- [5] Spectroscopic data for **1**: ^1H NMR (300.1 MHz, $\text{C}_6\text{D}_6\text{N}$, 25 °C): δ = 6.03 (br. s, 4H; OH), 3.85 (t, J = 4.5 Hz, 8H; OCH_2), 2.49, 2.41 (br., 24H; NCH_2), 1.51 (q, J = 81.3 Hz, 4H; BH_4); ^{13}C NMR (75.4 MHz, CD_3CN , 25 °C): δ = 51.17 (NCH_2), 56.45 (NCH_2), 58.96 (OCH_2); ^{11}B NMR (96.23 MHz, CD_3CN , 25 °C, $\text{B}(\text{OCH}_3)_3$): δ = –54.21 (quintet, J = 81.4 Hz; BH_4); ^{11}B MAS NMR (128.33 MHz, 4013 Hz, 25 °C, $\text{B}(\text{OH})_3$): δ = –45.4 ($\Delta\nu_{1/2}$ = 2198 Hz); ^{23}Na MAS NMR (105.81 MHz, 6028 Hz, 25 °C, NaCl): δ = –12.3 ($\Delta\nu_{1/2}$ = 1440 Hz); IR (KBr): $\tilde{\nu}$ = 2298, 2225 cm^{-1} (B–H); elemental analysis calcd for $\text{C}_{16}\text{H}_{40}\text{N}_4\text{O}_4\text{BNa}$ (%): C 49.74, H 10.36, N 14.51; found: C 49.37, H 10.87, N 14.80; m.p. 180–181 °C.
- [6] A Siemens SMART CCD diffractometer with MoK_α radiation (λ = 0.71073) was used for the X-ray single-crystal analyses in this study. The structures were solved by direct methods and refined by full-matrix least-squares on F^2 using the SHELXTL package (Structure Analysis Program 5.1, Bruker AXS, Inc., Madison, WI, **1997**). Absorption corrections were applied using SADABS. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located from the difference Fourier maps and refined isotropically. For the borohydride ions in **1**, however, only the hydridic hydrogen atoms involved in dihydrogen bonding could be found; the remaining H atoms from BH_4^- were calculated and placed in idealized positions. The observed O–H and B–H distances appear typically shorter than the ideal values of 0.96 and 1.21 Å, and were not corrected. Crystal data for **1**: crystal dimensions: $0.23 \times 0.18 \times 0.13$ mm; T = 173 K; orthorhombic, I_{222} ; a = 8.8240(3), b = 14.1344(6), c = 17.3533(7) Å, V = 2164.34(15) Å³, Z = 4; ρ_{calcd} = 1.186 g cm^{-3} ; $2\theta_{\text{max}}$ = 56.4°; 12 854 reflections collected, 2608 unique; 200 parameters; R_1 = 0.0487, wR_2 = 0.1221 for $I > 2\sigma(I)$; residual electron density: 0.488 e Å^{–3}. Crystal data for **3**: crystal dimensions: $0.47 \times 0.39 \times 0.29$ mm; T = 173 K; monoclinic, $P2_1/n$; a = 8.7696(1), b = 13.0571(1), c = 17.7724(2) Å, β = 96.36°, V = 2022.50(4) Å³, Z = 4; ρ_{calcd} = 1.301 g cm^{-3} ; $2\theta_{\text{max}}$ = 56.5°; 20 248 reflections collected, 4840 unique; 462 parameters; R_1 = 0.0352, wR_2 = 0.0921 for $I > 2\sigma(I)$; residual electron density: 0.261 e Å^{–3}. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-143751 and CCDC-143752. 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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NMR and Theoretical Study of Acid Sites Formed by Adsorption of SO_3 onto Oxide Surfaces**

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The structure and function of acid sites formed by the treatment of oxide surfaces with sulfur compounds is a current problem in heterogeneous catalysis.^[1] While many studies have considered hydrogen sulfate surface complexes as the locus of solid acidity in such materials, other acidic surface sites have also been proposed, including those formed from adsorbed SO_3 . Haase and Sauer recently reported a periodic ab initio theoretical study of sulfuric acid adsorbed on low-index surfaces of ZrO_2 .^[2] One of the stable structures they found on $\text{ZrO}_2(001)$ was a tridentate-adsorbed SO_3 species. This conclusion was consistent with an experimental study of Babou and co-workers, who on the basis of infrared studies

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concluded that adsorbed SO_3 formed on sulfated zirconia prepared by using sulfuric acid as the sulfur source.^[3] Yamaguchi and co-workers found that generation of strong acid sites on Fe_2O_3 was independent of the sulfur source including sulfate, SO_2 , and SO_3 .^[4]

We treated various oxide materials with SO_3 and studied them using a combination of solid-state NMR spectroscopy and theoretical methods.^[5] Here we report results obtained for a material prepared from silica gel ($\text{SO}_3/\text{silica}$) and one prepared from zirconia ($\text{SO}_3/\text{zirconia}$). Figures 1a and 1b show the ^1H MAS NMR spectra of activated silica and $\text{SO}_3/\text{silica}$ following evacuation at 473 K, respectively. Activated

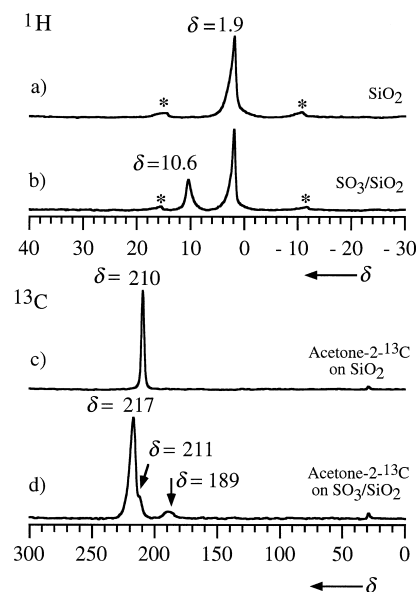


Figure 1. 359.7 MHz ^1H MAS NMR spectra and 50.1 MHz ^{13}C MAS NMR spectra characterizing activated silica and $\text{SO}_3/\text{silica}$: a) ^1H MAS spectrum of activated silica; b) ^1H MAS spectrum of $\text{SO}_3/\text{silica}$; c) ^{13}C MAS spectrum of acetone-2- ^{13}C on silica; d) ^{13}C MAS spectrum of acetone-2- ^{13}C on $\text{SO}_3/\text{silica}$. Asterisks (*) denote spinning side bands. All spectra were acquired at 298 K.

silica displays isolated silanol groups at $\delta = 1.9$, but $\text{SO}_3/\text{silica}$ has a new resonance signal at $\delta = 10.6$. The ^{13}C isotropic chemical shift of adsorbed acetone is a useful spectroscopic probe of relative acid strength.^[6] Figure 1c shows the ^{13}C MAS NMR spectrum of acetone-2- ^{13}C on activated silica; the ^{13}C shift at $\delta = 210$ indicates hydrogen bonding but negligible acidity. Figure 1d shows the ^{13}C MAS NMR spectrum of acetone-2- ^{13}C on $\text{SO}_3/\text{silica}$. The peak due to unreacted acetone is at $\delta = 217$; this shift indicates acidity, but of slightly lower strength than zeolite HZSM-5, which induces an acetone shift at $\delta = 223$. Figure 1d also shows small peaks at $\delta = 211$ and 189, due to mesityl oxide, which forms from the acid-catalyzed reaction of acetone.^[6]

We probed the nature of the acid sites formed by SO_3 treatment by measuring the ^{15}N MAS NMR spectra of ^{15}N -pyridine on both materials.^[7] The ^{15}N spectrum of pyridine on silica (Figure 2a) shows only hydrogen-bonded pyridine ($\delta = -88$) and a small amount of “free” pyridine ($\delta = -65$). Figure 2b shows that much of the pyridine on $\text{SO}_3/\text{silica}$ is protonated by Brønsted sites ($\delta = -174$). Figure 2c is the ^{15}N

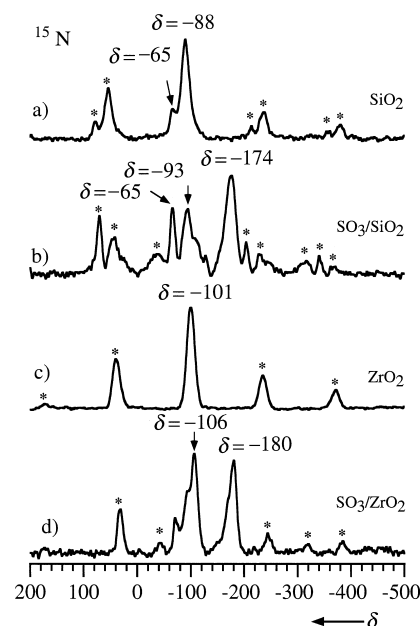


Figure 2. 36.5 MHz ^{15}N CP/MAS NMR spectra of ^{15}N -pyridine on the four oxide materials (0.3 mmol g^{-1} on silica materials and 0.2 mmol g^{-1} on zirconia materials). a) Silica; b) $\text{SO}_3/\text{silica}$; c) zirconia; d) $\text{SO}_3/\text{zirconia}$. All spectra were acquired at 123 K. Asterisks (*) denotes spinning side bands.

spectrum of pyridine on zirconia; the pyridine is complexed to Lewis sites on this sample and resonates at $\delta = -101$. The thermal stability of $\text{SO}_3/\text{zirconia}$ is remarkable; much of the SO_3 initially adsorbed remains on the surface after evacuation to 773 K. Figure 2d shows that this material contains Brønsted ($\delta = -180$) as well as Lewis ($\delta = -106$) sites. The latter are presumably the adsorbed SO_3 species described by Hasse and Sauer.

We applied theoretical methods to model the Brønsted sites formed by treatment of silica with SO_3 . We used cluster models based on β -cristabolite to approximate the catalysts and density functional theory to optimize the geometries of the reaction products. Initial calculations used a cluster of stoichiometry $\text{Si}_4\text{O}_{13}\text{H}_{10}$, fixing the terminal OH groups in crystallographic positions^[8] and optimizing the inner nine atoms of the cluster and the adsorbed SO_3 . Final energies were calculated by using a cluster to which an additional shell of atoms was added ($\text{Si}_{13}\text{O}_{13}\text{H}_{28}$). We considered both the interaction of SO_3 with the silanol lone pairs in a dative fashion, and as an insertion complex which creates an ester of hydrogen sulfate bound to the silica surface (Figure 3). The reaction of SO_3 with the surface silanol to form a dative complex is exothermic by $5.1 \text{ kcal mol}^{-1}$. Formation of the hydrogen sulfate ester is further exothermic by $5.0 \text{ kcal mol}^{-1}$. The theoretical deprotonation enthalpy of the ester is $294.4 \text{ kcal mol}^{-1}$, whereas it is $290.2 \text{ kcal mol}^{-1}$ for the dative complex. For comparison, the deprotonation enthalpy of the unactivated silanol group is $326.3 \text{ kcal mol}^{-1}$, in good agreement with the experimental value of $332.2 \pm 6.0 \text{ kcal mol}^{-1}$.^[9] The ester is predicted to be a much stronger acid than the unactivated surface silanols. In fact, the calculated acidity of the ester is comparable to that of a similar model of zeolite H-ZSM5 at the same level of theory ($294.1 \text{ kcal mol}^{-1}$). These

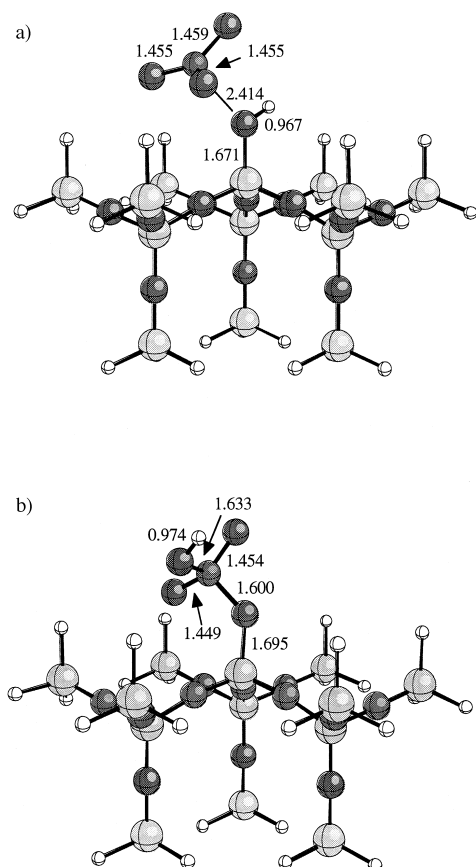


Figure 3. Optimized B3LYP/DZVP2 geometry of the hydrogen sulfate ester formed by reaction of SO_3 on β -cristabolite.

theoretical values compare to experimental deprotonation enthalpies for ZSM-5 of 291–300 kcal mol⁻¹.^[10]

In summary, NMR experiments confirm that adsorption of SO_3 onto oxide surfaces introduces acid sites. On SO_3/ZrO_2 , both Lewis and Brønsted sites are present, and theoretical modeling, in agreement with NMR studies, supports the formation of Brønsted sites on $\text{SO}_3/\text{silica}$.

Experimental Section

Electronic grade silicon(IV) oxide (Puratrem, 99.999 %) was obtained from Strem. This was evacuated at 873 K, cooled to 298 K, and then contacted with SO_3 (0.8 mmol g⁻¹). $\text{SO}_3/\text{silica}$ samples were evacuated at 473 K prior to investigation. Zirconia was prepared by the hydrolysis of zirconyl chloride octahydrate (98 %, Aldrich), followed by washing and drying the gel. All zirconia samples were first calcined at 773 K for two hours in flowing air. SO_3 (1.43 mmol g⁻¹) was adsorbed at 298 K and all samples of $\text{SO}_3/\text{zirconia}$ were further evacuated at 773 K before study. ¹H and ¹⁵N MAS NMR spectra were measured at 9.4 Tesla on a Chemagnetics CMX-360 spectrometer and ¹³C MAS NMR spectra were measured at 4.7 Tesla on a home-built spectrometer.

Theoretical calculations were performed by using Gaussian 94^[11] and the B3LYP^[12] exchange-correlation functional and the DZVP2^[13] basis set. The terminal O and H atoms were held fixed in crystallographic positions, with the OH bond lengths set to 0.9675 Å. Frequency calculations on $\text{H}_3\text{SiOH}_2\text{SO}_3$ and $\text{H}_3\text{SiOSO}_3\text{H}$ were used to estimate the zero-point and thermal energy contributions for the determination of the deprotonation enthalpies of the larger models.

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Syntheses, Structures, and Magnetic Properties of Two Gadolinium(III)–Copper(II) Coordination Polymers by a Hydrothermal Reaction**

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Studies on the syntheses, structures, and properties of lanthanide–transition metal complexes are currently of great interest because they are good models to investigate the nature of the magnetic exchange interactions between 3d and 4f metal ions in magnetic materials that contain rare earth metals.^[1–5] Many such studies focused on discrete complexes, which were synthesized from conventional, self-assembly

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reactions in solution.^[1–13] Although several infinite lanthanide–transition metal complexes containing organic ligands have been obtained by conventional solution syntheses,^[14–17] gadolinium(III)–copper(II) polymeric complexes have been poorly explored.^[18] Recently, we tried to use organic ligands to design magnetic complexes that comprise lanthanide and transition metal ions, especially the Gd^{III}–Cu^{II} couple, with infinite structures and we hoped to prepare a useful model of the magnetic exchange in magnetic materials. The key step to form infinite structure architectures is to select appropriate bridging ligands; a rigid, multidentate ligand is helpful. Based on the coordination behaviors of Gd^{III} and Cu^{II}, we chose 2,5-pyridinedicarboxylate (pydc) as the bridging ligand. Unfortunately, the reaction of Gd^{III}, Cu^{II}, and pydc by a conventional solution method, an uncharacterized polymer, insoluble in most common solvents, was obtained. It was apparent that the reaction speed must be reduced to avoid the rapid polymerization and an unusual synthetic method should be employed to obtain single crystals suitable for X-ray analysis. The hydrothermal synthetic method is well known, due to its ability to produce metastable compounds which may not be accessible by the conventional method and thereby promote crystal growth.^[19] After introducing a hydrothermal synthesis, along with using gadolinium oxide and pyridine-2,5-dicarboxylic acid (H₂pydc) as initial reagents, two Gd^{III}–Cu^{II} complexes, $[\text{Gd}_2\text{Cu}_3(\text{pydc})_6(\text{H}_2\text{O})_{12}] \cdot 4\text{H}_2\text{O}$ (**1**) and $[\text{Gd}_4\text{Cu}_2(\text{pydc})_8(\text{H}_2\text{O})_{12}] \cdot 4\text{H}_2\text{O}$ (**2**) with infinite structures were isolated and characterized.

The reaction of Gd₂O₃, Cu(OAc)₂, H₂pydc, and H₂O in a molar ratio of 1:2:4:290 at 140°C yielded crystals of **1** suitable for X-ray diffraction analysis. The crystallographic analysis^[20] of **1** revealed that its crystal structure is an one-dimensional (1D) chain polymer. As shown in Figure 1, the chain consists

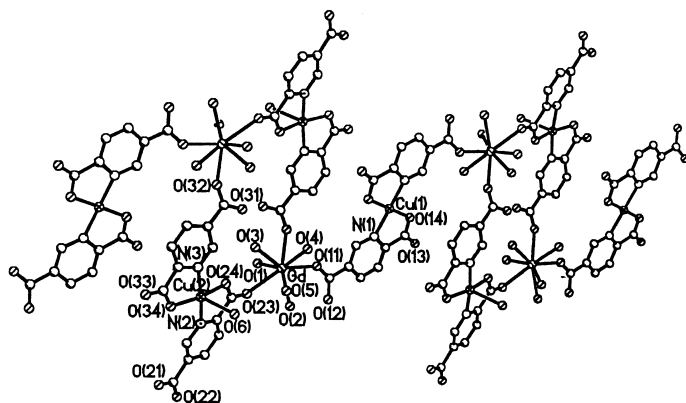
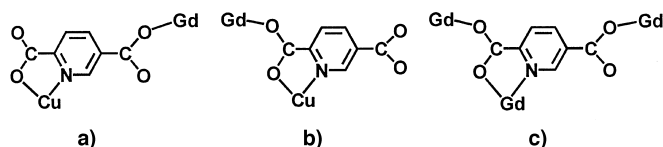


Figure 1. Structure of **1**, showing the 1D organization.

of two building blocks, $[\text{Gd}_2\text{Cu}_2(\text{pydc})_4(\text{H}_2\text{O})_{12}]$ and $[\text{Cu}(\text{pydc})_2]$, which are linked to each other by pydc ligands through Gd–O bonds. The chain can also be viewed as two building blocks, $[\text{Cu}(\text{pydc})_2]$ and $[\text{Cu}(\text{pydc})_2(\text{H}_2\text{O})]$, connected by Gd^{III} linkers, in which each Gd^{III} linker connects one $[\text{Cu}(\text{pydc})_2]$ and two $[\text{Cu}(\text{pydc})_2(\text{H}_2\text{O})]$ units. Every Gd^{III} center is coordinated by eight oxygen atoms, three from pydc and five from coordinated water molecules. There are two types of environments for Cu^{II} centers: One in $[\text{Cu}(\text{pydc})_2]$ is

chelated by two pydc ligands through its nitrogen and oxygen atoms in an exactly square planar geometry; the other in $[\text{Cu}(\text{pydc})_2(\text{H}_2\text{O})]$ is surrounded by two nitrogen and two oxygen atoms from the pydc ligands and one oxygen atom from a coordinated water molecule in a square-pyramidal geometry. The coordination modes of the pydc ligands in **1** are shown in Schemes 1 a and b, in which mode b) is seldom found



Scheme 1. Coordination modes of the pydc ligand.

in multicarboxylate ligands, especially in the lanthanide systems. The 1D chains are linked by the hydrogen bonds to form layer structures which are further linked by hydrogen bonds to form the three-dimensional (3D) structure. The Gd–O bond lengths range over 2.329–2.490 Å, while the Cu–O and Cu–N distances in the square plane range over 1.951–1.973 and 1.931–1.966 Å, respectively. The Cu–O_{water} distance (2.381 Å) is rather long, indicating the Cu–O_{water} bond is weak. Gadolinium–gadolinium, gadolinium–copper, and copper–copper distances between the adjacent units are 6.290, 5.801, and 5.119 Å, respectively, indicating a lack of directed M–M interactions.

In an attempt to prepare similar polymers with different Gd:Cu ratios, the reactions of Gd₂O₃, Cu(OAc)₂, H₂pydc, and H₂O at different Gd:Cu:H₂pydc molar ratios were carried out, however, polymer **1** was always obtained. Through the replacement of Cu(OAc)₂ by CuO, we succeeded in the isolation of **2** in high yield. The reaction of Gd₂O₃, CuO, H₂pydc, and H₂O in a molar ratio of 1:2:4:290 at 140°C yielded blue crystals of **2** suitable for X-ray analysis. The structure^[20] of **2** is a 3D polymer, which consists of $[\text{Gd}_4\text{Cu}_2(\text{pydc})_8(\text{H}_2\text{O})_{12}]$ units. Through Gd–O_{pydc} bonds, the units are linked head-to-tail to form infinite zigzag chains (Figure 2), where two neighboring chains are linked to generate wave-like, two-dimensional (2D) structures which are again connected to each other by pydc ligands to produce the 3D structure, as shown in Figure 3. The 2D wave can also be

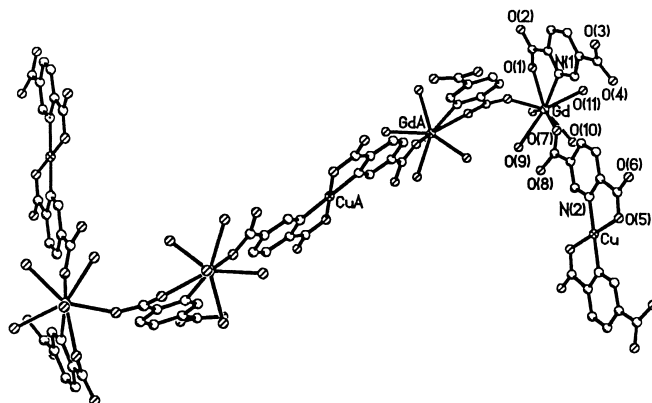


Figure 2. Wavelike structure of **2**; for clarity, only one zigzag chain is shown.

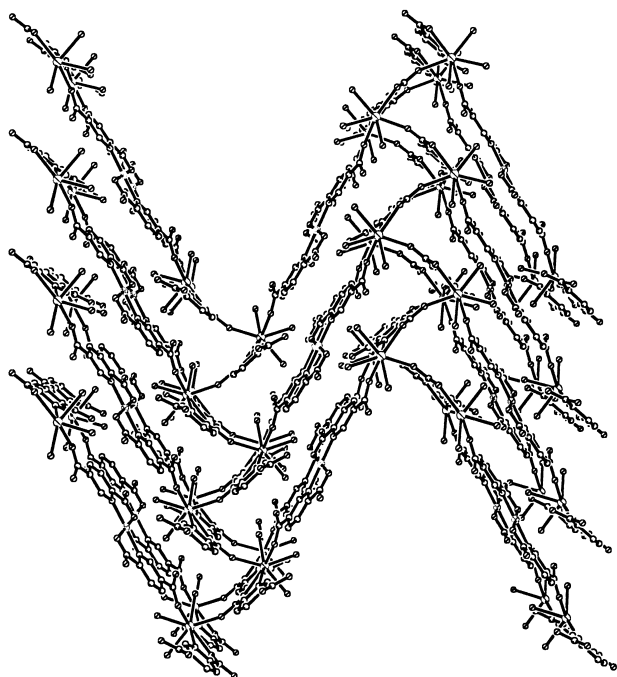


Figure 3. Structure of **2**, showing the packing along the *a* axis.

viewed as Gd–Cu ladders, in which the rungs are formed by [Cu(pydc)₂] species and the sidepieces by Gd^{III} chains; the sidepieces of the neighboring ladders are linked by pydc ligands through Gd–O bonds to yield the wave. Each Gd^{III} center is coordinated by one nitrogen and four oxygen atoms from four pydc ligands and three oxygen atoms from coordinated water molecules. Each Cu^{II} atom is chelated by two pydc ligands through its nitrogen and oxygen atoms in an exactly square planar geometry. The coordination modes of pydc ligands in **2** are also given in Schemes 1a and c. The Gd–O bonds range over 2.338–2.434 Å, while the Gd–N bond is 2.575 Å. The Cu–N and Cu–O bonds are 1.970 and 1.946 Å, similar to those in **1**. Gadolinium–gadolinium, gadolinium–copper, and copper–copper distances between the adjacent units are 6.399, 6.081, and 7.779 Å, respectively.

Interestingly, although the molar ratio of the reagents and the reaction conditions for the syntheses of **1** and **2** are the same, the ratios of Cu/Gd, the structures, the coordination environments of metal centers, and coordination modes of pydc ligands in the two compounds are quite different. For **1**, free Cu^{II} ions are used as reagent (in reaction with Gd₂O₃) and these readily coordinate to the nitrogen atoms of pydc ligands; thus all nitrogen atoms are coordinated by Cu^{II} giving rise to a high Cu/Gd ratio of 3/2. For **2**, the reaction rate of CuO may be comparable with that of Gd₂O₃, thus the nitrogen atoms of pydc are bound simultaneously by Gd^{III} and Cu^{II}, and a low Cu/Gd ratio of 1/2 is obtained. This result may provide a new way to adjust the metal:metal ratio and to control the coordination mode of organic ligands in the lanthanide–transition metal reaction systems which are useful for the designing of model complexes for magnetic materials.

The $\chi_M T$ versus *T* plots of temperature-dependent magnetic susceptibility data for **1** and **2** are shown in Figure 4. For **1**, the value of $\chi_M T$ is 16.44 cm³ K mol^{−1} at 300 K and remains essentially constant to 50 K. It increases slightly below 50 K and reaches its maximum value of 16.87 cm³ K mol^{−1} around

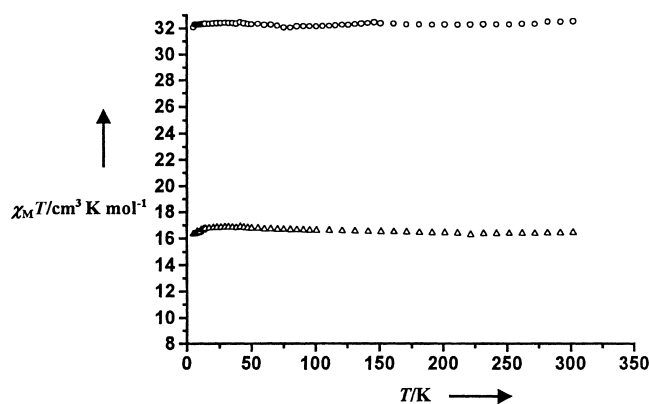


Figure 4. Experimental $\chi_M T$ versus *T* curves for complexes **1** and **2**.

10.7 K, then decreases as *T* is lowered further. For **2**, the values of $\chi_M T$ are essentially constant from 300 to 5 K (the average value is 32.33(20) cm³ K mol^{−1}). The $\chi_M T$ values of **1** at high temperature (*T* > 50 K) and **2** are compared with the calculated values using the Equation (1), which is based on the free-ion approximation for Gd^{III} and Cu^{II}, where *N_A*, β, and *k* are the Avogadro number, the Bohr magneton, and the Boltzmann constant, respectively.

$$\chi_M T = \frac{N_A \beta^2}{3k} \{ \sum g_{\text{Gd}^{\text{III}}}^2 (S_{\text{Gd}^{\text{III}}} (S_{\text{Gd}^{\text{III}}} + 1)) + \sum g_{\text{Cu}^{\text{II}}}^2 (S_{\text{Cu}^{\text{II}}} (S_{\text{Cu}^{\text{II}}} + 1)) \} \quad (1)$$

The magnetic behaviors for **1** and **2** are different from those of discrete gadolinium–copper complexes which normally show ferromagnetic interactions.^[4–9] Detailed studies on magnetic properties and the other polymeric lanthanide–transition metal complexes are underway and will be reported later in a full paper.

Experimental Section

Synthesis of 1: A mixture of Gd₂O₃ (0.090 g, 0.25 mmol), Cu(OAc)₂ (0.100 g, 0.5 mmol), 2,5-pyridinedicarboxylic acid (0.167 g, 1.0 mmol), and H₂O (14.0 mL) in a mole ratio of about 1:2:4:290 was heated in a 25-mL stainless-steel reactor with a Teflon liner at 140°C for 72 h. Green-blue crystals of **1** formed. Yield 20%. Elemental analysis for C₄₂H₅₀N₆O₄₀·Cu₃Gd₄ (%) calcd: C 28.28, H 2.82, N 4.71; found: C 28.54, H 2.80, N 4.74; IR (KBr pellet): $\tilde{\nu}$ = 3385(br, vs), 1612(vs), 1391(s), 1358(s), 1284(m), 1038(m), 768(s), 517(m) cm^{−1}.

Synthesis of 2: An analogous procedure was followed to prepared **2** except Cu(OAc)₂ was replaced by CuO. A mixture of Gd₂O₃ (0.090 g, 0.25 mmol), CuO (0.040 g, 0.50 mmol), 2,5-pyridinedicarboxylic acid (0.168 g, 1.0 mmol), and H₂O (14.0 mL) in a mole ratio of about 1:2:4:290 was heated in a 25-mL stainless-steel reactor with Teflon liner at 140°C for 72 h. Blue crystals of **2** formed. Yield 75%. Elemental analysis for C₃₆H₅₆N₈O₄₈·Cu₂Gd₄ (%) calcd: C 28.44, H 2.39, N 4.74; found: C 29.14, H 2.47, N 4.95; IR (KBr pellet): $\tilde{\nu}$ = 3385(br, vs), 1610(vs), 1392(s), 1358(s), 1286(m), 1038(m), 766(s), 513(m) cm^{−1}.

The magnetic susceptibility data were collected from polycrystalline samples at an external field of 1 kG on a PPMS Model 6000 magnetometer

(Quantum Design) in the temperature range 5 to 300 K. The output data were corrected for the diamagnetism of the sample holder and of the samples, calculated from their Pascal's constants.

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Experimental Observation of Pentaatomic Tetracoordinate Planar Si- and Ge-Containing Molecules: MAI_4^- and MAI_4^{*}

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The chemistry of silicon and germanium is dominated by their tendency to form tetracoordinate tetrahedral structures, just as their more famous congener, the tetracoordinate tetrahedral carbon atom. The tetrahedral carbon idea, proposed by van't Hoff and Le Bel 125 years ago, has been the most important stereochemistry concept in understanding organic and biomolecules. The tetracoordinate tetrahedral structure of C, Si, and Ge atoms is rationalized elegantly by the concept of sp^3 hybridization. However, in the last 30 years chemists have attempted to make unusual compounds that contain *planar* tetracoordinate C, Si, and Ge atoms. In particular, substantial efforts and progress have been made in the design and synthesis of tetracoordinate planar carbon molecules by overcoming the inherent preference for tetrahedral bonding.^[1–6] Tetracoordinate planar silicon molecules have also been studied theoretically^[7] and experimentally.^[8]

We are interested in the generation of planar carbon, silicon, germanium, tin, and lead species in the gas phase and the investigation of their electronic structure and chemical bonding by using a combined experimental and theoretical approach. We recently reported the first observation of a *pentaatomic* tetracoordinate planar carbon molecule (CAI_4^-) which has a planar structure and represents the smallest species to contain a tetracoordinate planar carbon.^[9] Interestingly, however, its neutral CAI_4 parent molecule is tetrahedral. We showed that planarity in CAI_4^- is achieved through ligand–ligand bonding interactions in its HOMO. We also found a general rule, that to achieve planarity in pentaatomic species composed of a first-row central atom and second- or

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third-row ligand atoms, these species should possess 17 or 18 valence electrons.^[9–11]

It is, however, not clear if the planar structure will be preserved when the first-row central atom is substituted by a second- or third-row atom. These molecules would be isoelectronic with the species that contain a first-row central atom, but the central cavity may be too small to accommodate the second- or third-row atom in the planar square structure. Herein we address this question by investigating SiAl_4^- and GeAl_4^- and their corresponding neutral molecules.

The experiments were performed with a magnetic-bottle time-of-flight photoelectron spectroscopy apparatus with a laser vaporization cluster source.^[12, 13] The SiAl_4^- and GeAl_4^- ions were produced by laser vaporization of the corresponding mixed targets (Si/Al and Ge/Al) with pure helium carrier gas. The 532 and 355 nm spectra of SiAl_4^- and GeAl_4^- are shown in Figure 1. The 532 nm spectrum of SiAl_4^- (Figure 1a) revealed a partially vibrationally resolved band with an

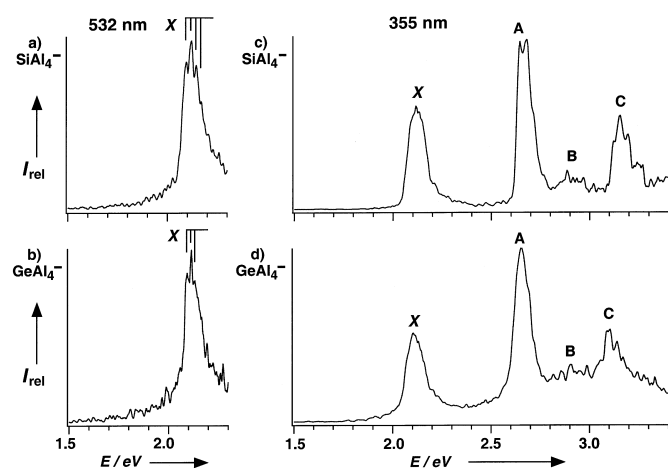


Figure 1. Photoelectron spectra of SiAl_4^- and GeAl_4^- at 532 nm (2.331 eV) and 355 nm (3.496 eV), plotted in increasing electron binding energy. The four observed detachment channels are labeled (X, A, B, and C). The vertical lines in (a) and (b) indicate vibrational structures.

average spacing of 220 cm^{-1} . The adiabatic electron detachment energy (ADE) of SiAl_4^- was determined from the 0-0 transition to be 2.094 eV and its vertical electron detachment energy (VDE) was measured to be 2.121 eV. At 355 nm (Figure 1c), several more features were observed. The feature at 2.7 eV (labeled A) shows a splitting which does not seem to be a vibrational progression. According to our theoretical result (see below), this splitting corresponds to a possible triplet–singlet splitting caused by detachment of a $3b_2$ electron. A broad feature (B) was observed around 2.9 eV, followed by a feature (C) at 3.16 eV. The spectra of GeAl_4^- are nearly identical to those of SiAl_4^- , which indicates that the anions were correctly selected, as these two isoelectronic species are expected to have similar electronic and molecular structures. The 532 nm spectrum (Figure 1b) of GeAl_4^- also revealed a partially vibrationally resolved band with a smaller vibrational spacing of 160 cm^{-1} . The ADE of GeAl_4^- is identical to that of SiAl_4^- within our experimental accuracy. The VDE of GeAl_4^- was measured to be 2.114 eV. At 355 nm

(Figure 1d), again several more features (A–C) were observed, similar to those of SiAl_4^- . All the observed ADEs and VDEs for SiAl_4^- and GeAl_4^- are given in Table 1.

Table 1. Experimental and theoretical VDE and ADE of SiAl_4^- and GeAl_4^- .

State	Experimental ADE [eV]	Experimental VDE [eV]	Detachment channel ^[a]	Theoretical VDE [eV] ^[b]	Theoretical ADE [eV] ^[b]
SiAl_4^-					
X	2.121 (0.020)	2.094 (0.020)	$5a_1 (^1A_1)$	2.00	2.00
A	2.65 (0.03)	2.65 (0.03)	$3b_2 (^3B_2)$	2.57	
B	≈ 2.9	≈ 2.8	$4a_1 (^3A_1)$	3.01	
C	3.16 (0.03)	3.11 (0.03)	$1b_1 (^3B_1)$	3.47	
GeAl_4^-					
X	2.114 (0.020)	2.094 (0.020)	$5a_1 (^1A_1)$	2.03	2.02
A	2.66 (0.06)	2.66 (0.06)	$3b_2 (^3B_2)$	2.56	
B	≈ 2.9	≈ 2.8	$4a_1 (^3A_1)$	3.00	
C	3.10 (0.03)	3.08 (0.04)	$1b_1 (^3B_1)$	3.35	

[a] $4a_1$ is the ligand–ligand bonding MO; the other three orbitals are ligand-centered nonbonding lone-pair MOs (see Figure 3). The final state is given in parentheses. [b] The $5a_1$ MO is singly occupied in the anions. Thus, the first detachment channel corresponds to a singlet final state (ground state of the neutral) and all the other detachment channels correspond to triplet final states. All data are at the CCSD(T)/6-311 + G(2df)//MP2/6-311 + G* level of theory.

In our ab initio calculations,^[14, 15] we considered in detail neutral SiAl_4 and GeAl_4 molecules and their anions. We performed a complete search for the global minimum of SiAl_4 and its anion. For GeAl_4 , we assumed that it would have a similar global minimum to that of SiAl_4 , based on their nearly identical photoelectron spectra (Figure 1). The lowest energy anion structures for each cluster are shown in Figure 2. Indeed, both SiAl_4^- and GeAl_4^- were found to be planar and contain tetracoordinate Si and Ge atoms (valence electronic configuration: $1a_1^2 1b_2^2 2a_1^2 3a_1^2 2b_2^2 1b_1^2 4a_1^2 3b_2^2 5a_1^1$). As expected, the small cavity formed by an Al_4 square was too small to accommodate Si or Ge atoms and the molecules are distorted into C_{2v} symmetry. Surprisingly and in contrast to

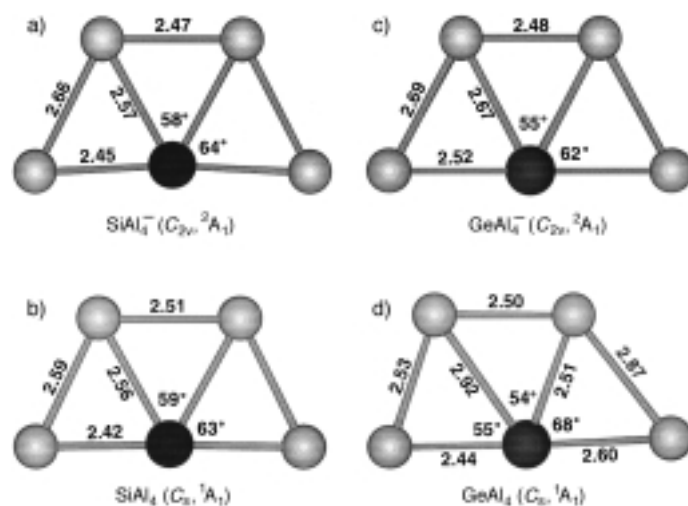


Figure 2. Ab initio structures of SiAl_4^- , SiAl_4 , GeAl_4^- , and GeAl_4 optimized at the MP2/6-311 + G* level of theory and showing the bond lengths [Å] and angles [°]. Note that SiAl_4 (b) is not planar, the two Al atoms appearing to be on the same line as Si are actually each 11° above the plane, giving a $158^\circ \angle \text{Al-Si-Al}$ angle, which cannot be seen in the two-dimensional plot.

CAI_4 , the neutral SiAl_4 and GeAl_4 molecules are not tetrahedral.^[9] The GeAl_4 molecule remains planar, whereas SiAl_4 is quasi-planar with the two Al units at the apices each being out of plane by 11° (Figure 2). However, the barrier to planarity for SiAl_4 after the zero-point energy (ZPE) corrections was found to be just $0.26 \text{ kcal mol}^{-1}$ (MP2). We did find T_d local minima for SiAl_4 and GeAl_4 , but they are 27 kcal mol^{-1} (MP2) higher in energy than the structures shown in Figure 2.

In Table 1 we present results of our calculations of the four lowest-lying vertical one-electron detachment processes from the tetracoordinate planar global-minimum structures of SiAl_4^- and GeAl_4^- (that is from the $5a_1$, $3b_2$, $4a_1$, and $1b_1$ orbitals, respectively) and compare them with the experimental data. Removal of the single electron from the $5a_1$ orbital results in the $^1A_1$ ground state for the neutral species. The calculated ADE and VDE of this detachment channel agree well with the experimental values (Table 1). Detachment from the $3b_2$, $4a_1$, and $1b_1$ orbitals will each result in both triplet and singlet excited states for the neutral species. Only the triplet final states are considered here, because the final singlet excited states require multiconfigurational references and cannot be treated by the CCSD(T) method used here. The calculated VDEs for the triplet excited states agree well with the major experimental features (A–C). The singlet excited states are likely to be close in energy to the corresponding triplet states. The splitting observed for the feature A in the 355 nm spectrum of SiAl_4^- is probably because of the singlet state. Both the features B and C are relatively broad and may conceivably contain the singlet states as well. The overall excellent agreement between the theoretical and experimental results of the global-minimum tetracoordinate planar structures provide solid evidence that we have indeed identified the first *pentaatomic* tetracoordinate planar silicon and germanium molecules in their anion states.

Our previous work provided the tetracoordinate planar carbon anion in CAI_4^- , whereas the neutral CAI_4 has a tetrahedral structure. Our previous experimental observation of an extremely broad $(X, \text{CAI}_4^-) \rightarrow (X, \text{CAI}_4)$ transition in the photoelectron spectra of CAI_4^- provided conclusive evidence for the large geometry changes from the planar square structure of CAI_4^- to the tetrahedral structure of CAI_4 .^[9] Interestingly, in the current work both the $(X, \text{SiAl}_4^-) \rightarrow (X, \text{SiAl}_4)$ and the $(X, \text{GeAl}_4^-) \rightarrow (X, \text{GeAl}_4)$ transitions are relatively sharp. These observations agree well with the relatively small geometry changes between the anionic and neutral molecules that contain Si and Ge centers. The planar structure of CAI_4^- was attributed to the occupation of the peripheral ligand–ligand bonding orbital (HOMO) by a single electron. When this electron is removed from the HOMO of CAI_4^- the corresponding neutral CAI_4 species becomes tetrahedral. In the case of SiAl_4^- and GeAl_4^- , the peripheral ligand–ligand bond is no longer the HOMO, but the HOMO-2 ($4a_1$), which remains doubly occupied in both the anion and neutral ground states (Figure 3). Feature B in the photoelectron spectra corresponds to the removal of an electron from this peripheral bond, and the broad nature of feature B is consistent with the bonding nature of this orbital. Therefore, it is understandable that the neutral SiAl_4 and

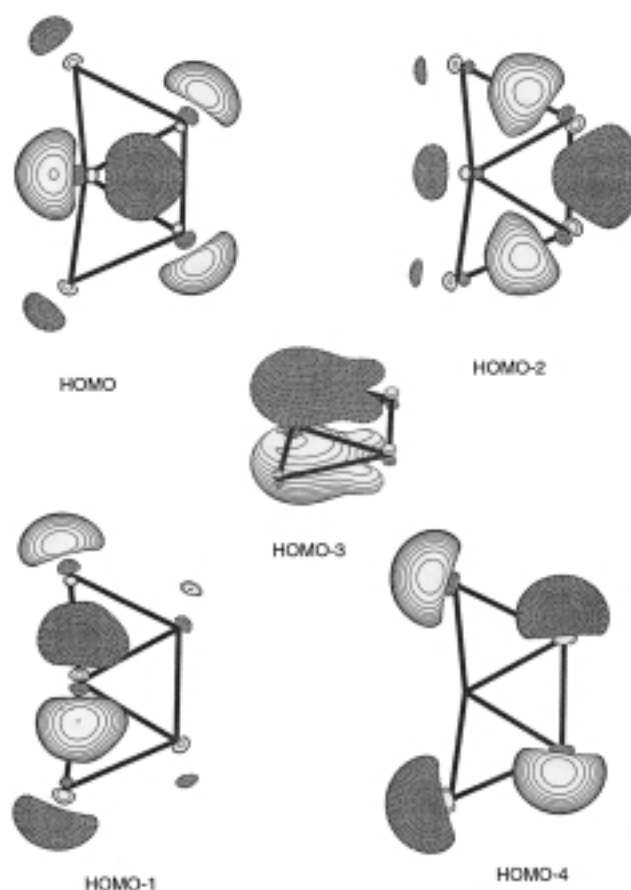


Figure 3. Molecular orbital diagram^[16] showing the HOMO ($5a_1$), HOMO-1 ($3b_2$), HOMO-2 ($4a_1$), HOMO-3 ($1b_1$), and HOMO-4 ($2b_2$) of SiAl_4^- . Note that the ligand–ligand bonding MO is the HOMO-2 ($4a_1$), and not the HOMO as in CAI_4^- .^[9]

GeAl_4 compounds remain planar, unlike their lighter congener, CAI_4 , which is tetrahedral. These results again reinforce the concept and importance of the ligand–ligand bonding in the stabilization of the planar structures in tetracoordinate C, Si, and Ge species.

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Control of Architecture in Block-Copolymer Vesicles**

Hongwei Shen and Adi Eisenberg*

It has been known for some years that highly asymmetric amphiphilic block copolymers can self-assemble in solution to form crew-cut aggregates of an extremely wide range of morphologies.^[1] The various morphologies include spheres, rods, bicontinuous structures, lamellae, vesicles, tubes, large compound vesicles (LCVs), hexagonally packed hollow hoops (HHHs), large compound micelles (LCMs), and many others. In such aggregates, the relatively long block forms the core, while the short one forms the corona. A number of other groups have also studied block-copolymer aggregates of various morphologies in recent years.^[2, 3] The appeal of this field is, in part, because of the many potential applications of

these nano-sized structures with their different morphologies, and in part academic curiosity.

Among the various morphologies, vesicles, including liposomes,^[4] are of special interest because of their potential application as encapsulation agents. However the stability of liposomes has been a concern because of the high mobility of the component molecules under ambient conditions; therefore, many attempts have been made to stabilize these liposomes. As polymers have higher stability and lower mobility than small-molecule amphiphiles most of these stabilization strategies consist of the polymerization of the liposomes or the addition of polymeric materials to the liposomes.

Block-copolymer vesicles can be equilibrium structures under some conditions, but can also be kinetically frozen during preparation.^[5] The block-copolymer vesicles are robust^[3] and are very stable (samples have remained unchanged since 1997) in aqueous solution.^[5] Because the studies on block-copolymer vesicles are still in the early stages, only a few attempts at encapsulation have been made.^[3, 6]

To utilize block-copolymer vesicles in any given application, the precise control of vesicular parameters, such as vesicle size, wall thickness, number of walls, and space between the walls, is crucial. In our previous studies on the phase diagrams of block-copolymer systems we found that under some conditions the vesicles can be under thermodynamic control.^[5] It is believed that the parameters which control the aggregate morphologies, that is the core chain stretching, interfacial tension, and corona repulsion, are also responsible for the control of the vesicular architecture. Thus, variation of these factors allows the properties of the vesicles to be tuned.^[1]

A special type of vesicle, the so-called dense “onions”, which are found in bulk block-copolymer blends were reported over a decade ago.^[7] In block-copolymer solutions, onionlike micelles have been prepared by the stepwise aggregation of two diblocks, first AB and then BC.^[8] Onionlike structures have also been reported in small molecule surfactant systems.^[9] Recently, it was found that shear can induce the formation of onions from a lamella phase in surfactant systems.^[10] By the same strategy, onionlike structures could also be formed in Pluronic systems,^[11] and, very recently, onions were prepared from block-copolymer solutions.^[12]

Herein we report a new morphology for block copolymers in solution, this consists of concentric vesicles with uniform spacing between the walls; we also describe the preparation in solution of multi-lamella onions in which there is no spacing between the walls, and discuss the control of these morphologies in the context of the other observed vesicular architectures (see Table 1).^[1, 5, 13] Our understanding of this subject is still qualitative, but the evidence obtained suggests that one can exercise control over a variety of vesicular morphologies. Noteworthy is that equally spaced concentric vesicles have been observed in small molecule amphiphiles.^[4, 9]

Figure 1 shows concentric vesicles with uniform spaces between the walls, which we refer to as “onions with spaces”. From the shadow of the vesicular structures seen by transition electron microscopy (TEM) observation (not shown here),

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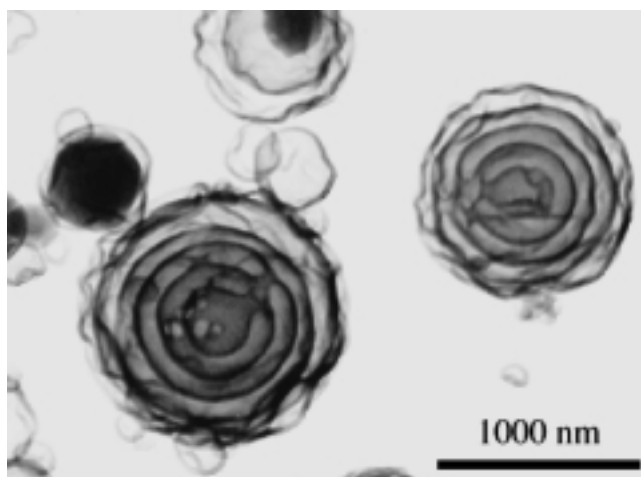


Figure 1. Concentric vesicles with uniform spaces between the walls. These vesicles were prepared from 10 wt % $\text{PS}_{132}\text{-}b\text{-PAA}_{20}$ in a dioxane/water mixture with 40 wt % water.

these structures are clearly spherical. The onions with spaces can consist of up to six layers, and their overall diameters can exceed $1\text{ }\mu\text{m}$ (Table 1). The average wall thickness is $22 \pm 2\text{ nm}$ and the spacing between the walls is $58 \pm 11\text{ nm}$ (95 % confidence), based on thirty measurements. The internal layers are fairly smooth, the outer layers are more rugged, probably because this layer is more rapidly dried, compared to those in the interior, during the sample preparation for electron microscopy. Imperfections can be seen between some of the internal wall spaces, for example the connecting regions, which probably helps to maintain the spacing between the walls. The total surface area affected by these imperfections is relatively small.

Figure 2 shows another type of concentric vesicle in which there is no spacing between the walls. These aggregates have a compact multi-lamella vesicular structure and we refer to them as “solid onions”. Shadowing indicates that these structures are spherical. The observed onions can be up to 500 nm in diameter and consist of up to eight layers. Because quaternized poly (4-vinyl pyridine) (P4VP), which makes up the corona, has a higher electron density than polystyrene (PS), the corona layer appears as the darker regions, which are $13 \pm 1\text{ nm}$ thick. The PS domains appear as gray regions, which are $24 \pm 2\text{ nm}$ thick (based on fifty measurements).

The mechanistic aspects of the formation of the solid onions and onions with spaces need to be discussed and compared with those that lead to the formation of other types of vesicles.

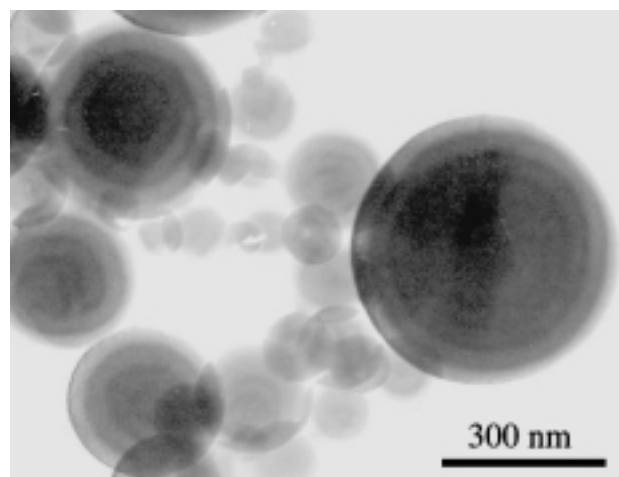


Figure 2. Solid onions prepared from 1 wt % $\text{PS}_{260}\text{-}b\text{-P4VPDecI}_{70}$ in THF by dropwise addition of water.

First, the formation of solid onions is probably similar to the formation of the lamella phase in bulk. Solid onions will be observed when both layers shrink at comparable rates, and the polymer has a composition that would yield the lamella phase in bulk. The small size of the structure also prevents the formation of large flat lamellae, and thus results in the onionlike architecture. Size limitations lead to the formation of curved structures to avoid the thermodynamic penalty of end-cap or rim formation.^[1, 7] The polymer $\text{PS}_{260}\text{-}b\text{-P4VPDecI}_{70}$ (4VPDecI: 4-vinyl pyridine decyl iodide) has a molecular weight ratio of 50:50, which places it in the lamella region for most bulk systems (another onion-forming polymer, $\text{PS}_{380}\text{-}b\text{-PCEMA}_{210}$ (CEMA: 2-cinnamoyl ethyl methacrylate) has a molecular weight ratio of 45:55^[12]). The process is thus similar to that encountered in the earliest preparation of onions in diblock systems.^[7] Hence, the process is governed largely by the same thermodynamics that lead to the formation of lamellae in bulk.

In contrast to the above phenomenon, the formation of the onions with spaces is a solution process. The polymer $\text{PS}_{132}\text{-}b\text{-PAA}_{20}$ (AA: acrylic acid) is highly asymmetric and would not yield lamellae in bulk. At relatively high polymer concentrations and with the progressive increase of the solvent water content, large vesicles form in solution because of the incompatibility between the core-forming block and the water and in the balance of forces operating in solution self-assembly. As the solvent diffuses out and water diffuses into the large vesicles, progressively smaller vesicles form inside

Table 1. Formation of various vesicular morphologies.

Polymer	M_w/M_n [a]	Conc. [wt %]	Solvent	Type of vesicle	Dimensions [nm]	
					diameter	wall
$\text{PS}_{410}\text{-}b\text{-PAA}_{20}$	1.05	2.0	DMF	uniform unilamella [b]	80–100	≈ 25
$\text{PS}_{410}\text{-}b\text{-PAA}_{20}$	1.05	3.0	DMF	polydisperse unilamella [b]	100–500	≈ 25
$\text{PS}_{200}\text{-}b\text{-PAA}_{18}$	1.05	3.0	DMF/THF	entrapped [b]	200–300	≈ 23
$\text{PS}_{132}\text{-}b\text{-PAA}_{20}$	1.07	10.0	dioxane	onions with spaces	500–1200	22 ± 2
$\text{PS}_{260}\text{-}b\text{-P4VPDecI}_{70}$	1.07	1.0	DMF	solid onions [b]	100–500	24 ± 2

[a] Polydispersity (weight average molecular weight over number average molecular weight, M_w/M_n) of the block copolymers as determined by size-exclusion chromatography. [b] Aggregates were prepared by the addition of water to polymer solutions, followed by dialysis.

the large vesicles by the self-assembly of the entrapped polymer under conditions similar to those which led to the formation of the outermost vesicle. The variation in the number of layers depends on the polymer concentration and the size of the outermost vesicles. This formation process may resemble that occurring in small-molecule amphiphile systems.^[9] The entrapped vesicles found previously^[13] are probably formed by the same solution process.

The onions with spaces and solid onions described above are the latest additions to the family of block-copolymer vesicles (Table 1). The various vesicular structures can be controlled by thermodynamic parameters through such external factors as the diblock composition, the polymer concentration, the type of common solvents, the water content, and the presence of additives. The wall thickness, which affects the strength and the ability of small molecules to penetrate the vesicle walls, is tunable by varying the core-forming block length and the type of common solvent. The shorter the core-forming block, the thinner the wall. The size of the vesicles is mainly controlled by the swelling of the core and by the polymer concentration (Table 1).

Different vesicles are of interest for applications in drug delivery, encapsulation, or in cosmetics. Small vesicles with a narrow size distribution can be targeted at applications that involve size limitations, while the larger vesicles can increase the capacity of encapsulation. Entrapped vesicles may slow down the delivery of some of encapsulated species, but may not be able to function as time-release devices because they lack multiple layers. The presence of chambers between layers allows the onions with spaces to be considered as potential time-release devices which would function by the progressive breaking or erosion of the layers, or by gradual diffusion through the walls. The newly found onions with spaces are unique for block copolymers and extend the range and the control of vesicular structures.

Experimental Section

Block copolymers, PS₁₃₂-*b*-P(*t*BuA)₂₀ (*t*BuA: *tert*-butyl acrylate) and PS₂₆₀-*b*-P4VP₇₀, were synthesized by anionic polymerization.^[5] After the hydrolysis of the P(*t*BuA) blocks, the PAA systems were fractionated to remove any homopolystyrene.^[5] P4VP blocks were quaternized by using decyl iodide.^[14] Onions with spaces were prepared by dissolving 10 wt % PS₁₃₂-*b*-PAA₂₀ in dioxane, then adding water over a three-month period until the water content was 40 wt %, and finally freeze-drying a drop of the colloidal solution on copper grids for TEM observation.^[5] Solid onions were prepared by dissolving 1 wt % PS₂₆₀-*b*-P4VPDecI₇₀ in THF, then adding water dropwise at a rate of 1 wt % per minute until the water content was 50 wt %, and finally dialyzing the resulting solution against water. A similar preparation method was also used for preparing other types of vesicles (Table 1) and has been described.^[13, 14]

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Hydrogen-Bonded Hexamolybdenum Clusters: Formation of Inorganic–Organic Networks**

Nicholas Prokopuk, Charles S. Weinert, David P. Siska, Charlotte L. Stern, and Duward F. Shriver*

The increasing importance of solid materials in catalysis, nonlinear optics, chemical sensors, separation technology, and electronics has created a demand to control the chemical and physical properties of solids.^[1] Solution chemists typically approach this problem by synthesizing discrete molecules that spontaneously self-assemble into extended arrays.^[2] The significance and impact of self-assembly are demonstrated by the porous zeolites that combine the acidic properties of the aluminosilicate with the channels and pores of the

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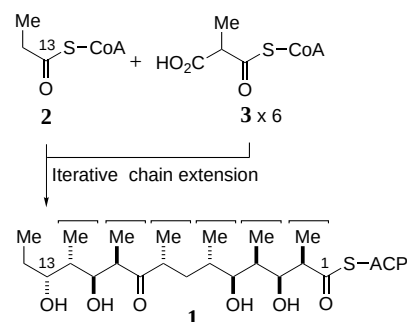
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A Combinatorial Approach to Polyketide-Type Libraries by Iterative Asymmetric Aldol Reactions Performed on Solid Support**

Ian Paterson,* Monica Donghi, and Kai Gerlach

The polyketides represent a rich reservoir of structurally complex, bioactive, natural products, with many having therapeutic importance (as antibiotics, anticancer agents, antifungals, antiparasitics, immunosuppressants, and cardiovascular agents).^[1] As a source of pharmaceutically relevant, molecular diversity, they are attractive targets for developing a combinatorial approach to library generation, particularly if this can be accomplished on solid phase, where purification procedures are simplified and automation becomes feasible. Solid-phase synthesis, routinely applied to the preparation of peptides and oligonucleotides, has been adapted in recent years to oligosaccharides and small molecules.^[2] However, the synthesis of elaborate polyketide sequences (e.g. **1**, the acyclic precursor of the erythromycin antibiotics, Scheme 1) involving the controlled formation of multiple stereocenters remains a challenge,^[3, 4] requiring the transfer of more sophisticated chemistry for achieving asymmetric carbon-carbon formation to solid phase.

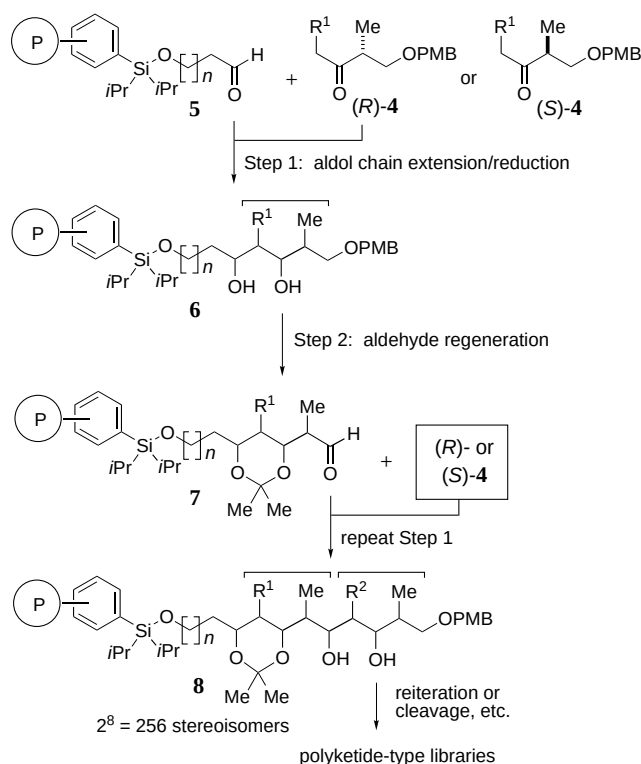
In the archetypal case of erythromycin, the heptaketide precursor **1** is assembled biosynthetically by the polyketide synthase from a starter unit **2** and six extender units **3**, with the growing chain bound to the acyl carrier protein (ACP).^[5, 6] By mimicking this processive mechanism in the laboratory using a greater variety of chain extending units, a combinatorial synthetic approach might be developed, leading to much greater molecular diversity. As part of studies towards this



Scheme 1. Biosynthesis of the heptaketide precursor **1** of erythromycin. ACP = acyl carrier protein. CoA = coenzyme A.

goal,^[3] herein we demonstrate the utility of the chiral ketones **4** (see Scheme 2) for performing efficient polyketide synthesis on solid support, providing wide-ranging opportunities for structural and stereochemical diversification.

In this new approach to expanding polyketide diversity (Scheme 2), a suitable aldehyde starter unit, such as **5**, is attached to a polystyrene support (which functions as a



Scheme 2. Solid-phase synthesis of polyketide-type libraries using iterative chain extension of resin-supported aldehydes **5** and **7** with ketones (*R*)- or (*S*)-**4**. P = polystyrene resin (styrene-1% divinylbenzene, 200–400 mesh), PMB = *p*-methoxybenzyl.

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surrogate for the ACP) through a silyl ether linker. By using stereoregulated aldol chemistry, employing the reagents (*R*)- or (*S*)-**4** for chain extension and a subsequent ketone reduction to produce the 1,3-diol **6** (Step1), followed by regeneration of the aldehyde functionality in **7** (Step2), repetition provides the more elaborate 1,3-diol **8**. This leads progressively to the synthesis of polyketide-type sequences of

increasing complexity, where the spacer ($\text{HOCH}_2\text{CH}_2\text{CH}_2$ for $n=2$) is incorporated into the final product. After a single iteration, the resulting sequence **6** has four contiguous stereocenters (16 possible stereoisomers), while a second iteration to give **8** introduces eight contiguous stereocenters (corresponding to 256 possible stereoisomers for $\text{R}^1 = \text{R}^2 = \text{Me}$). By varying the substitution on the reagent module **4** in a combinatorial sense, many additional opportunities for structural diversification in **6** and **8** are possible, leading potentially to even larger libraries.

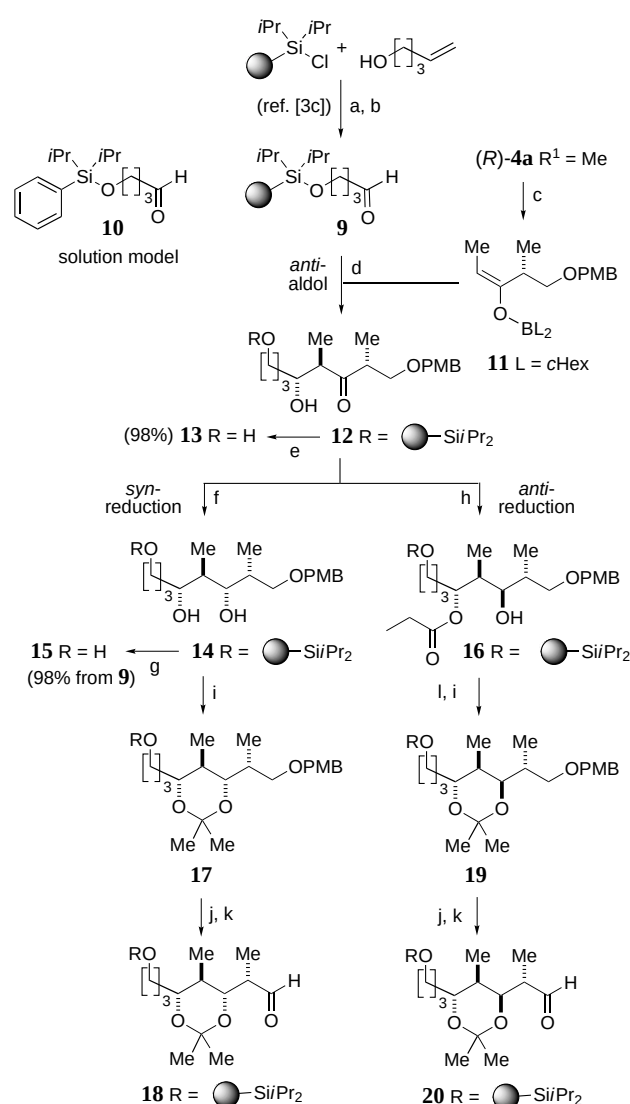
As shown in Scheme 3, the viability of performing stereocontrolled aldol reactions and reductions in an iterative fashion on a suitable solid-supported aldehyde was first examined. The resin-bound aldehyde **9** was prepared^[7] by attachment of 4-penten-1-ol to chlorodiisopropylsilyl polystyrene, followed by ozonolysis with a reductive workup (Ph_3P , sonication); its loading was determined as 0.75 mmol g^{-1} using the method described previously.^[3c] To assist the ^{13}C NMR and FT-IR characterization of the polymer-supported intermediates, aldehyde **10** was selected as a solution model,^[3c] which also allowed a direct comparison of the reaction yields and diastereoselectivities.

In initial studies, aldehyde **9** was allowed to react with the (*E*)-enol dicyclohexylborinate **11** (5 equiv), derived from the dipropionate reagent (*R*)-**4a**,^[8,9] by shaking in Et_2O to form the *anti-anti* isomer **12** after oxidative boron removal. A loading of 0.51 mmol g^{-1} (i.e. 68 % yield of isolated product) was determined by subsequent cleavage from the resin with HF/pyridine , leading to generation of the diol **13** with similarly high diastereoselectivity ($\geq 97\% \text{ ds}$) to that in the solution-phase reaction with **10**. An essentially quantitative conversion of **9** to **12** could be obtained, as determined by ^{13}C NMR analysis and cleavage to give **13** in 98 % yield, by adopting a two-cycle protocol. Thus, 20 h after the first addition of enolate **11**, the resin was washed with Et_2O and dried, fresh enolate solution was again added, then after a further 16 h an oxidative workup (H_2O_2 , MeOH , DMF , pH 7 buffer) was performed, followed by a final washing and drying of the resulting resin **12**.

To achieve efficient *syn*-selective reduction on solid phase, a novel modification of the Narasaka reduction protocol^[10] was developed. Treatment of the resin-supported ketone **12** with $(\text{cHex})_2\text{BCl}$ and Et_3N regenerated the dicyclohexylboron aldolate,^[11] which was reduced efficiently by LiBH_4 to give the 1,3-*syn*-diol **14**. Gratifyingly, through this sequence of aldol addition, reduction, and cleavage from the resin using tetrabutylammonium fluoride (TBAF), the triol **15** was obtained in excellent yield (98 %) with high diastereoselectivity (95 % *ds*), comparable to that obtained from **10** using solution-phase chemistry (91 %, $\geq 97\% \text{ ds}$).

Diversification in the β -hydroxy ketone reduction step was achieved by using a complementary *anti*-selective protocol. We found that the Evans–Tishchenko reduction^[12] can be adapted successfully to solid phase, provided SmI_2 is employed stoichiometrically to achieve complete conversion. In this way, the ester **16** was prepared with $\geq 97\% \text{ ds}$, which gave the corresponding 1,3-*anti*-diol on reaction with LiBH_4 .

Having installed four stereocenters on the growing chain anchored to the resin support, some functional group

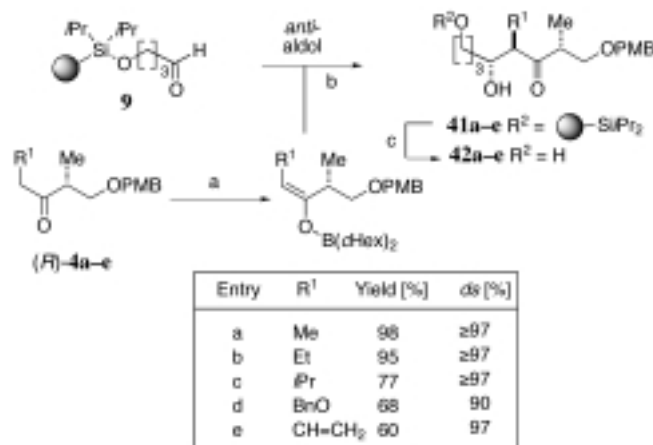


Scheme 3. Solid-phase synthesis of aldehydes **18** and **20** from **9** (loading = 0.75 mmol g^{-1}) using *anti*-aldol addition and *syn* or *anti* reductions. a) $i\text{Pr}_2\text{NET}$, DMAP , CH_2Cl_2 , 20°C , 48 h; b) O_3 , CH_2Cl_2 , -78°C ; Ph_3P , 20°C , sonication, 16 h; c) $(\text{cHex})_2\text{BCl}$, Et_3N , Et_2O , 0°C , 4 h; d) 1) **11**, Et_2O , $-78 \rightarrow 0^\circ\text{C}$, 20 h; filter and wash (two cycles); 2) H_2O_2 (30 % aq), MeOH , DMF , pH 7 buffer, 0°C , 1 h; e) HF/py , py , MeCN , 20°C , 16 h; f) 1) $(\text{cHex})_2\text{BCl}$, Et_3N , Et_2O , 0°C , 4 h; 2) LiBH_4 , Et_3N , -78°C , 4 h; 3) H_2O_2 (30 % aq), MeOH , NaOH (10 % aq), CH_2Cl_2 , 20°C , 16 h; g) TBAF, THF , 20°C , 4 h; h) EtCHO , SmI_2 , THF , $-10 \rightarrow 0^\circ\text{C}$, 20 h; i) $(\text{MeO})_2\text{CMe}_2$ or MeOC(Me)=CH_2 , CSA , CH_2Cl_2 , 20°C , 48 h; j) DDQ, pH 7 buffer/ CH_2Cl_2 (20/1), 20°C , 3 h; k) $\text{py} \cdot \text{SO}_3$, DMSO , Et_3N , CH_2Cl_2 , 0°C , 6 h; l) LiBH_4 , THF , $-78 \rightarrow 20^\circ\text{C}$, 16 h. *cHex* = cyclohexyl, *CSA* = camphorsulfonic acid, *DMF* = *N,N*-dimethylformamide, *DDQ* = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, *DMAP* = 4-(*N,N*-dimethylamino)-pyridine, *DMSO* = dimethyl sulfoxide, *py* = pyridine.

manipulation was now required to regenerate the aldehyde functionality, thus enabling access to extended polyketide sequences. These transformations were selected to be compatible with the resin support and orthogonal to the silyl linker.^[13] First, the diol **14** was converted into the *syn*-acetone **17**, which had the expected ^{13}C NMR resonances ($\delta = 97.6$, 30.0 , and 19.5).^[14] The aldehyde was then introduced by deprotection of the PMB ether using DDQ,^[15] followed by Parikh–Doering oxidation^[16] using $\text{py} \cdot \text{SO}_3$ and DMSO to

these 1,3-diols into the corresponding acetonides **37** and **38** allowed characterization by ^{13}C gel-phase NMR spectroscopy.^[14] Subsequent cleavage from the resin led to the isolation of the stereochemically pure alcohols **39** (79%) and **40** (62%) after chromatography. Since the enantiomeric ketone (*S*)-**4a** and (–)-Ipc₂BOTf are available, it should be possible to access 12 out of the 16 possible stereotetrads per iteration with high diastereoselectivity.^[20]

Finally, having demonstrated stereochemical permutations in the aldol addition and reduction steps, the introduction of further diversity by combinatorial variation of the chain extension reagent was addressed (Scheme 6). Five different



Scheme 6. Solid-phase synthesis of a library of β -hydroxy ketones **42a–e**. a) (cHex)₂BCl, Et₃N, Et₂O, 0 °C (–78 °C for **4d**), 4 h; b) 1) Et₂O, –78 → 26 °C, 16 h (two cycles); 2) H₂O₂ (30% aq), MeOH, DMF, pH 7 buffer, 0 °C, 16 h; c) HF/py, py, MeCN, 20 °C, 16 h.

ketones (*R*)-**4a–e** (R¹ = Me, Et, *i*Pr, BnO, CH=CH₂, respectively)^[21] were chosen for library generation. Resin **9** was split into batches, to which each was added a different boron enolate, and two cycles of enolate addition were performed. After oxidative workup, cleavage of the resin-supported adducts **41a–e** with HF/pyridine gave the *anti*-configured β -hydroxy ketones **42a–e** with high diastereoselectivity.

In conclusion, we have demonstrated that boron-mediated aldol reactions of the chiral ketones **4** can be used for the efficient, solid-phase synthesis of novel polyketide-type sequences. Notably, better yields and comparable diastereoselectivities can usually be achieved in comparison with the conventional solution-phase reactions, which opens up the possibility of automation. While this approach mimics the processive mechanism of chain growth operating in the biosynthesis of polyketides, it enables much greater structural and stereochemical diversification through variation of the chain extension reagents, as well as in the stereochemistry of the aldol and reduction steps. In principle, a large variety of starter units,^[7] immobilized on a solid support, and a wide range of chain extending units can be employed in a combinatorial fashion, leading to large, structurally diverse, libraries of polyketide-type sequences. Thus, this approach complements the combinatorial generation of new polyketide structures based on the genetically engineered reconstruction of biosynthetic pathways.^[5]

Experimental Section

Representative procedure for *anti*-aldol reaction of **4a** with resin **9**:^[3c] To a cooled solution (0 °C) of (cHex)₂BCl (1.80 mL, 8.5 mmol) in dry Et₂O (23 mL) under Ar was added Et₃N (1.25 mL, 9.0 mmol). After 10 min (*R*)-**4a** (1.18 g, 5.0 mmol) was added and the reaction mixture stirred for a further 4 h. The resulting solution of enolate **11** was added by cannula to the resin **9** (1.0 g) cooled to –78 °C and shaking was maintained for 1 h, before storing in the freezer (–26 °C, 16 h). After shaking for a further 4 h at 0 °C, the enolate solution was filtered off and the resin washed with Et₂O and dried under reduced pressure. A second cycle was then carried out. To the resin cooled to –78 °C, a solution of enolate **11**, prepared as above, was added and shaking was maintained for 1 h, before storing in the freezer (–26 °C, 16 h). The resin was filtered and washed sequentially with pH 7 buffer, H₂O, H₂O/THF, THF, and MeOH. To the resin at 0 °C were added DMF (4 mL), MeOH (2 mL), pH 7 buffer (4 mL), and H₂O₂ (30%; 12 mL) and shaking was continued for 1 h before storing in the freezer (–26 °C) for 16 h. After filtering, the resin was washed with H₂O, H₂O/THF, THF, CH₂Cl₂, and MeOH and dried under reduced pressure at 50 °C for 3 h. This gave the pale yellow resin **12**. IR (KBr disk): $\tilde{\nu}$ = 3440, 1708, 1098 cm^{–1}; ^{13}C NMR (100.6 MHz, CD₂Cl₂): δ = 217.8, 159.4, 130.2, 129.4, 113.8, 73.5, 73.0, 72.3, 64.0, 55.3, 52.2, 45.8, 31.3, 29.1, 17.4, 13.6, 13.4, 12.6, 12.2.

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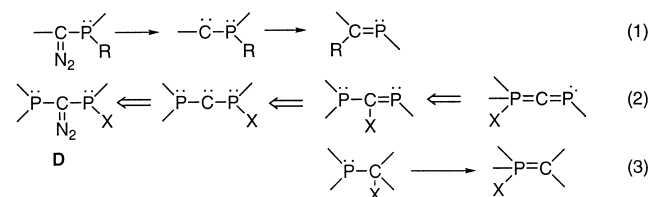
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and Guy Bertrand*



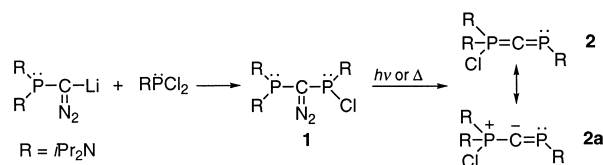


(two different types of phosphorus–carbon “double” bond and a lone pair at one of the phosphorus atoms) has only been postulated as an intermediate, but never observed spectroscopically.^[3]

Our synthetic strategy was based on two consecutive rearrangements: the well-established 1,2-migration reaction of singlet carbenes,^[4] which operates for phosphinocarbenes [Eq. (1)],^[5] and the 1,2-halogen shift associated with α -halogenophosphanes [Eq. (3)].^[6] Combining these two 1,2-migration reactions, a possible precursor for the preparation of the desired $1\sigma^4, 3\sigma^2$ -diphosphaallene can be identified as the (phosphino)(chlorophosphino)diazomethane of type **D** [Eq. (2)].



Exactly this type of derivative, namely [bis(diisopropylamino)phosphino][chloro(diisopropylamino)phosphino]diazomethane (**1**), is readily available in one step by addition of the lithium salt of [bis(diisopropylamino)phosphino]diazomethane^[7] to dichloro(diisopropylamino)phosphane (Scheme 1).



Scheme 1.

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The photolysis (300 nm) or thermolysis (70 °C) of a solution of the diazo derivative **1** in toluene leads to the formation of the cumulene **2**, as only one isomer, in near quantitative yield (according to ^{31}P NMR spectroscopy).

The $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of **2** showed two doublets at $\delta = 302.8$ and 61.2 ($^2J(\text{P},\text{P}) = 240.1$ Hz) in the regions expected for a σ^2 - and a σ^4 -phosphorus atom, respectively.^[8] The signal for the dicoordinate carbon atom appears as a doublet of doublets at $\delta = 169.9$ ($^1J(\text{P},\text{C}) = 36.5$, 9.7 Hz), with a chemical shift approximately halfway between the values observed for carbodiphosphoranes **A** and diphosphaallenes **B**.^[9] The molecular structure of **2** was unambiguously established by an X-ray diffraction study (Figure 1).^[10] The C1–P1 bond length

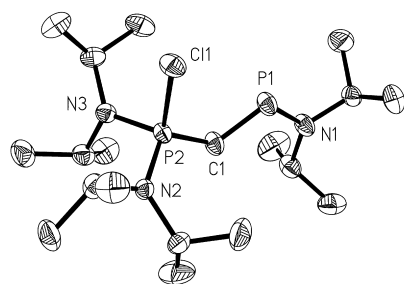
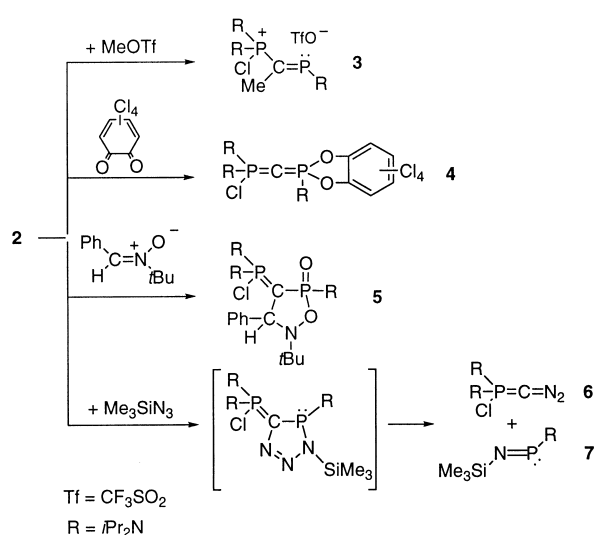


Figure 1. Molecular structure of **2**. Selected bond lengths [Å] and angles [°]: C1–P1 1.6429(13), C1–P2 1.6503(13), P1–N1 1.6707(11), P2–N2 1.6393(11), P2–N3 1.6627(11), P2–Cl1 2.1267(4); C1–P1–N1 108.05(6), P1–C1–P2 120.88(9), C1–P2–Cl1 115.99(5), C1–P2–N2 113.91(6), C1–P2–N3 116.48(7).

(1.64 Å) is markedly shorter than an isolated C=P bond,^[11] but comparable with the value observed for a diphosphaallene of type **B**.^[2] The planarity of the N1–P1–C1–P2–Cl1 fragment (maximum deviation 0.0169 Å) and the value of the P1–C1–P2 angle of 120.88° are in agreement with an sp^2 -hybridized carbon atom. As expected, the molecule has an *E* configuration. The presence of a lone pair of electrons at C1 (form **2a**; Scheme 1) interacting with P2 is confirmed by the short P2–C1 bond length (1.65 Å) and the P2–Cl1 bond in an eclipsed position to the C1–P1–N1 fragment. This so-called negative hyperconjugation^[12] transfers the electron density from the occupied sp^2 orbital on the C1 atom to the energetically low-lying σ^* orbital of the P2–Cl1 bond.^[13] Therefore, derivative **2** can be considered as an heterocumulene (Scheme 1).

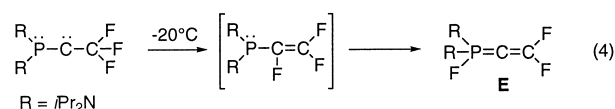
Derivative **2** is a versatile and selective reagent as illustrated by the reactions given in Scheme 2. Owing to the presence of an ylidic carbon atom, **2** reacts with an alkylating agent such as methyl trifluoromethanesulfonate to give the expected C-phosphoniophosphaalkene **3** as only one isomer (59% yield).^[14] The lone pair of electrons at the phosphorus center can be used for a formal [1+4] cycloaddition with tetrachloro-*o*-benzoquinone to afford the carbodiphosphorane **4**, which was isolated in 62% yield. Note that the alternative [2+4] cycloadduct involving the P=C bond was not observed.^[15] However, the phosphorus–carbon π bond remains an excellent dipolarophile as shown by the reaction with a nitron. Heterocycle **5**, resulting from a diastereoselective [2+3] cycloaddition, followed by a subsequent oxida-



Scheme 2.

tion by the excess of nitron, was isolated in 85% yield and fully characterized both spectroscopically and by a single-crystal X-ray diffraction study.^[9b)] A [3+2] cycloaddition reaction also occurs with trimethylsilyl azide, but the resulting adduct fragments cleanly into the iminophosphane **6** and the diazomethylenephosphorane **7**.^[16] This is an attractive synthetic route for this very unique stable diazocumulene.^[17]

The formation of the $1\sigma^4,3\sigma^2$ -diphosphaallene **2** from **1**, along with the recently described synthesis of the σ^4 -phosphaallene **E** [Eq. (4)],^[18] strongly suggest that the sequence of two successive 1,2-halogen migrations should become a general route for the preparation of a variety of hitherto unknown heterocumulenes featuring a σ^4 -phosphorus center.



Experimental Section

All operations were carried out under argon using Schlenk tube techniques.

1: A solution of dichloro(diisopropylamino)phosphane (3.10 g, 15.3 mmol) in THF (20 mL) was added to a solution of the lithium salt of [bis(diisopropylamino)phosphino]diazomethane^[7] (15.3 mmol) in THF (20 mL) at -78°C . The reaction mixture was allowed to warm to 0°C over a period of 1 h. After evaporation of the solvent under vacuum at 0°C , the residue was quickly extracted with pentane (20 mL) and filtered. After the volume of solvent had been reduced to 5 mL, orange crystals of **1** (4.02 g, 60%) were obtained at -20°C ; m.p. 30°C (decomp). $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): $\delta = 110.6$, 59.0 (d, $^2J(\text{P},\text{P}) = 239.6$ Hz); IR (pentane): $\tilde{\nu} = 2039$ cm^{-1} (CN_2).

2: A solution of the diazo derivative **1** (0.044 g, 0.1 mmol) in toluene (1 mL) was heated at 70°C for 4 h. ^{31}P NMR spectroscopy indicated the quantitative formation of **2**. After evaporation of the solvent, **2** was obtained as pale yellow crystals from a saturated pentane solution at 0°C (0.037 g, 90%); m.p. 73 – 74°C .

The experiments used to prepare **3**–**5** were performed with a solution of freshly prepared cumulene **2** (0.041 g, 0.1 mmol) in pentane or toluene (1 mL).

3: Methyl trifluoromethanesulfonate was added to a solution of **2** at -78°C and the alkylated compound **3** precipitated as a white powder from the

solution mixture (0.034 g, 59 %); m.p. 136–137 °C. $^{31}\text{P}\{^1\text{H}\}$ NMR (CDCl_3): $\delta = 309.7, 79.9$ (d, $^2J(\text{P,P}) = 192.5$ Hz); ^1H NMR (CDCl_3): $\delta = 2.23$ (dd, $^3J(\text{P,H}) = 6.8, 23.0$ Hz, 3H; C-CH₃); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): $\delta = 18.8$ (dd, $^2J(\text{P,C}) = 3.4, 3.7$ Hz, C-CH₃), 109.7 (dd, $^1J(\text{P,C}) = 131.3, 64.5$ Hz, PCP).

4: Tetrachloro-*o*-benzoquinone was added to a solution of **2** at –78 °C and the resulting carbodiphosphorane **4** was crystallized from a toluene/pentane solution at –20 °C as white crystals (0.031 g, 48 %); m.p. 143–144 °C. $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6): $\delta = 25.5, 21.5$ (d, $^2J(\text{P,P}) = 126.4$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6): $\delta = 38.3$ (dd, $^1J(\text{P,C}) = 149.4, 187.2$ Hz, P=C=P).

5: The nitron $\text{Ph}(\text{H})\text{C}=\text{N}(\text{O})\text{tBu}$ was added to a solution of **2** at room temperature and the solution was heated at 55 °C for 15 days. The cycloadduct **5** precipitated from an $\text{Et}_2\text{O}/\text{CH}_3\text{CN}$ solution at –20 °C as colorless crystals (0.051 g, 85 %); m.p. 160–161 °C. $^{31}\text{P}\{^1\text{H}\}$ NMR (CDCl_3): $\delta = 71.0, 46.8$ (d, $^2J(\text{P,P}) = 67.8$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): $\delta = 40.0$ (dd, $^1J(\text{P,C}) = 131.4, 17.5$ Hz, P-C-P), 72.2 (dd, $^2J(\text{P,C}) = 14.7, 6.4$ Hz, Ph-C-H).

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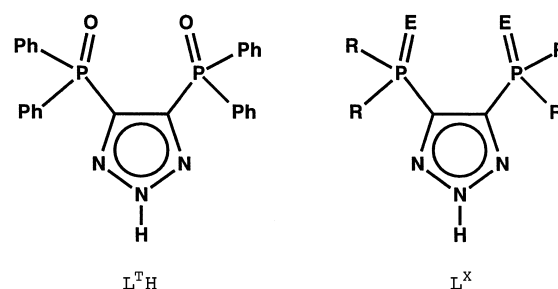
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4,5-Bis(diphenylphosphinoyl)-1,2,3-triazole: A Powerful New Ligand That Uses Two Different Modes of Chelation

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We report a novel and powerful ligand that is thermally, oxidatively, and hydrolytically very stable. It avidly forms complexes with cations through the adoption of two entirely different chelation modes, allowing the facile extraction of metal ions from aqueous systems. This novel ligand is the anion $[\text{L}^{\text{T}}]^-$ of 4,5-bis(diphenylphosphinoyl)-1,2,3-triazole $\text{L}^{\text{T}}\text{H}$, which combines the features of the coordinating ability

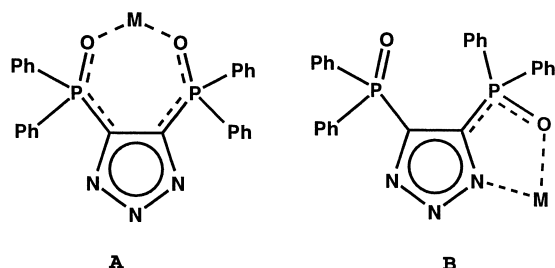


of the $\text{P}(\text{O})\text{Ph}_2$ groups with the nitrogen donor atoms of the triazole ring. Moreover, it is the prototype of a much broader family of ligands with the general structure L^{X} , in which E can

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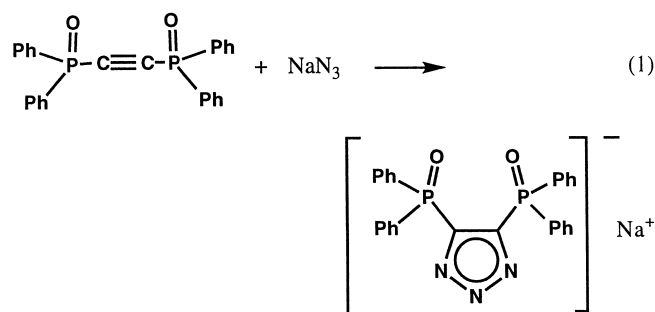
be S, Se, NR, or NAr, while R can be a variety of alkyl or aryl groups.

As can be seen from structures **A** and **B** there are two possible modes of chelation for $[L^T]^-$: a symmetric one (C_{2v}), through the two O termini, forming a seven-membered



chelate ring (**A**), and an asymmetric one, through one O and the nearest N, resulting in a five-membered chelate ring (**B**). In addition, nucleophilic reactivity is also possible through the central nitrogen. These chelation modes of $[L^T]^-$ are quite different from the six-membered chelate rings formed by the known $P(O)Ph_2$ -containing β -diketonate analogues, such as those derived from the anions $HN[P(O)Ph_2]_2$,^[1–3] $HC[P(O)Ph_2]_2$,^[4] or 4-diphenylphosphinoyl-3-methyl-1-phenylpyrazolone.^[5] Each of the chelating modes for L^T has been demonstrated, and pertinent key structures of type **A** and **B** have been established by X-ray crystallography.

The sodium salt of L^TH , $Na[L^T]$, was obtained directly by the reaction of NaN_3 with bis(diphenylphosphinoyl)acetylene in methanol [Eq. (1)],^[6] and this solution could be used directly for the preparation of metal chelates.



Acidification of the $Na[L^T]$ solution with HCl yielded the free acid L^TH , which melted sharply at 333–334 °C. It formed a halocarbon-soluble triethylamine salt, $[L^T][Et_3NH]$, used for reactions of the L^T ligand in organic solvents.

In an exploratory screening of the coordination behavior of $[L^T]^-$ with first-row transition metals, we found that Fe^{II} , Co^{II} , Ni^{II} , and Cu^{II} were readily extracted from aqueous solutions into chloroform or dichloromethane. The isolated solid chelates, the colors of which were suggestive of octahedral coordination, were also soluble in hot aromatic hydrocarbons, but crystals suitable for X-ray crystallographic determination could not be grown from these solvents. Considering that, regardless of the mode of chelation, there are still three additional donor atoms available from each L^T ligand, we

assumed the presence of extended oligomeric or polymeric structures for all these species. The cobalt complex $[Co(L^T)_2]$ was recrystallized from pyridine, yielding the structurally characterized compound **1** (Figure 1) of composition $[Co(L^T)_2(py)_2] \cdot 2py$ (py = pyridine). In **1**, the cobalt ion was N,O-bonded in a square-planar configuration to two L^T ligands, and also to two apical pyridine N donors. The two L^T ligands were coordinated in *trans* fashion.

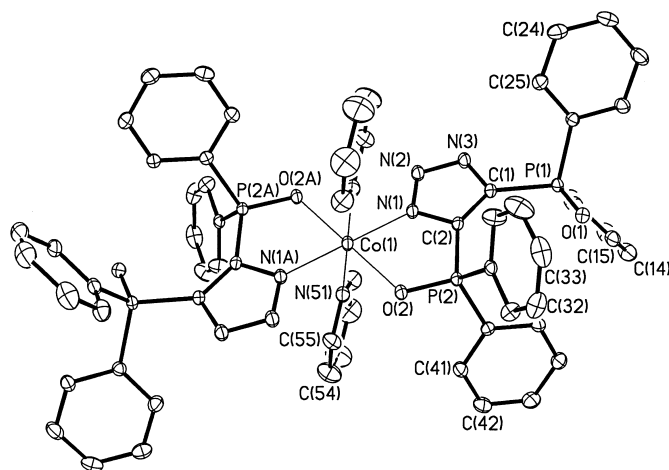


Figure 1. Molecular structure of $[Co(L^T)_2(py)_2] \cdot 2py$ (**1**) (30% thermal ellipsoids). Selected bond lengths [Å] and angles [°]: Co(1)–N(1) 2.122(2), Co(1)–O(2) 2.121(1), Co(1)–N(51) 2.203(2); N(1)–Co(1)–O(2) 82.17(6), N(1)–Co(1)–O(2a) 97.83(6), N(51)–Co(1)–O(2) 88.85(7), N(51)–Co(1)–N(1) 92.24(7); *trans* angles at Co(1) 180.

The same κ^2 N,O-mode of chelation was found in other structurally characterized complexes, such as $[Rh(L^T)(cod)]$ (**2**; cod = 1,5-cyclooctadiene; Figure 2), produced by the reaction of $[L^T][Et_3NH]$ with $[Rh(cod)Cl]_2$, as well as in the heteroleptic five-coordinate complexes $[Co(L^T)(Tp^{aNi})]$ (**3**; Figure 3), and $[Ni(L^T)(Tp^{iPr,4Br})]$ (**4**; Figure 4), obtained by the reaction of $[L^T][Et_3NH]$ with $Co[TP^{aNi}]Cl$ and $Ni[TP^{iPr,4Br}]Cl$, respectively.^[7]

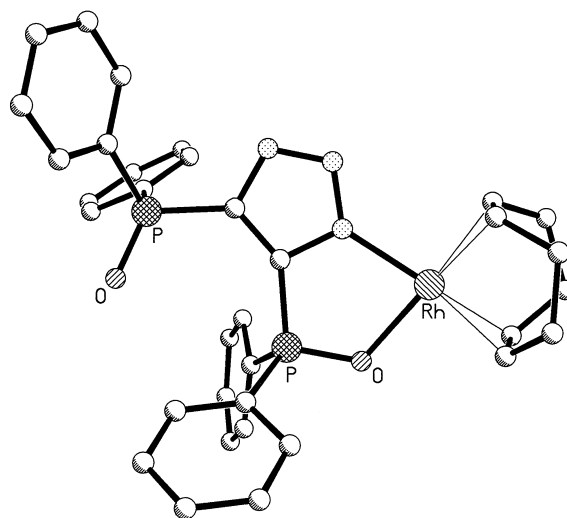


Figure 2. The structure of $[Rh(L^T)(cod)]$ (**2**).

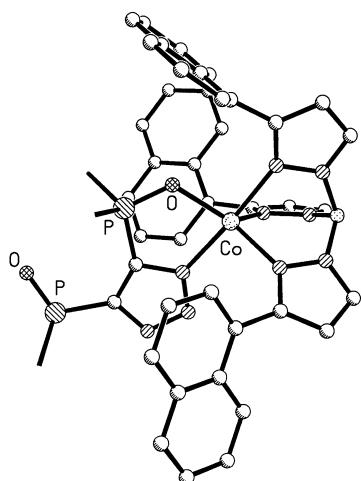


Figure 3. The structure of $[\text{Co}(\text{L}^{\text{T}})(\text{Tp}^{\text{nBu}})]$ (**3**). The phenyl groups are not shown for the sake of clarity.

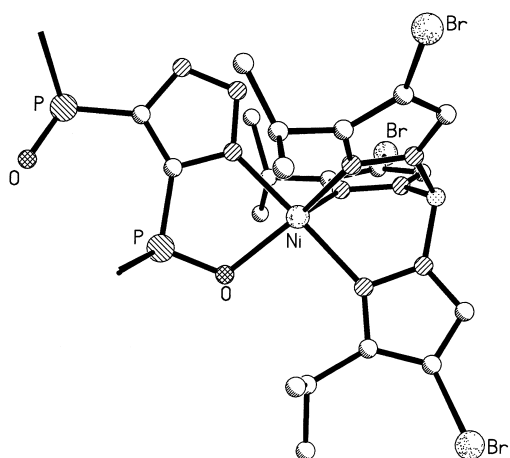


Figure 4. The structure of $[\text{Ni}(\text{L}^{\text{T}})(\text{Tp}^{\text{Pr,4Br}})]$ (**4**). The phenyl groups are not shown for the sake of clarity.

There was practically no difference in the triazolyld C–P bond lengths in the coordinated and the free POPh_2 substituents: they were all in a narrow 1.78–1.81 Å range. On the other hand, P–O bond lengths in the coordinated POPh_2 substituents were slightly longer than in the uncoordinated ones.

Anticipating that O,O-bonding might be achieved with more oxophilic metal ions, in spite of the sterically disfavored seven-membered ring, we synthesized the uranium complex, obtained as a DMF monosolvate, $[\text{UO}_2(\text{L}^{\text{T}})_2(\text{dmf})]$ (**5**; Figure 5), in which both L^{T} ligands were O,O-bonded, and the uranyl ion was in a pentagonal-bipyramidal seven-coordinate environment.

Further structurally characterized examples of L^{T} acting in κ^2 O,O-bonding fashion were also obtained. One such example was the lanthanum(III) complex $[\text{La}(\text{L}^{\text{T}})_3]$ (**6**; Figure 6), which was recrystallized from DMF. In **6**, all three L^{T} ligands were O,O-coordinated, along with two additional O-bonded DMF molecules, placing La^{III} in an eight-coordinate environment. Another example was the O,O-chelated octahedral $[\text{Mg}(\text{L}^{\text{T}})_3]^-$ ion **7**, obtained by the reaction of

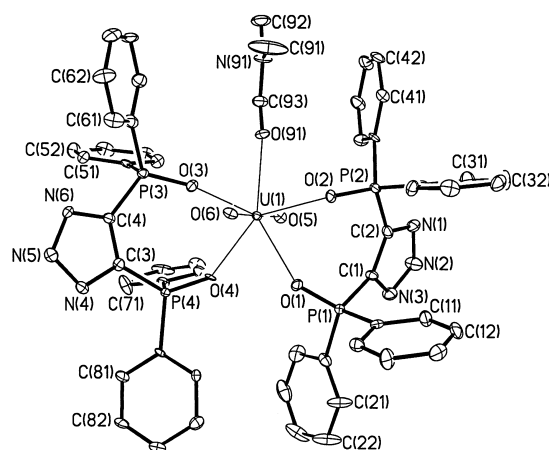


Figure 5. Molecular structure of $[\text{UO}_2(\text{L}^{\text{T}})(\text{dmf})] \cdot \text{DMF}$ (**5**) (30 % thermal ellipsoids). Selected bond lengths [Å] and angles [°]: U(1)–O(1) 2.380(6), U(1)–O(2) 2.372 (6), U(1)–O(3) 2.348(6), U(1)–O(4) 2.352(5), U(1)–O(5) 1.779(6), U(1)–O(6) 1.775(6), U(1)–O(91) 2.407(6); O(1)–U(1)–O(2) 71.6(2), O(3)–U(1)–O(4) 73.8(2), O(5)–(1)–O(6) 178.0(3), O(1)–U(1)–O(4) 71.2(2), O(2)–U(1)–O(3) 143.2(2).

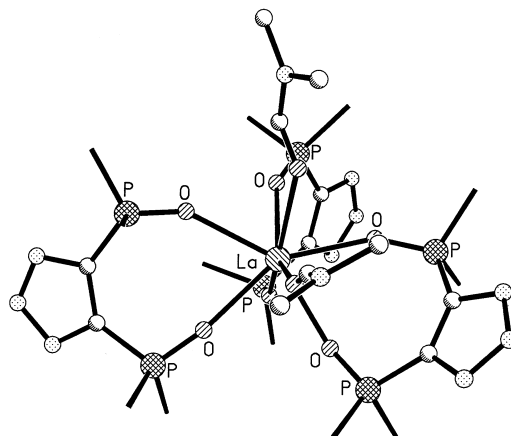


Figure 6. The structure of $[\text{La}(\text{L}^{\text{T}})_3(\text{dmf})_2]$ (**6**). The phenyl groups are not shown for the sake of clarity.

MgSO_4 with $[\text{L}^{\text{T}}][\text{Et}_3\text{NH}]$ in chloroform, even in the presence of excess MgSO_4 . This anion was isolated and structurally characterized as the Et_3NH salt (Figure 7).

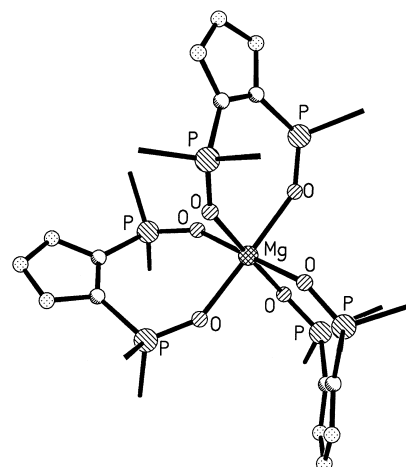


Figure 7. The structure of the anion $[\text{Mg}(\text{L}^{\text{T}})_3]^-$ in $[\text{Mg}(\text{L}^{\text{T}})_3][\text{Et}_3\text{NH}]$ (**7**). The phenyl groups are not shown for the sake of clarity.

The average U–O distances in $[\text{UO}_2(\text{L}^{\text{T}})_2(\text{dmf})] \cdot \text{DMF}$ (2.378 Å) were comparable to those in $[\text{UO}_2(\text{acac})_2(\text{H}_2\text{O})]^{[8]}$ and in $[\text{UO}_2(\text{acac})_2(\text{py})]^{[9]}$ while the O–M–O angles were marginally larger than those in related acac complexes such as $[\text{UO}_2(\text{acac})_2(\text{py})]^{[9]}$ and $[\text{UO}_2(\text{acac})_2(\text{H}_2\text{O})]^{[8]}$. In $[\text{La}(\text{L}^{\text{T}})_3(\text{dmf})_2]$ the average O–La–O angle was 71.5° , as compared with 68.5° in $[\text{La}(\text{acac})_3(\text{H}_2\text{O})_2]^{[10]}$. Finally, the average O–Mg–O angles for $[\text{Mg}(\text{L}^{\text{T}})_3]^-$ and $[\text{Mg}(\text{acac})_2(\text{H}_2\text{O})_2]$ were 90.0 and 82.7° , respectively; the very large difference is probably due to the steric crowding of twelve phenyl groups of the three coordinated L^{T} ligands around the small Mg^{2+} ion.^[11]

Nucleophilic reactivity was demonstrated for the central nitrogen atom of L^{T} in the methylation reaction of the $[\text{L}^{\text{T}}]^-$ ion with methyl iodide, which yielded 2-methyl-4,5-bis(diphenylphosphinoyl)-1,2,3-triazole, as implied by its ^1H and ^{31}P NMR spectra which were indicative of C_{2v} symmetry. This neutral compound should be capable of the same reactions as the $[\text{L}^{\text{T}}]^-$ ion, except that the resulting complexes will be cationic. The relationship between $[\text{L}^{\text{T}}]^-$ and $[\text{L}^{\text{T}}]\text{-2-Me}$ is reminiscent of that between $[\text{HB}(\text{pz})_3]^-$ ($=\text{Tp}$; pz = pyrazolyl) and its neutral $\text{HC}(\text{pz})_3$ analogue.

In summary, we have reported the synthesis of 4,5-bis(diphenylphosphinoyl)-1,2,3-triazole, $\text{L}^{\text{T}}\text{H}$, a powerful novel ligand which is the prototype of a new ligand class, and have demonstrated examples of its two different modes of coordination. This versatile chelating agent has potential not only as metal extractant or as a stable ligand core in diverse types of catalysis, but also as a possible model for N_2PO , and $(\text{PO})_2$ bonding modes for, for example, magnesium in biological systems. We are currently working at defining the boundaries for the different coordination modes of L^{T} as a function of the metal ion, and exploring the coordination chemistry of several variants of the L^{T} ligand system.

Experimental Section

Bis(diphenylphosphinoyl)acetylene was prepared by the literature method.^[6] Satisfactory elemental analyses were obtained for all of the compounds mentioned below. NMR spectra were determined in CDCl_3 .

$\text{L}^{\text{T}}\text{H}$: To a slurry of bis(diphenylphosphinoyl)acetylene (42.6 g) in methanol (200 mL) was added in one portion NaN_3 (7.0 g). The solid dissolved exothermally, yielding a solution of $[\text{Na}[\text{L}^{\text{T}}]]$, which could be used directly for the preparation of L^{T} complexes. This was done by mixing aliquot portions of the $[\text{Na}[\text{L}^{\text{T}}]]$ solutions with an aqueous solution of the appropriate metal perchlorate or nitrate salt. After dilution with a tenfold volume of water, the products were extracted with dichloromethane, passed through a short alumina column, and the eluate was evaporated. The free ligand $\text{L}^{\text{T}}\text{H}$ was obtained by pouring the NaL^{T} solution into aqueous HCl , and purifying the filtered, water-washed, and dried precipitate by recrystallization from boiling DMF. M.p. $333\text{--}334^\circ\text{C}$.

$[\text{L}^{\text{T}}][\text{HNEt}_3]$: To a slurry of $\text{L}^{\text{T}}\text{H}$ in methanol was added excess triethylamine, and the resulting solution was evaporated to dryness. The residue was stirred with acetone and filtered. This yielded $[\text{L}^{\text{T}}][\text{HNEt}_3]$, which gradually darkened and decomposed from 330°C . This salt is very soluble in dichloromethane and chloroform. ^1H NMR: $\delta = 7.69$ (m, 8H; Ph), 7.31 (m, 4H; Ph), 7.20 (m, 8H; Ph), 3.13 (quart., 6H; Et), 1.13 (t, 9H; Et); ^{31}P NMR: $\delta = 19.2$.

$[\text{L}^{\text{T}}]\text{-2-Me}$ was prepared by adding a slight excess of MeI to a solution of $[\text{L}^{\text{T}}]\text{Na}$, and stirring the solution overnight. Addition of water and chloroform led to two phases, and the product was isolated from the organic phase. M.p. $210\text{--}211^\circ\text{C}$. ^1H NMR: $\delta = 7.65$ (m, 8H; Ph), 7.44 (m, 4H; Ph), 7.29 (m, 8H; Ph), 4.17 (s, 3H; Me); ^{31}P NMR: $\delta = 18.68$.

Crystallographic data for $[\text{Co}(\text{L}^{\text{T}})_2(\text{py})_2] \cdot 2\text{py}$ (**1**) and $[\text{UO}_2(\text{L}^{\text{T}})(\text{dmf})] \cdot \text{DMF}$ (**5**) were collected on a Bruker P4/CCD diffractometer. For **1**: $\text{C}_{72}\text{H}_{60}\text{CoN}_{10}\text{O}_4\text{P}_4$, triclinic, $P\bar{1}$, $\phi_w = 1312.11$, $a = 10.37830(8)$, $b = 13.3831(2)$, $c = 13.5025(2)$ Å, $\alpha = 113.0332(9)$, $\beta = 98.0195(7)$, $\gamma = 97.1090(7)^\circ$, $V = 1675.97(6)$ Å³, $Z = 1$, $\rho_{\text{calcd}} = 1.300$ g cm⁻³, $\mu(\text{MoK}\alpha) = 4.08$ cm⁻¹, $T(\text{min}/\text{max}) = 0.776, 0.998$; 10866 data collected, 7059 independent, 412 parameters refined, $R(1) = 0.0482$ ($I = 2\sigma(I)$), $wR2 = 0.1042$, $T = 213(2)$ K. For **5**: $\text{C}_{36}\text{H}_{36}\text{N}_8\text{O}_8\text{P}_4\text{U}$, monoclinic, Pn , $\phi_w = 1353.00$, $a = 15.4055(1)$, $b = 10.6835(1)$, $c = 18.6390(2)$ Å, $\beta = 111.80(2)^\circ$, $V = 2848.26(3)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.578$ g cm⁻³, $\mu(\text{MoK}\alpha) = 30.23$ cm⁻¹, $T(\text{min}/\text{max}) = 0.464, 0.864$; 10302 data collected, 7054 independent, 712 parameters refined $R(1) = 0.0331$ ($I = 2\sigma(I)$), $wR2 = 0.0992$, $T = 193(2)$ K, absolute structure parameter = 0.023(6). Both samples were mounted on fine glass fibers. Compound **1** was found to be triclinic and the centrosymmetric alternative was initially chosen based on its greater frequency of occurrence. Compound **5** was found to be monoclinic; of the two space groups in agreement with the systematic absences, Pn and $P2_1/n$, the former was chosen due to a lack of rotational symmetry in the complex. Both choices were supported by the results of refinement which yielded narrow ranges for chemically related bond parameters. The data were corrected for absorption using SADABS.

Both structures were solved by direct methods and completed from subsequent difference maps. The asymmetric unit for **1** consists of one half of the Co complex residing on an inversion center and a molecule of pyridine, and for **5**, one U complex and a molecule of DMF. All non-hydrogen atoms were anisotropically refined and hydrogen atoms were placed in idealized locations. All software and sources of scattering factors were contained in the SHELXTL (5.1) library of programs (G. Sheldrick, Bruker-AXS, Madison, WI). 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-143802 (**1**) and CCDC-143803 (**5**). 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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